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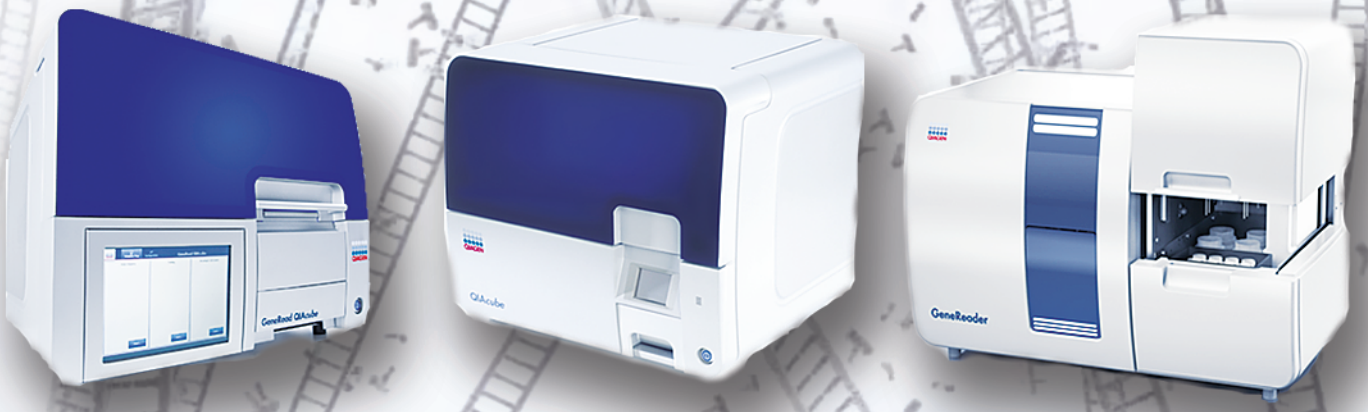

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PHILIPPINE JOURNAL OF PATHOLOGY

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

Welcome to the 2nd issue of the Philippine Journal of Pathology or PJP. Congratulations to the editorial team and the PSP Board of Governors for a job well done.

Though our journal has gone a long way from its inception, many challenges remain. We are fully aware of the fact that establishing a viable scholarly journal takes time and effort. We certainly cannot succeed without the understanding and support of all our PSP members.

We are delighted that you are joining us as readers and hope you will also join us as contributors. We look forward to welcoming your submissions.

Let us look forward to many more issues. More power to the PSP and PJP!

Januario D. Veloso, MD, FPSP
President, Philippine Society of Pathologists

It Takes A Village

With this first issue for 2017, we are glad to announce that the Philippine Journal of Pathology is now officially a member of the Philippine Association of Medical Journal of Editors (PAMJE). Established in 2011 through the Philippine Council for Health Research Development of the Department of Science and Technology, the PAMJE *strives to raise the quality of medical and health-related journal publishing in the Philippines.*[1] It aims to ensure the quality and dissemination of health-related information published in medical journals, utilized for the purposes of better-decision making and effective delivery of health services. With the PJP's inclusion, it aspires to further build capacity of its editorial team through participation in the association's activities.

My first job straight off pathology residency back in 2010 was as editorial coordinator for a regional subspecialty journal on endocrinology which was being planned to be revived. It was to be funded through society support from ASEAN member states and hosted in Manila. I remember fidgeting during my panel interview, sitting nervously in the warmly lit *sala* of an old house-turned-restaurant in Binondo, face to face with the future editor-in-chief and a few senior specialists who would be part of the journal's first editorial board, armed only with my amateur experience and driven by my interest in publishing.

Over the course of six years, I was provided a unique opportunity to understand and appreciate the evolving landscape of medical journals, how scientific knowledge is shared, cited and built upon by other scientists, the concept of open access, peer review and editorial deliberation, the tools that can be used to improve journal visibility and searchability, and the resources that are available to ensure that the quality of information is ethical and truthful.

Along the way, I learned how establishment and implementation of solid editorial policies based on international standards will spell the difference between a high quality medical journal and a mediocre one, and how crucial and powerful a factor online presence is in this digital age. Moreover, I realized that being trusted by readers and would-be authors, being eligible for indexing, and being taken seriously by funders, take not just continued, consistent efforts, but also patience and time.

Ultimately though, the valuable lessons I learned in that journal convinced me that we, Filipino pathologists, could actually replicate the publication's success, if we follow the same open-access, society support-driven paradigm which aspires to achieve international publishing standards without short-changing the quality offered by the journal. Having learned from this journal's journey, the PJP is fortunate to start on the right track.

I am overwhelmed at, and humbled by, the financial support being provided by the Philippine Society of Pathologists, Inc for the successful revival of the PJP. This kind of support from the present leadership is encouraging and it actually takes us halfway to sustainability. To complement this, the PJP is looking into partnering with industry through sponsorships, but maintaining our stance against levying article processing fees to authors or asking for payment for subscriptions or downloading of scientific content.

In addition, the society governors and committees can update the country's research agenda for pathology and laboratory medicine, and look into creative ways of encouraging research initiatives, through research grants and calls for proposals, for example. Residency training officers can look into building capacity for the next generation of pathologists on research methods, project management, and promote submission of manuscripts. The society members, for their part, may expand support for its own journal, not only by reading published articles, providing constructive feedback, sharing their expertise as peer reviewers and editors, but more so, through submission.

All of us are enjoined to support the PJP through our different ways, through our different contributions. This second issue, the first for 2017, is dedicated to you.

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

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Development and Pilot Implementation of a Ladderized Biosafety Training Program in a Specialty Infectious Disease Hospital and Research Institute

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ABSTRACT

Biosafety is the application of laboratory practices, use of safety equipment and implementation of procedures in laboratory facilities when working with potentially infectious microorganisms to protect not only the laboratory worker, but also the general public and the environment. Biosafety training specifically structured based on risk is vital to establish a safe working environment to reduce the risks of unintentional exposure and/or intentional release of infectious microorganisms. In 2016, a ladderized 3-step biosafety training program was established by the Research Institute for Tropical Medicine, a specialty infectious disease hospital and National Reference Laboratory in the Philippines. The training program includes 1) Biosafety 101, offered to all new RITM employees; 2) Applied Biosafety training, especially designed for laboratory personnel; and 3) Advanced Biosafety training, focused on developing Biosafety Officers and infectious disease outbreak responders. A 30% increase in awareness on biosafety has been achieved among participants of the first two steps of the program, with the third module to be implemented in 2017.

Key words : biosafety, biosafety training program, biosecurity

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INTRODUCTION

In the past, laboratory practices are directly mentored by senior staff as an orientation procedure prior the conduct of laboratory work. To be able to work independently, individuals must demonstrate successful and acceptable behavior within the laboratory according to the evaluation and trust of senior and experienced laboratory personnel.

Emergence of antimicrobial resistance and highly infectious viral pathogens including MERS CoV, Ebola and Zika virus, comprise health threats that challenge both the public health sector and biosafety experts worldwide. Collection, handling, testing, transportation, storage and disposal of clinical specimens from cases suffering from emerging infectious diseases present significant risks to health care personnel, the community and the environment.

Biosafety is the discipline that addresses the safe handling and containment of infectious microorganisms and hazardous biological materials.[1] The goal of biosafety is to minimize the risk of infection to individual laboratory workers and reduce the accidental or unintentional release of pathogens from the laboratory to the community.[2] It is influenced by the impact of emerging and re-emerging infectious diseases while serving the interest of global public health and protecting laboratory workers all at the same time. Ensuring safe working conditions in laboratories should be based on identified risks, among which are the lack of biosafety and biosecurity awareness and weak implementation of standard biosafety practices.[3] As such, there is a necessity to develop and implement a comprehensive biosafety training program that not only accounts for the risks posed by pathogens and pathogenic materials handled by the laboratory, and its existing facilities and biosafety controls, but also the capability of personnel in identifying and mitigating these risks.

Risk classification for laboratories

Laboratory manipulation of microorganisms can be safely conducted in either a basic laboratory, containment facility, or maximum containment facility based on risk assessment. The World Health Organization (WHO) and US National Institutes of Health (NIH) has recommended pathogens classification for laboratory use that describes the four general risk groups (1-4) based on its individual characteristics and the route of transmission of the natural disease in relation to biosafety levels (Table 1). Laboratory facilities are divided into four categories. Biosafety Level 1 and Biosafety Level 2 (basic), Biosafety Level 3 (containment), and Biosafety Level 4 (maximum containment). Biosafety level designations are based on a combination of design features, construction, containment facilities, equipment, practices and operational procedures required for working with agents from the various risk groups.[1,4] The biosafety level requirement for a specific laboratory task shall be based on risk assessment and professional judgment rather than by risk group classification. Work with novel and dangerous pathogens has to be conducted in an appropriate containment facility by trained and competent laboratory personnel.

Biosafety and laboratory biorisk management

Laboratory biorisk management is the analysis of risks and development of strategies to minimize the likelihood of the occurrence of biorisks, with the overall aim of reducing the risk of accidental exposure to or release of biological hazards.[5] Biosafety training plays a vital role in this risk management strategy. It provides an effective approach to increase biosafety and biosecurity capacity of laboratory workers in a containment laboratory.[2] A well-defined and structured biosafety training enables the application of these concepts and skills in a formal and systematic manner against emerging highly infectious agents.[6] Biosafety professionals working in individual institutions are encouraged to develop and implement site specific and standardized biosafety training programs appropriate for their own laboratory facilities.

The CEN Workshop Agreement (CWA 15793: 2011) on Biorisk Management, WHO Biosafety Guidelines, US BMBL and the Canadian Biosafety Standard and Guidelines highlight the importance of biosafety training requirement to ensure biological safety and security.

BACKGROUND OF THE INSTITUTE

The Research Institute for Tropical Medicine (RITM) was established in 1981 thru Executive Order No. 674 to undertake research activities in the diagnosis, control and prevention of tropical diseases that are major causes of mortality and morbidity in the Philippines; conduct clinical trials aimed at better understanding and control of tropical diseases; conduct regular training courses for medical and paramedical personnel in the control of common tropical diseases in the country; and provide high quality tertiary care to both in-patients and out-patients suffering from tropical diseases included within the scope of the Institute's research activities.

RITM was constructed and equipped by the Japanese government through a Grant-in-Aid of the Japan International Cooperation Agency (JICA) in a 31,000 square meters lot located inside the Filinvest Corporate City, Alabang, Muntinlupa City. Further grant assistance was extended by JICA to RITM for the construction of the Animal Research Laboratories in 1985, Training Center and Residence Hall in 1989, and eventually the National TB Reference Laboratory Building in 2002.

In 1999, production of biologicals and vaccines was added to the mandate of RITM through Executive Order 102, in which the Department of Health Biologicals Production Service (BPS) was merged with the institute.

In November 2000, the DOH, thru the issuance of Department Order No. 393-E, in addition to the functions stated above, designated the Research Institute for Tropical Medicine as the National Reference Laboratory (NRL) for dengue and other arboviruses, influenza and other respiratory viruses, tuberculosis and other Mycobacteria, malaria and other parasites, bacterial enteric diseases, measles and other viral exanthems, mycology, polio and other enteroviruses, antimicrobial resistance, emerging bacterial diseases, and confirmatory testing of blood units. RITM is also the designated National Referral Center for Management of Severe Acute Respiratory Syndrome (SARS) and Other Emerging and Re-emerging Infectious Diseases (DOH-Department Order No. 698 s. 2004). In 2014, RITM was officially designated as the Philippine National Influenza Center (PNIC). The following year, RITM was officially designated as National Reference Laboratory for Rotavirus and other Enteric viruses. Through the DOH Committee on NRLs of the National Health Laboratory Network Initiative, RITM shall be further designated as NRL for Schistosomiasis, Neglected Tropical Parasitic Diseases, Rabies and other Lyssaviruses, Special Pathogens, Invasive Bacterial Vaccine Preventable Diseases, and Public Health Entomology. Specialized laboratories were also established to further improve the service of the institute in response to emerging and re-emerging infectious pathogens, like the National Tuberculosis Reference Laboratory (NTRL), Special Pathogens Laboratory (SPL) and the Molecular Biology Laboratory (MBL).

With these highly-specialized facilities, it is imperative that the institute lay down and implement stringent biosafety and biosecurity policies.

RITM BIOSAFETY TRAINING PROGRAM

Biosafety training has previously been dependent on each laboratory department's separate orientation and training programs. With no standard training material, there is a potential for inconsistencies

Table 1. WHO and NIH risk group classification in relation to biosafety levels, practices and equipment

Risk Group	Biosafety Level	Laboratory Type	Laboratory Practices	Safety Equipment
1	Basic-Biosafety Level 1	Basic teaching, research	Good microbiological techniques (GMT)	None; open bench work
2	Basic-Biosafety Level	Primary Health Services, diagnostic services, research	GMT plus protective clothing, biohazard sign	Open bench plus biological safety cabinet (BSC) for potential aerosols
3	Containment-Biosafety Level 3	Special diagnostic services, research	Level 2 plus special clothing, controlled access, directional airflow	BSC and/or other primary devices for all activities
4	Maximum containment-Biosafety Level 4	Dangerous pathogen units	Level 3 plus air lock entry, exit showers, special waste disposal	Class III BSC or positive pressure suits with Class II BSCs, double ended autoclaved (through the wall), filtered air

on the level of awareness and competence. As the number of individuals working with infectious microorganism increases, standardized introductory biosafety training may be helpful, thus, a sustainable, centralized, and reproducible system needs to be developed and improved. A ladderized training program has been set to create different levels of training and awareness among RITM employees depending on their specific role within the institute.

An institutional biosafety committee previously existed under the Assistant Director's Office which served to establish biosafety and biosecurity policies. However, the committee suffered attrition in recent years due to retirement of key staff. In 2016, the Director's Office established the Biorisk Management Office (BRMO). The BRMO, with the vision of making RITM the leader of applied biosafety and biosecurity in the Philippines, is tasked to implement policies, monitor their implementation, conduct risk assessments, and evaluate biosafety and biosecurity programs within the institute.

The BRMO developed a new institutional ladderized biosafety training course, designed as a 3-ladder step program which includes the following: an introductory course offered to all new employees, regardless of whether they are engaged in any laboratory activities or not. In-house guidelines have also been developed to familiarize workers on biosafety and biosecurity; the Applied biosafety training program, the second step of the program, is designed for in house laboratory personnel tasked to conduct routine and special laboratory procedures, such as, collection, handling, testing, storage and disposal of specimens, isolates and biologicals; the third part of the program is the Advanced Biosafety training, which focuses on developing biosafety officers and infectious disease outbreak responders. A regular monthly meeting of biosafety officers is conducted to provide a proper platform for the sharing of newly acquired skills and knowledge, as well as discussion of relevant concerns.

As reference for the development of the ladderized training program, the office incorporated the following critical elements for an effective biosafety training program according to the WHO laboratory biosafety manual of 2004:

- **Needs assessment:** Determination of tasks to be carried out as well as the proper approach for each task.
- **Establishment of training objectives:** Identification of observable behaviors, capabilities and level of proficiency that are expected to be demonstrated by the trainees at the end of the program.
- **Specification of training content and media:** Design of media to be used in conducting training. It must be comprehensive and efficient to be able to provide the intended knowledge and skills to the trainees.
- **Accounting for individual learning differences:** Incorporation of different training approaches in developing a biosafety training program is vital since each individual has different learning capabilities.
- **Specifying learning conditions:** Selection of a mode of instruction is important in passing the required information to the trainees. The different approaches to be used should deliver the expected outcomes of the training.
- **Training evaluation:** This should determine the effectivity of the training as measured by the achievement of the overall goal.
- **Training revision:** The use of questionnaires and survey methods to evaluate the overall training process are necessary to improve future training programs.

DEVELOPMENT OF THE PROGRAM

The development of the Biosafety training aims to adapt a centralized biosafety and biosecurity orientation that will be applicable to all RITM personnel. The training shall be conducted by both traditional lecture and non-traditional training methods customized to the adult learning process and strategies. The flow chart on Figure 1 shows the detailed step by step process on the creation of this ladderized biosafety training program.

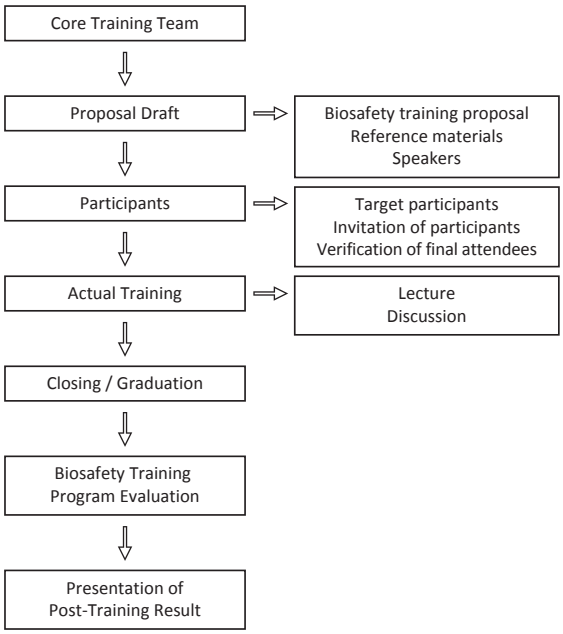

Figure 1. Process flow for the development of the Ladderized Biosafety Training Program.

First, the creation of the core training team which is composed of consultants and Biosafety officers. The team is responsible for the planning, organization and to facilitate the execution of the whole program. A draft proposal was then conceptualized with the use of RITM biosafety manuals with the aid of the previously mentioned international biosafety manuals as reference materials. After which the target speakers which are all biosafety officers, will then be tapped for the program. Guidelines for determination of participants were also drafted. Each Biosafety training level targets different levels of participants. The actual training program will then be carried out and was evaluated based on the evaluation guidelines that were prepared.

Initial Assessment

Participants took a written examination before the start of the Applied Biosafety training. The test aimed to assess the knowledge of each participant on the concepts on biosafety and biosafety hazards associated with the facility as well as to determine the training needs and approaches to be used. The set of questionnaires is based on the content that will be covered in the entire program.

Level 1: Biosafety 101

Biosafety 101 is an introductory course open for all employees regardless of its role in a laboratory containment facility. It was established in the premise that as one institution, employees work towards one mission and therefore share one risk. The information on how to mitigate the risk must therefore be understood by all. Biosafety 101 aims, not only to raise awareness on the basic and fundamental principles of biological safety and biosecurity but to direct employees' perception of the risk that this institution shares

when fulfilling its role in infectious disease outbreak investigation and national health emergencies.

Biosafety 101 is presented and facilitated by trained and experienced biosafety officers and professionals through a comprehensive and informative didactic, lecture and question and answer at the end of each session. The course covers the following topics:

- Fundamentals of Biosafety / Biosecurity, Code of Practice and Best BSL2 Practices
- Biological Risk Management
- Risk Assessment
- Personal Protective Equipment
- Biological Safety Cabinet
- Infectious Substance Shipping and Specimen Transport
- Biological Waste Disposal
- Animal Biosafety
- Arthropod Biosafety
- Infection Control
- Chemical Safety
- Emergency Response

Biosafety 101 as the initial step in the ladder aims to establish the employees' baseline awareness on the basic principles of biological safety and security that could facilitate employees understanding in its role in the mission of the institute of providing safe and secured working environment.

Level 2: Applied Biosafety Training

Applied biosafety training is dedicated for employees who are directly involved in collection, handling, testing, transport, storage and disposal of clinical specimen, isolates and biological materials. It aims to ensure compliance with the standard biosafety practices based on risk assessment. This uses the adult learning teaching strategies to ensure active participation and maximize hands on learning experience. The training is not limited to power point slide presentation, lecture, and question & answer portion alone. Participants experience applied biosafety through its sessions that are filled with hands on laboratory activities that will stimulate the audience active participation and maximize learning capability. This includes demonstrations and return demonstration of best laboratory practices, video presentations on how safety equipment works, case study scenario, actual risk assessment process, performance evaluation process, group exercises, reporting and open discussion. Applied biosafety training is facilitated by home grown biosafety officers, invited biosafety professionals and experts who are trained in facilitating adult learning and teaching strategies. The Applied Biosafety Training is a three day workshop covering the following topics:

- Review of the basic principles of Biosafety and Biosecurity
- Microbiological Risk Assessment
- Engineering Mitigation Control: Facility, Biosafety Cabinet and other Safety Equipment
- Personal Protective Equipment
- Operational Practices
  - BSL-1, 2 and 3 Practices
  - Sterilization and Disinfection
  - Infectious Substance Shipping based on IATA regulation
  - Emergency Response
    - Biological Spill Drill and other emergencies related and limited to biosafety
- Biorisk Performance Evaluation
  - Biosafety Audit and Inspection
- Establishing and Understanding Biological Safety Culture in an Institution

Level 3: Advanced Biosafety Training

Advanced biosafety training is of two forms, the Advanced Biosafety Training program for Biosafety Officers and the Advanced Biosafety Training program for laboratory outbreak responders.

The Advanced Biosafety Training for Outbreak Responders is limited for Laboratory Research Division appointed personnel who will be task to be first line, second and third line responders at the event of an emerging disease outbreak. Appointed LRD personnel shall undergo three whole day intensive biorisk management session. The training program ensures risk assessment based approach in selecting the most appropriate control measures at a given situation at the time of outbreak and biological emergency. This will enable the participants to review and apply best biosafety practices to reduce the risk of exposure. This training program aims to strengthen camaraderie and coordination among all biosafety officers nationwide as well as to promote professional biosafety judgment.

Advanced biosafety training for biosafety officers is exclusive for the selected/appointed laboratory personnel of each department who undergone meticulous screening selection process, biosafety train the trainer programs and completed the first two steps of this ladderized program. Biosafety Officers will serve for a minimum of 2 years- on the job-training program upon the release of office order. Biosafety Officers training program is focused on biorisk management system according to CEN Workshop Agreement 15793:2011. Biosafety Officers shall deal with the implementation of Biosafety/Biosecurity administrative controls. This includes institutional biosafety risk assessment and management, hazard communication and biosafety protocol review. In addition, monitoring of biorisk performance through audit and inspection and facilitating biosafety training programs are also included. Each biosafety officers are expected to promote a biosafety program under the Biorisk Management Committee that will strengthen the culture of safety in the institute. Upon completion, successful biosafety officers will be qualified to be listed as Registered Biosafety Professionals. RITM Registered Biosafety Professional will be the official members of the Biorisk Management Committee who will ensure continuous improvement of the biorisk management system of the institute.

The sessions for this training include:

- Updates on emerging threats
- Review of laboratory procedure and work flow
- Biological Risk Assessment
- Biorisk Management Plan
  - Engineering Control
- Rational Use and Selection of Personal Protective Equipment
  - Respiratory Fit Testing
- Use of Biological Safety Cabinets
- Decontamination Procedures
- Response for Biological Spill and other Emergencies

Participants will spend 60% of the program in a laboratory setting. Responders are expected to master the standard flow of specimen, personnel and wastes, proper donning and doffing procedures, inspection of engineering controls and directional air flow, use of biosafety cabinets and material placement, spill response and procedures in the event of an emergency. Twenty percent (20%) of the time will be a workshop on risk assessment and risk management plan. Participants will be provided with different realistic scenarios. The group will develop constructive risk assessment based approach to different biosafety challenges at

the time of response. The remaining 10% of the time will be spend for listening and participating to discussion on essential biosafety topics and updates from invited biosafety experts and professionals At the end of the training, appointed responders will be equipped with professional judgment and advanced biosafety skills necessary to reduce the risk and step over the challenges of an emerging biological threat.

Final Assessment

The final assessment is accomplished through a written examination consisting of the same set of questionnaires that were given during the initial assessment. After the post-test, discussions on the answers were done focusing on the items where the most number of participants performed poorly. Those who fail to take the post-test are required to take a refresher course prior to re-examination. Completion certificates will be issued to successful participants upon completion of each training.

PILOT IMPLEMENTATION

A total of 118 RITM employees were enrolled for a one-day Biosafety 101 training workshop. All divisions and offices under the institution have cooperated and sent representatives, Janitorial Services, Engineering, Security Support Service, Surveillance Unit, Administrative Office, Clinical Trial Personnel under the Clinical Research Division and the Laboratory Research Division. Majority of the participants are under the laboratory division. This aims to train all employees of RITM, laboratory workers or not. Because of different educational backgrounds, experiences and job description level of familiarity with biosafety guideline varies. Eighty-seven (87) out of 118 trainees composed of the Laboratory Research Division which can be considered more familiar with biosafety guidelines compared to 31 participants from the non-laboratory divisions. A graph representation of the division of the participants is shown on Figure 2.

Applied biosafety training serves as a higher level of biosafety training. The second step of this ladderized program, which prioritizes applied biosafety practices and drills, is especially designed for laboratory workers. It serves a refresher course to maintain previously acquired skills and knowledge as well as to keep updated to new biosafety concepts after the Biosafety 101 training. A total of 40 participants advanced to this training workshop, which is composed of laboratory personnel from the Laboratory Research Division (LRD). Figure representation of the attendees is shown at Figure 3.

As of this publication, the Biorisk Level 3, Advanced Biosafety training is still in process for implementation. Target participants are those who have already completed both Biosafety 101 and Applied Biosafety Training courses. At the end of this 3-step ladder program, it is expected that the successful participants can serve as responders during infectious disease outbreak and related emergencies.

Evaluation of the biosafety training program

Post-training test scores of all trainees were above the passing mark. A paired t-test was run on the pre- and post-test scores of the participants of the Biosafety training course to determine if there is a statistically significant mean difference between the two sets of exams. As seen in the statistical output generated using the computer tool Stata 13, there is a statistically significant increase of 30.07 (95% CI, $p<0.05$) (Figure 4).

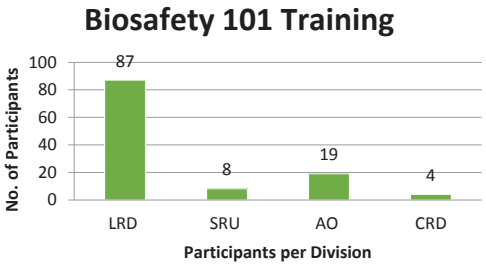

Figure 2. Biosafety 101 training distribution of participants (LRD – Laboratory Research Division; SRU – Surveillance and Response Unit; AO – Administrative Office; CRD – Clinical Research Division).

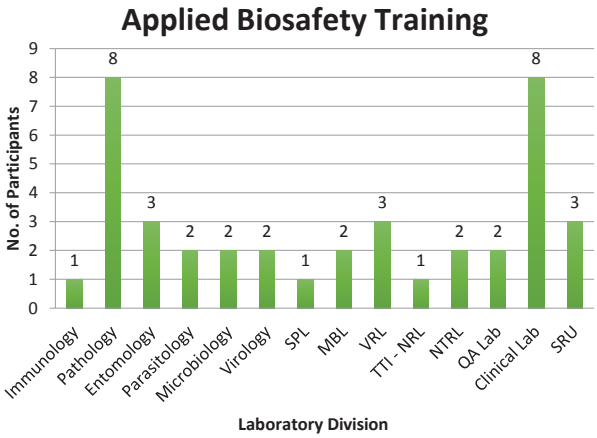

Figure 3. Applied Biosafety training distribution of participants (SPL- Special Pathogens Laboratory; MBL – Molecular Biology Laboratory; VRL – Veterinary Research Laboratory; TTI-NRL – Transfusion Transmitted Infections National Reference Laboratory; NTRL – National Tuberculosis Reference Laboratory; QA – Quality Assurance; SRU – Surveillance and Response Unit).

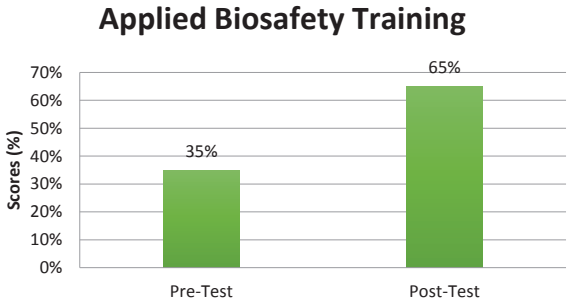

Figure 4. Mean pre-training and post-training scores.

RECOMMENDATIONS

The authors suggest breaking down the introductory Biosafety 101 course into a series of sessions focusing on 1 to 2 topics per session to improve attendance and allow more in-depth discussion of all the topics, instead of conducting it as a one-day activity. A standard, fit-for-purpose method on the provision of pre- and post-tests for all the 3 levels of the training should be implemented. Demonstration of standard biosafety skills and practices, and its evaluation, should be incorporated under the Applied Biosafety training. Finally, the authors recommend holding an annual refresher course complete with biosafety spill drills, and a module type refresher every two years, to ensure that all participants are up to date.

ACKNOWLEDGMENTS

The authors thank the Certified Biosafety Officers of the Philippine Advanced Biorisk Officers Training (PhABOT 3) who facilitated the Biosafety 101 training. They also thank the RITM Department Biosafety Officers for their participation as biosafety trainers for the Applied Biosafety Training. They are grateful to the World Health Organization (WHO) TDR-Supported Regional Training Center and the RITM Laboratory Research Division, Head and staff for their continued support to the program. They extend special thanks to Ms. Rowena Capistrano, head of the Surveillance Unit and to Ms. Johanna Beulah Sornillo, Department of Epidemiology and Biostatistics – RITM for her assistance in this paper's statistical analysis.

AUTHOR DISCLOSURE

The authors declared no conflicts of interest.

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SAMPLE AND REAGENT DISPENSING UNIT

Liquid handling	1 syringe of 1-mL capacity
Disposable tips	Carbon, 300 or 1000 µL, automatically managed by the software
Pipetting area	4-plate handling
Precision (Sample & Reagent)	CV < 8.0% with 10 µL CV < 2.5% with 10 µL
Level sensor system	Electronic
Clot detection	Yes
Mixing	Yes (for predilution tube & microplate)
Multidispensing	Yes (sample, control & reagent)
Sample dispensing time	< 18 min/96 well (100 µL/well)
Reagent dispensing time	< 4 min/96 well (100 µL/well)
Carryover	None

SAMPLE IDENTIFICATION UNIT

Identification	Barcode scanner to primary tubes, controls & reagents Barcode scanner for microplate(optional) manual barcode gun (optional, connected in emulation keyboard)
Tubes	11-12.5 mm diameter, 55-100 mm height 16 mm diameter, 100 mm height
Labels	Interleaved 2 of 5, UPC A & E, IATA 2 of 5, Industrial 2 of 5, EAN 8 or 13, Code 128, EAN 128, Pharmacode, EAN Addendum 2 or 5
Capacity	Up to 240 sample tubes

INCUBATION UNIT

Capacity	4 independt chambers
Temperature range	5°C above room temperature to 50°C
Accuracy	± 0.7°C mean of plate
Uniformaity	± 0.7°C accross plate
Shaking	Longitudinal

WASHING UNIT

Capacity	Up to 4 wash biffers
Wash head	1 x 8
Dispensing volume	200-2500 µL/well, managed per assay
Precision	± 3% CV with 300 µL
Residual volume	< 0.5 µL in U-shaped wells < 2 µL in flat-bottomed wells
Buffers level sensor	Yes
Waste tank level sensor	Yes
Wash cycles	1 to 9, managed per assay
Soak time	1 to 999 sec, managed per assay
Dispensing pressure	Adjustable per assay

READING UNIT

Reading	Vertical with photodiodes, absorbance or kinetics
Channels	8
Method	Single, double or double beam with overrange filter
Spectrum	400-700 nm
Filters	Up to 8 positions available, 5 already on board (405, 450, 492, 550, 620 nm)
Reading time	less than 10 sec
Dynamic range	-0.100 to 3.000 absorbance units
Linearity	0-2.000 absorbance units ± 1.0 %
Accuracy	± 0.005 absorbance units or 2.5%

MANAGEMENT SYSTEM

Computer	Pentium III, 500 MHZ, 64 Mbytes RAM
Floppy Drive	3.5"
Hard disk	6.4 GBytes
Keyboard	Alphanumeric
Mouse	Standard
Monitor	15" colour
Printer	Standard Ink jet

SOFTWARE FEATURES

Operating system	Windows XP, Windows 2000 or 95 32-bit application
Language	Multilanguage
Plate capacity	4 up to 7, in continuous loading
Multiple assays per plate	Yes, up to 12 assays
Data reduction	Cut-off (qualitative) 4 parameters, point-to-point, linear regression, cubic, spline, etc
QA analysis	Mean, SD, CV, Levey-Jennings
Protocols storage	Related to HD capacity
Result printout	Definable per assay and per patient
Patient archive	Yes
Plate loading	Per plate, managed with time scheduling
Process in control	Yes (on-line log evebt/error file)
I/O Interface	ASTM and Flexible ASCII
Patient sample archiving	Yes, plate and tube

DIMENSIONS

Width	1130 mm
Depth	760 mm (880 mm including the pipette waste bag)
Height	1000 mm
Weight	130 kg

ELECTRICAL REQUIREMENTS

Universal a.c. input	90-260 VAC, 47-62 Hz
Power	Typically 500 VA max

SAFETY REQUIREMENTS

CE mark according to Directives 89/336/EEC and 73/23/EEC

BC- 6800

PRINCIPLES

SF Cube cell analysis technology for WBC, 5-Part diff, NRBC, RET and PLT-O
Focusing Flow-DC method for RBC and PLT
Cyanide free hemoglobin measurement

PARAMETERS

33 reportable parameters(whole blood):WBC, Lym%, Mon%, Neu%, Bas%, Eos%, Lym#, Mon#, Neu#, Eos#, Bas#, RBC, HGB, HCT, MCV, MCH, MCHC, RDW-CV, RDW-SD, RET%, RET#, IRF, LFR, MFR, HFR, NRBC#, NRBC%,PLT, MPV, PDW, PCT, P-LCR, P-LCC
14 research parameters(whole blood): HFC#, HFC%, IMG#, IMG%, WBC-R, WBC-D, WBC-B, WBC-N, RBC-O, PLT-I, PDW-SD, InR#, InR%

7 reportable parametes (body fluid)*: WBC-BF, TC-BF#, MN#, MN%, PMN#, PMN%, RBC-BF
5 research parameters (body fluid)*:WBC-BF, TC-BF#, MN#, MN%, PMN#, PMN%,RBC-BF

2 histograms for RBC and PLT
3 scattergrams (3D) for DIFF, NRBC and RET
6 scattergrams (2D)for DIFF, BASO, NRBC, RET, RET-EXT,PLT-O

PRINTOUT

Various printout formats and user-defined formats available

PERFORMANCE

Parameter	Linearity Range	Precision	Carryover
WBC	0-500x10^9/L	≤ 2.5% (≥4x10^9/L)	≤1.0%
RBC	0-8x10^9/L	≤ 1.5% (≥3.5x10^{12}/L)	≤1.0%
HGB	0-250g/L	≤ 1.0% (110-180g/L)	≤1.0%
HCT	0-75%	≤ 1.5% (30%-50%)	≤1.0%
PLT	0-500x10^9/L	≤ 4.0% (≥100x10^9/L)	≤1.0%
RET#	0-0.8x10^{12}/L	≤ 15% (RBC≥3x10^{12}/L; 1%≤RET%≤ 4%)	/

SAMPLE VOLUME

Predilute mode (capillary blood), Open vial	40 µL
Manual mode (whole blood), Open vial	150 µL
Autoloader mode (whole blood) Closed vial	200 µL
Manual mode (body fluid) Open vial	150 µL

THROUGHPUT

Up to 125 samples per hour (CBC+DIFF)
Up to 90 samples per hour (CBC + DIFF + RET)
Up to 40 samples per hour (body fluid)

LOADING CAPACITY

Up to 100 sample tubes

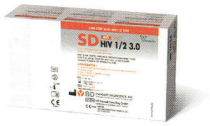
DATA STORAGE CAPACITY

Up to 100 patient results including all numerical and graphical information

OPERATING ENVIRONMENT

Temperature: 15°C~32°C
Humidity: 30%~85%

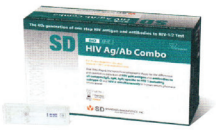
SD HIV 1/2 3.0



One Step HIV 1/2 Antibody Test

HIV (human immunodeficiency virus) is the virus that causes AIDS. This virus may be passed from one person to another when infected blood, semen or vaginal secretions come in contact with an uninfected person's broken skin or mucous membrane. In addition, infected pregnant women can pass HIV to their baby during pregnancy or delivery as well as through breast-feeding

SD HIV Ag/Ab Combo



The 4th generation of one step HIV P24 antigen and antibodies to HIV-1/2 Test

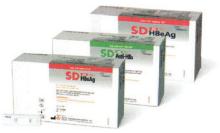
HIV (human immunodeficiency virus) is the virus that causes AIDS. This virus may be passed from one person to another when infected blood, semen or vaginal secretions come in contact with an uninfected person's broken skin or mucous membrane. In addition, infected pregnant women can pass HIV to their baby during pregnancy or delivery as well as through breast-feeding

SD HIV/Syphilis Duo

Simultaneous Detection of HIV-1/2 and Syphilis Antibodies Test

HIV and Syphilis are the major public health problems affecting women and their newborn infants in the world. Over a million women and families are having to face the trauma of repeated pregnancy loss, stillbirth, or childborn infected with and suffering from HIV and Syphilis

SD HBV

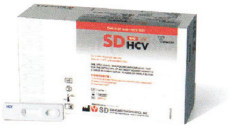


One Step Hepatitis B Virus Test

HBsAg, Anti-HBs, HBeAg

Hepatitis B is a widespread and serious liver disease. Hundreds of millions of people, most of them in regions with poor medical care, are chronically infected with the virus and face an elevated risk of acquiring liver cancer.
The hepatitis B virus (HBV) is made up of an inner core surrounded by an outer capsule. The outer capsule contains the HBsAg(surface antigen). HBeAg is also found within the core. The detection of anti-HBs has become important in the follow up of patients with the Hepatitis B virus (HBV). It is also important when monitoring the recipients of vaccination with recombinant and natural anti-HBs

SD HCV



One Step Hepatitis C Virus Antibody Test

The Hepatitis C virus (HCV) is recognized as a major agent of chronic hepatitis, transfusion acquired non-A, non-B hepatitis and liver diesease throughout the world. HCV diagnostic kits detect the presence of HCV antibodies in human serum, plasma or whole blood by immunoassay. For diagnosis of HCV infection, recombinant proteins (Core, NS3, NS4 and NS5 protein) were used as capture materials and coated on the membrane of an immunochromatographic (rapid) test.

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OPEN ACCESS – ORIGINAL ARTICLE

Is FNA Still a Useful Tool in the Diagnosis of Breast Masses? A 5-Year Review with Cytohistopathologic Correlation*

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[2]*Southwestern University – Matias H. Aznar Memorial, College of Medicine, Cebu City, Philippines*

ABSTRACT

Introduction. Breast cancer is the most common cancer among women worldwide. In the Philippine National Cancer registry, 1 in every 13 Filipino women is likely to suffer from breast cancer. Fine needle aspiration cytology (FNAC) is a safe, accurate, fast and economical technique practiced worldwide in breast cancer diagnosis.

Objective. To assess the value of FNAC as a rapid diagnostic tool in the local setting with the expectation to provide an immediate and highly reliable diagnosis in more than 90% of breast lesions.

Methodology. From January 2010 to December 2014, there were 306 out of 1465 breast FNAC documented cases with histopathological correlation. The FNAC smears were retrieved, retrospectively reviewed blindly and reclassified into 5 categories (C1- C5). All FNAC were performed by pathology residents, pathologists and cytopathologist. Smears were fixed in 95% ethyl alcohol and stained with Papanicolaou method.

Results. The FNAC findings showed: 13 (4.25%) unsatisfactory (C1); 160 (52.29%) benign (C2); 23 (7.52%) atypical (C3); 9 (2.94%) suspicious (C4) and 101 (33.01%) malignant cases (C5). There were 120 (39.22%) malignant and 186 (60.78%) benign lesions. There were 3.92% (12/306) false negative and 0.65% (2/306) false positive cases.

The FNAC had 90% sensitivity, 99% specificity, 98% positive predictive value, 99% negative predictive value and 95% accuracy. The risks of malignancy for each category were: C1=15%; C2=4%; C3=13%; C4=78% and C5=100%.

Conclusion. Despite the increasing preference for core needle biopsy among surgeons, FNAC continues to be an acceptable, affordable, quick and valuable tool contributing significantly to early breast cancer diagnosis and treatment, particularly in developing countries like the Philippines. Owing to its high sensitivity and specificity, it can be used as a screening and confirmatory diagnostic tool. Malignant and benign interpretations of breast FNAC give highly accurate prediction of outcomes but must be correlated with clinical and mammographic findings.

Key words : fine needle aspiration cytology, breast, cytohistopathological correlation

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INTRODUCTION

Breast cancer is the most common cancer in the Philippines, comprising 16 percent of the 80,000 cancer cases in 2010. The country has the highest incidence of breast cancer in Asia and an estimated 3 out of 100 Filipino women will have the disease before age 75 with mortality rate of 1 out of 100 according to the Philippine Society of Medical Oncology in 2012.[1] It has become so common that one out of every thirteen Filipina is expected to have this disease. Early detection and accurate diagnosis of breast lesions is imperative for the appropriate medical management.

Fine needle aspiration (FNA) cytology has become a widely used and cost-effective tool in the assessment of breast masses.[2,3] The current practice is to classify cytological results into one of the five categories ranging from C1 (insufficient material); C2 (benign); C3 (atypical); C4 (suspicious) and C5 (malignant).[4] This system helps the cytopathologist define uncertain areas and for clinicians to properly manage their patients. The standardization for reporting breast cytology cases was initiated by Britain's National Health Service Breast Screening Programme.[5] It serves as a common language among all health care professionals involved in breast care management.

Specifically, C1 refers inadequate aspirate smear due to hypocellularity, aspiration, smearing or staining errors. The exact definition of what constitutes an adequate aspirate and whether or not a diagnosis could be confidently made with the quantity and quality of the aspirate remain a subjective issue and are best resolved by the pathologist. C2 category is for smears that are usually cellular, showing the characteristic patterns of different benign lesions. No atypical or malignant features are present. Usually, duct configurations, myoepithelial cells, and bipolar nuclei are visible. An inflammatory background is commonly encountered. In contrast, C3 and C4 are gray zones. C3 represents the characteristics of a smear with benign features that are not usually seen in clearly benign specimens such as cellular crowding, pleomorphism, and discohesion. C4 is reserved for aspirates where atypical features are obvious but factors such as poor preservation, hypocellularity, or components of a benign smear are present, thus precluding a firm diagnosis of malignancy. This ambiguity shows the importance of correlation with other disciplines. C5 category consists of cellular aspirates with evidently malignant cytologic features.[6]

The Pathology Department of Governor Celestino Gallares Memorial Center, provides diagnostic service on cytological studies to the entire province of Bohol. On average, the department conducts 800 – 1100 fine needle aspiration cytological examination of masses from different sites per year. The data profile of breast cases in our institution has not been fully established. Studies on FNA cases focusing on the breast alone have not been done to assess the diagnostic performance and accuracy of the procedure.

REVIEW OF RELATED LITERATURE

Diagnostic Performance of fine needle aspiration cytology

Fine needle aspiration cytology has become widely accepted as a reliable diagnostic tool for diagnosis breast masses. It is a simple and safe method which yields high diagnostic performances.[7,8] In 2009, a study in Thailand reviewed diagnostic performances of FNA in breast lesions; the sensitivity of the test was 87.6-94.8% with a specificity of 85.9-94.5% and positive predictive value of 83.4-92.8% negative predictive value of 90.4-97.4% and accuracy of 87.6-94.8% with false positive and false negative rates of 5.5% and 3.3% respectively.[9] Another recent meta-analytical review, including 25 studies of FNA, has shown that FNA cytological analysis of palpable breast masses is highly accurate to differentiate benign from malignant tumors.[10] Core needle biopsy has mostly replaced FNA in Europe and the United States.[11,12] However, it is still commonly used in Asia and other developing countries with low financial resources.[13,14]

Breast Cancer Experience in the Philippines

Breast cancer has been consistently the most common cancer among Filipino women. With an age-standardized incidence rate (ASR) of 47.7 per 100,000 women (1998), it was second only to lung cancer when both male and female cancers were considered. ASRs had increased (1980 – 1992), female residents in highly urbanized cities in Metro Manila were experiencing similar rates in Europe, South America and Oceania. One out of 28 Filipinos who live up to 64 years, and one of 19 who live up to 74 will have breast cancer.[15]

In 2000, a local clinical practice guideline from the Philippine College of Surgeons was created recommending patients with a palpable breast mass and in which cancer is suspected. Fine needle aspiration cytology (FNAC) is the initial diagnostic procedure in patients with palpable breast mass. If the FNAC results are benign but clinical findings are highly suspicious for breast cancer, either a core needle or an open biopsy is done. Philippine standards on FNAC have not been established.[16]

OBJECTIVES

General objective:

- To assess the value of FNAC as a rapid diagnostic tool in the local setting with the expectation to provide an immediate and highly reliable diagnosis in more than 90% of breast lesions.

Specific objectives:

- To determine the sensitivity and specificity of FNAC as a diagnostic tool.
- To determine the risk of malignancy of the individual categories (C1 – C5).

METHODOLOGY

Fine needle aspiration biopsy (FNAB) is done by pathology residents, pathologists, and cytopathologist in the Histopathology Section of the Governor Celestino Gallares Memorial Hospital, a research and training institution in Bohol, Philippines. These were palpation-guided using a G23 x 1" needle attached to a 5.0 ml syringe. Direct conventional smears were prepared from each pass and immediately fixed in 95% ethyl alcohol, then stained using the Papanicolaou method and read by the consultant pathologist on duty. Turnaround time of FNAB results varied from 30 minutes (routine cases) to 1 hour (difficult cases).

During the study period of January 2010 – December 2014, all breast fine needle aspiration cytology cases were retrieved from the cytology logbooks with approval from the research and ethics committee of the institution. Patient demographic data such as age, sex, clinical and FNA findings were included. Ultrasonography and mammogram data were not included in the study since not all patients had them. FNAC without final histopathological findings and histopathological specimens without prior FNAC were not included in this study. The FNA cytology cases were retrospectively reviewed and reclassified according to the National Health Service Breast Screening Programme (NHS-BSP) standards: unsatisfactory (C1), benign (C2), atypical (C3), suspicious (C4) and malignant (C5).

Fine needle aspiration biopsy results that were found to have inadequate or acellular findings such as benign cyst contents, suppurative material, bloody aspirate, abscess, inflammatory cyst, collagenized stroma with rare benign ducts, adipose tissue and fibrosis, and inflammatory lesions with suspicious granulomas were reclassified as C1. C2 were diagnoses of benign conditions such as epidermal inclusion cyst, fibroadenoma, fibroadenoma with fibrocystic changes, fibrocystic changes alone, non-proliferative fibrocystic lesion, non-proliferative breast lesion, proliferative breast lesion including those with lactational changes with minimal atypia and without atypia; proliferative fibrocystic lesion including those without atypia, papillary neoplasm, spindle cell tumor, and suppurative granulomatous mastitis. Proliferative breast lesions and fibrocystic lesions with atypia were reclassified as C3.

Results favoring a more malignant diagnosis were reclassified as C4 or C5. Cases with results such as suspicious for phyllodes tumor, mammary carcinoma, ductal carcinoma in situ or malignant lymphoma were reclassified as C4. C5 were cases with clear cut malignant findings such as ductal, lobular or papillary carcinoma, high grade sarcoma or carcinoma, mucinous carcinoma and carcinoma with ductal, papillary or mucinous features.

Corresponding available histopathological specimens of all FNAC were retrieved and compared. These specimens were primarily obtained from lumpectomies and mastectomies. Core needle biopsy specimens were used when no other sample was available. Concordance of findings between the FNAC and final histopathological outcome were investigated through blind microscopic rescreening of slides by a most senior pathologist. True negative results were cases which turned out to have benign outcomes on final tissue biopsy. True positive results were cases which had malignant outcomes. C1 – C3 Categories were considered to support a benign process while C4 – C5 Categories favor a malignant process. Accuracy, precision, sensitivity and specificity of the procedure were calculated.

RESULTS AND DISCUSSION

From January 2010 to December 2014, there were a total of 5043 fine needle aspiration biopsies done, of which 1465 (29%) were breast cases, composed of 1389 females and 76 males. The average age was 52 years (range, 10 - 88 years). A total of 13262 histopathological specimens were received by the pathology department but only 715 (5%) were breast related. There were 306 fine needle aspiration biopsies with corresponding final histopathology specimens (303 females and 3 males) available for the entire study duration (Table 1).

The majority of breast cytology results (160; 52.29%) were the benign C2 category. The malignant C5 was the second largest category at 101 (33.01%). The atypical C3 category ranked third with 23 cases (7.52%) while unsatisfactory C1 category had 13 cases (4.25%) and suspicious C4 category had 9 cases only (2.94%) (Figure 1). Individual diagnoses per category were tabulated in Table 2.

When the final histopathological outcomes were reviewed, there were 120 (39.22%) malignant and 186 (60.78%) benign lesions (Figure 2). Tumor diameters of the malignant and benign cases ranged from 0.8 cm to 10 cm. The maximum number of benign cases was in the age group of 20-24 years while the malignant cases peaked in the age group of 45 to 49 years (Table 3). For the individual categories, there were 2 out of 13 C1 cases, 7 out of 160 C2 cases, 3 out of 23 C3 cases, 7 out of 9 C4 cases and 101 out of 101 C5 cases that turned out to be malignant. The risks of malignancy for each category were as follows: 15% for C1, 4% for C2, 13% for C3, 78% for C4 and 100% for C5 (Figure 3). Individual histopathological results per category are listed in Table 4.

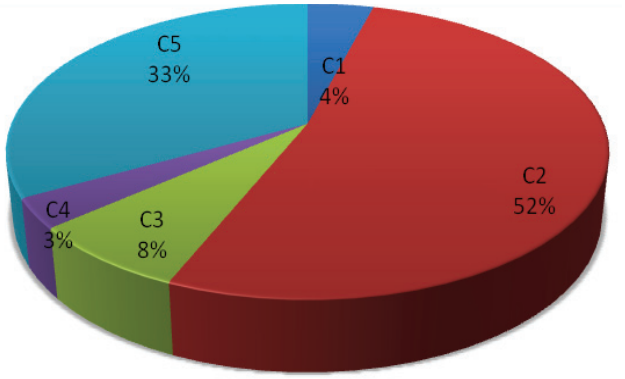

Figure 1. Percentage of breast fine needle aspiration cytology cases per category (C1- C5).

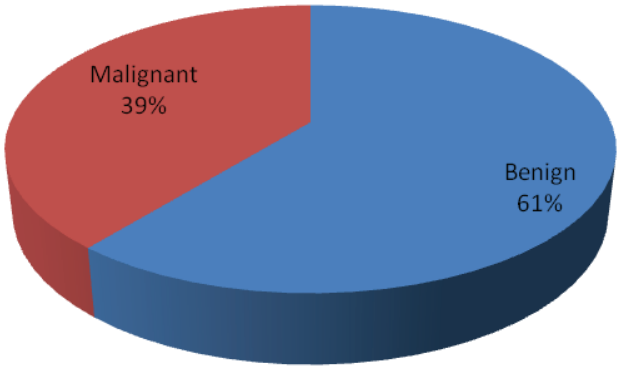

Figure 2. Final histopathological outcomes of submitted breast specimens for 2010 – 2014.

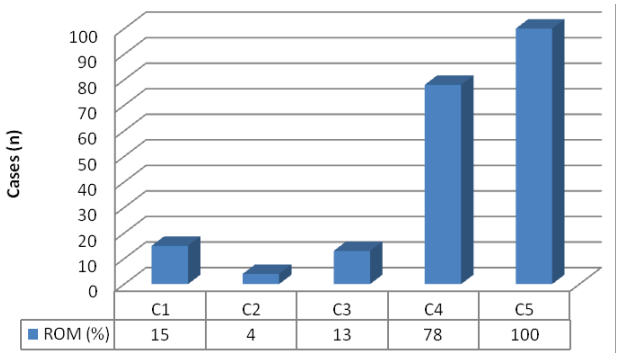

Figure 3. Risk of malignancy of the C1 – C5 categories.

Final Histopathological Outcomes

C1 unsatisfactory category

The final histopathological specimens of the 13 cases in the C1 category yielded 11 benign (5 fibroadenoma with fibrocystic changes, 5 fibrocystic changes and 1 ductal hyperplasia with intraductal papilloma) and 2 malignant (2 invasive ductal carcinomas) outcomes. The rescreen process of the FNA slides of one malignant case showed that there was a pre-analytical error of suboptimal sampling or missed sampling due to abundance of cyst fluid (more than 10 cc) with a deep seated malignant tumor that was not reached by the aspirating needle. The other case was missed because the cells that turned out to be malignant were obscured amidst severe inflammation, hemorrhage and necrotic background, reflecting the skill of the reader and the limitation of conventional smear.

Table 1. Total number of fine needle aspiration biopsies and histopathological specimens received per year (2010 – 2014) with corresponding number of breast cases

Total Number	2010	BREAST	2011	BREAST	2012	BREAST	2013	BREAST	2014	BREAST	TOTAL (BREAST)	F	M
FNA	827	264	1011	302	1079	319	1123	302	1003	278	1465	1389	76
HP	2441	124	2774	139	2726	172	2620	128	2701	152	715		
FNA+HP		**54**		**58**		**88**		**57**		**50**	**306**	**303**	**3**

FNA – Fine Needle Aspiration, HP – Histopathology, F – female, M – Male

Table 2. Diagnoses in 306 breast masses on FNAC

C1. UNSATISFACTORY SAMPLES (n=13)	
Benign cyst contents	11
Suppurative materials	2
C1. UNSATISFACTORY SAMPLES (n=13)	
Inflammatory breast lesions	
Mastitis	1
Breast abscess	1
Gynecomastia	1
Fibroadenoma	36
Fibroadenoma with fibrocystic changes	1
Fibroepithelial neoplasm	5
Non-proliferative breast lesion without atypia	3
Non-proliferative fibrocystic lesion without atypia	24
Papillary Neoplasm	5
Proliferative breast lesion with lactational changes	2
Proliferative breast lesion without atypia	51
Proliferative breast lesion with mild atypia	2
Proliferative fibrocystic lesion without atypia	24
Proliferative fibrocystic disease with mild atypia	1
Phyllodes tumor	1
Lipoma	1
Soft tissue tumor	1
C3. ATYPICAL CATEGORY (n= 23)	
Proliferative breast lesion with moderate atypia	1
Proliferative breast lesion with atypia	13
Proliferative fibrocystic lesion with atypia	5
Spindle cell tumor	4
C4. SUSPICIOUS CATEGORY (n=9)	
Suspicious for phyllodes tumor, high grade	2
Suspicious for mammary carcinoma	7
C5. MALIGNANT CATEGORY (n=101)	
Ductal carcinoma	94
Lobular carcinoma	2
Mucinous carcinoma	**2**
Carcinoma with mucinous features	**1**
Metastatic carcinoma	**2**

Experience and technique of the aspirator are the most important factors for specimen adequacy and interpretation of results.[17,18] Referred slides from biopsies done by clinicians are usually insufficient and often require repeating procedure by a trained pathologist.[19] The sensitivity of aspiration cytology of the breast has been reported to drop significantly (98.2% to 75%) when done by an untrained individual.[20,21] Technique plays an important role.[17] In this study, the pathologists had variable years of training. The senior pathologist had the least numbers of insufficient specimens and missed lesions.

Imaging such as sonographic and stereotactic guidance is another factor affecting diagnostic accuracy. The cystic, deep seated lesion in this study could have benefited from ultrasound guided - FNAC. Thus, the need to diagnose patients with a combination of physical examination, radiological studies and FNA (triple test) is emphasized.

Smearing, drying artifacts, background materials, thick smears and inadequate fixation were some of the drawbacks seen in conventionally prepared slides.[22] A liquid based preparation could have been used in this study to minimize background inflammation and concentrate cellular material.[23]

C2 benign category

Of the 160 C2 benign category cases, 153 were benign and 7 turned out to be malignant. Benign findings included 7 benign phyllodes tumor, 4 chronic granulomatous mastitis, 66 fibroadenoma, 38 fibroadenoma with fibrocystic changes, 28 fibrocystic changes, 1 galactocoele, 3 lipoma, 2 intracystic papilloma, and 4 cases of

Table 3. Age distribution of cases with benign and malignant breast disease on histology

	10-14	15-19	20-24	25-29	30-34	35-39	40-44	45-49	50-54	55-59	60-64	65-69	70-74	75-79
Benign	2	18	**36**	14	13	26	27	22	10	7	7	2	-	2
Malignant	-	-	-	2	3	8	19	**23**	17	19	5	11	10	3

FNA – Fine Needle Aspiration, HP – Histopathology, F – female, M – Male

Table 4. Histopathological diagnoses in 306 breast lesions based on C1-C5 categories

HISTOPATHOLOGICAL OUTCOMES	C1 (13)	C2(160)	C3 (23)	C4 (10)	C5 (101)	TOTAL
BENIGN	**11(TN)**	**153(TN)**	**20 (TN)**	**2 (FP)**		**186**
Acute and/or Chronic mastitis		4	**1**			
Benign phyllodes tumor		7	**3**			
Ductal hyperplasia with intraductal papilloma	1					
Fibroadenoma		66	**3**	2		
Fibroadenoma with fibrocystic changes	5	38	**2**			
Fibrocystic changes	5	28	**6**			
Galactocoele		1				
Gynecomastia		1				
Intracystic papilloma		2	**5**			
Lipoma		2				
Papillary Lesion		4				
MALIGNANT	**2(FN)**	**7(FN)**	**3 (FN)**	**7 (TP)**	**101 (TP)**	**120**
Ductal Carcinoma In Situ		2				
Invasive Ductal Carcinoma	2	3	**3**	4	89	
Invasive Ductal Carcinoma with lobular features					3	
Intracystic Papillary carcinoma		1				
Invasive Lobular Carcinoma					2	
Invasive Papillary Carcinoma		1		2	4	
Malignant phyllodes tumor				1		
Mucinous Carcinoma					2	
High grade carcinoma with sarcoma features					1	

TN – True Negative, TP – True Positive, FN – False Negative, FP – False Positive

Table 5. Diagnoses in 306 breast masses on FNAC

PARAMETER	Value (%)	PARAMETER	Value (%)
True Positive	108 (35%)	Accuracy	95%
True Negative	184 (60%)	Sensitivity	90%
False Positive	3 (1%)	Specificity	98%
False Negative	12 (4%)	Positive predictive value (PPV)	98%
		Negative predictive value (NPV)	99%

tissue fragments suggestive of a papillary lesion. There were 2 ductal carcinoma in situ, 3 invasive ductal carcinomas, 1 papillary carcinoma and intracystic papillary carcinoma.

On rescreening of the 7 false negative C2 cases, majority were pre-analytical errors of suboptimal sampling. The masses were seen to have minimal or rare atypia on FNA. This atypia was usually seen in hyperplastic changes. These patients were likewise young (<40 years old), favoring a more benign process. Another difficulty encountered was the interpretation of papillary lesions. The smears showed moderate cellularity composed of more uniform tall and columnar cells with elongated uniform small, deceptively bland- looking nuclei. The lack of cytomorphological clues of malignancy in these cases led to false negative findings. Indeed, the cytologic diagnosis of papillary lesions remains to be a challenge as experienced by other investigators.[24,25]

C3 atypical category

Of the 23 C3 atypical cases, 3 turned out to be malignant and 20 benign. There were three cases of invasive ductal carcinoma which were noted to have abundant atypical cells on FNA. Those with benign outcomes included 3 fibroadenomas, 2 fibroadenomas with fibrocystic changes, 6 fibrocystic changes, 3 benign phyllodes tumors, 5 intracystic papillomas and 1 acute on chronic mastitis.

This category has been overused by most pathologists when insufficient atypia is seen to fulfill criteria for a malignant diagnosis. In this study, the percentage of C3 cases that turned out to be malignant was 15%. The frequency of a diagnosis of a malignant lesion in this category is highly dependent on the skill and experience of the reader.

C4 suspicious category

Seven of 9 C4 suspicious category cases turned out to be malignant and were composed of 4 invasive ductal carcinomas, 2 invasive papillary carcinomas, and 1 malignant phyllodes tumor. Benign outcomes included 2 fibroadenomas with fibrocystic changes (FAFCC).

Rescreen of FNA slides of the FAFCC revealed scattered atypical cells with high nuclear to cytoplasmic ratio. The atypical cells appear slightly larger than the normal ductal epithelial cells and comprised more than 20% of the cell population. Pre-analytical and analytical error may have occurred due to missampling and wrong interpretation of the smears.

C5 malignant category

All FNAs that were categorized as C5 turned out to be malignant on their corresponding tissue specimens. The 101 histopathological outcomes were distributed among 89 invasive ductal carcinoma, 3 invasive ductal carcinoma with lobular features, 2 invasive lobular carcinoma, 4 invasive papillary carcinoma, 2 mucinous carcinoma and 1 high grade carcinoma with sarcomatous features.

The overall accuracy, precision, sensitivity, specificity, positive and negative predictive value were summarized in Table 5. Sensitivity (90%) and specificity (99%) values of this study were similar to other studies conducted.[26-29] The false positive (0.65%), false negative (3.92%) and accuracy (95%) of this study also fell within the target values as described in other studies.[6,30]

Both PPV (98%) and NPV (99%) of FNAC are high and are comparable to other larger studies reported in literature.[31,32] A high sensitivity and positive predictive value proves that a positive FNAC in the breast means having comparable diagnosis with the final histopathology report. The high specificity and negative predictive value for malignancy demonstrates the high accuracy in the diagnosis of malignancy in the breast using FNAC.

CONCLUSION

Fine needle aspiration cytology is still recognised as an important tool in the diagnosis of malignancy in palpable breast masses despite the increasing preference of clinicians for core needle biopsies. It remains a popular procedure due to its cost-effectiveness, rapidity and reliability especially to low income countries such as the Philippines. However, it is emphasized that FNAC requires clinical and radiologic correlation to reduce false positive and false negative results.

RECOMMENDATIONS

Mammography and breast ultrasound studies are strongly recommended to improve diagnostic accuracy especially for patients with large, cystic breast masses and young patients with strong family history for breast cancer.

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AUTHOR DISCLOSURE

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OPEN ACCESS – ORIGINAL ARTICLE

The Accuracy of Mean Neutrophil Volume Relative to Blood Culture for the Diagnosis of Sepsis: A Meta-analysis

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ABSTRACT

Background. Sepsis is difficult to diagnose clinically because the signs and symptoms are non-specific. Blood culture is the gold standard, but it has low sensitivity and it takes at least 24-48 hours before results are released. Cell population data such as mean neutrophil volume (MNV) has recently been shown to be significantly increased in septic patients both with high WBC and normal/low WBC count.

Objective. The aim of the present study was to conduct a meta-analysis of published papers on the accuracy of MNV in diagnosing sepsis relative to blood culture.

Methodology. Electronic databases including PubMed/Medline, Elsevier/Scopus, and Google Scholar were reviewed. Papers that were not retrieved in full text and papers that do not have data on MNV were excluded. The sensitivity and specificity were pooled, and the area under the receiver operating characteristic curve (AUROC) is computed.

Results. Seven studies including 994 participants were included in the meta-analysis. With a mean cut-off value of 153.15 fL [149.1315, 157.1685], the pooled sensitivity and specificity were 0.82 [0.71, 0.89], and 0.78 [0.68, 0.86] respectively. The AUROC is 0.87 [0.83-0.89].

Conclusions. MNV is a potential indicator for sepsis with high specificity and sensitivity, with moderate to high test accuracy. GRADE evaluation indicated a moderate quality of evidence: despite the large effect size, there is a serious risk of bias and high heterogeneity between the included studies.

Key words: Mean Neutrophil Volume, sepsis, accuracy, blood culture

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INTRODUCTION

Sepsis is difficult to diagnose because the signs and symptoms are non-specific. Clinical and laboratory findings are used for the diagnosis, and the gold standard is blood culture.[1] Blood culture has its shortcomings, such as low sensitivity, the need for sterile collection techniques to avoid contamination, and false positivity. There is a delay of at least a 24-48 hours before results of blood culture are available.[2] Early diagnosis and appropriate management are critical to reducing mortality.[3] Early diagnosis may be more effective in overall cost containment and outcome than a more specific but late diagnosis.[4] Therefore, a rapid, accurate, and cost-effective test is needed.[3]

However, early diagnosis of sepsis is difficult because the signs and symptoms of sepsis such as fever, are nonspecific and may be blunted or absent.[5] Peripheral blood smears can also yield important diagnostic information by identifying characteristic morphologic changes seen in reactive neutrophils.[4] Characteristic morphological changes regarding the size of the cell, the density of the nucleus, number of nuclear lobes, along with the presence of toxic granules, vacuolization and occasional Döhle bodies are evident in sepsis.[6] However, this approach is labor-intensive and time-consuming because it requires manual examination and an experienced medical technologist and pathologist. Furthermore, the results are subjective because they depend on human interpretation, and only a few hundred cells can be analyzed for any given sample.[4]

Reactive segmented neutrophils tend to be larger and have lower nuclear complexity than their normal "resting" counterparts.

This could be analyzed quantitatively by using an automated hematology analyzer with volume, conductivity, and scatter (VCS) technology.[7] The automated hematology analyzer with VCS technology can determine the traditional parameters, such as total white blood cell and differential counts, and also determine the intrinsic biophysical properties of over 8000 leukocytes as well as measure the degree of cell size variation. This is analogous to the microscopic evaluation of a peripheral blood smear but uses the most modern technology to refine the output. These measurements of cellular morphological properties are known as cell population data (CPD).[8] VCS technology is exclusive for Beckman-Coulter hematology analyzers, but other manufacturers may develop future models that generate cell population data as well.

Previous studies demonstrated that CPD such as mean neutrophil volume (MNV), measured in femtoliters (fL), and neutrophil volume distribution width (NDW), measured in fL, are significantly increased in septic patients both with high WBC and those with normal or low WBC counts.[9] The MNV and/or NDW show superior sensitivity and specificity for predicting sepsis when compared with WBC, neutrophil percentage, band counts, C-reactive protein, or procalcitonin, proving to be new and promising indicators for the diagnosis of early sepsis.[3]

In this study, we performed a meta-analysis to evaluate the accuracy of mean neutrophil volume in diagnosing sepsis. Specifically, we aimed to: (a) determine the pooled sensitivity and specificity of MNV in detecting sepsis, and to (b) determine the area under the curve or diagnostic accuracy.

METHODOLOGY

The data of this manuscript came from electronic databases and previous studies. Thus, it is not applicable to receive an ethics committee approval or follow the Declaration of Helsinki, and there is no need to get informed consent of patients.

Search strategy

Electronic databases including PubMed/Medline, Elsevier/Scopus, and Google Scholar were reviewed as of October 20, 2016, to select relevant studies on sepsis and mean neutrophil volume. Search terms ("Mean Neutrophil Volume" or MNV) AND (sepsis or infection) with limits: Published from 2000 to present, Human, English, were used for the initial screening. However, initial results showed MNV as murine norovirus. Thus another search was made with search terms ("Mean Neutrophil Volume" or MNV NOT murine) AND (sepsis or infection) with limits: Human, English.

Inclusion and exclusion criteria

Studies from the search were screened accordingly. We asked help from the Medical Library Librarian to retrieve papers without free access. The studies were included if they met the following criteria: (a) disease of interest is sepsis, (b) index test of MNV for diagnosis of sepsis, and, (c) reference test of positive blood culture as a criterion for inclusion in the sepsis group. Papers that were not retrieved in full text and papers that do not have the sensitivity and specificity of the MNV were excluded.

Data extraction

Using an electronic spreadsheet, the investigators extracted the following data – name of the first author, publication year, study region, age group, the number of samples for the sepsis and healthy groups, true positive, false positive, false negative, true negative, and cut-off points.

Risk of bias in individual studies

Individual studies were critically appraised based on QUADAS 2 assessment. Publication bias is evaluated by Deek's funnel test.

Statistical analysis

The statistical analysis was performed using STATA 13. In pooling the sensitivity and specificity, a bivariate mixed-effects regression framework was used because of the assumed heterogeneity in the study characteristics. This was evaluated by Q statistic.[10] A p-value <0.05 considered statistically significant heterogeneity is present. Multiple univariable bivariate meta-regression models were used as an exploratory analysis of threshold-related heterogeneity.[11]

RESULTS

Study selection

In the current study, we conducted a literature search published between the years 2000 and 2016 to identify studies relevant to investigating the utility of mean neutrophil volume in the diagnosis of sepsis. We searched publications in PubMed/Medline, Elsevier/Scopus and Google Scholar using the search terms ("Mean Neutrophil Volume" OR MNV NOT norovirus) AND (sepsis or infection), with limits: Human, and English; the manuscripts published were evaluated.

A total of 75 results were evaluated. The twenty-six duplications were removed. After investigating all titles and abstracts from these articles, 12 studies were taken into consideration.[1,2,4-9,12-15] Figure 1 describes the selection process done following the aforementioned inclusion and exclusion criteria. A total of seven studies were included in the meta-analysis.

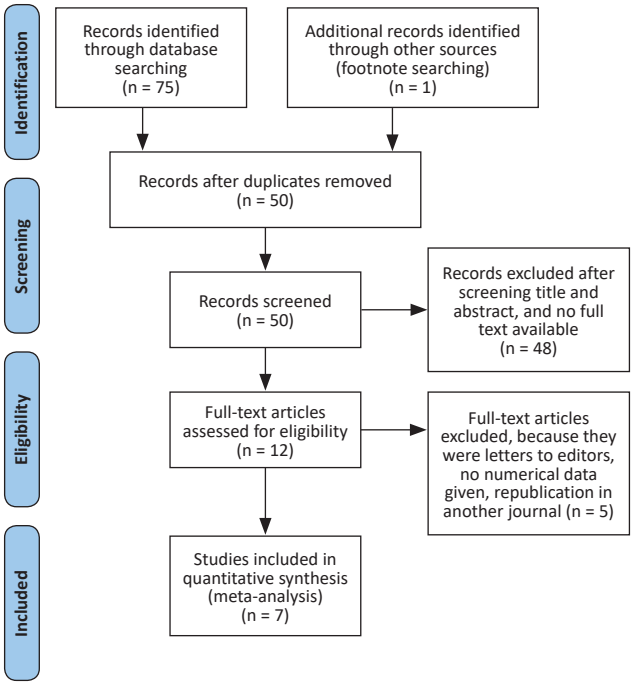

Figure 1. Process of study selection.

Table 1. Characteristics of the seven included studies

ID	Author	Year	Area	Analyzer	Age-group	n (sepsis)	TP	FP	n (control)	FN	TN	Cut-off
1	Chaves	2005	USA	LH750	Adult	69	48	21	35	3	32	150
2	Mardi	2009	Germany	LH750	Elderly	37	28	18	48	9	30	150
3	Celik	2012	Turkey	LH780	Neonate	76	60	18	98	16	80	157.15
4	Lee	2013	Korea	DxH800	Elderly	18	15	6	29	3	23	156.5
5	Zhu	2014	China	LH750	Adult	31	17	19	219	14	200	159.4
6	Purohit	2015	India	LH750	Adult	162	148	5	40	14	35	149
7	Suresh	2016	India	LH780	Elderly	36	26	14	46	10	32	150

Table 2. Signaling questions in critical appraisal of the included studies

Questions	Chaves	Mardi	Celik	Lee	Zhu	Purohit	Suresh
1. Was a consecutive or random sample of patients enrolled?	●	●	●	●	●	●	●
2. Was a case-control design avoided?	●	●	●	●	●	●	●
3. Did the study avoid inappropriate exclusions?	●	●	●	●	●	●	●
4. Were the index test results interpreted without knowledge of the results of the reference standard?	●	●	●	●	●	●	●
5. If a threshold was used, was it pre-specified?	●	●	●	●	●	●	●
6. Is the reference standard likely to correctly classify the target condition?	●	●	●	●	●	●	●
7. Were the reference standard results interpreted without knowledge of the results of the index test?	●	●	●	●	●	●	●
8. Was there an appropriate interval between the index test and the reference standard?	●	●	●	●	●	●	●
9. Did all patients receive the same reference standard?	●	●	●	●	●	●	●
10. Were all patients included in the analysis?	●	●	●	●	●	●	●

Legend: Blue – Yes, Tan – No

Table 3. The QUADAS-2 assessment of risk of bias of the included studies

Author	Risk of Bias				Applicabilty Concerns		
	Patient Selection	Index Text	Reference Standard	Flow and Timing	Patient Selection	Index Text	Reference Standard
Chaves	●	●	●	●	●	●	●
Mardi	●	●	●	●	●	●	●
Celik	●	●	●	●	●	●	●
Lee	●	●	●	●	●	●	●
Zhu	●	●	●	●	●	●	●
Purohit	●	●	●	●	●	●	●
Suresh	●	●	●	●	●	●	●

Legend: Blue – Low risk, Tan – High risk

Study Characteristics

The characteristics of the seven included studies are listed in Table 1. A total of 994 participants were involved in this meta-analysis. Five of the included studies were distributed in Asia while one study was conducted in Europe, and another in the USA. The patients who were positive for the blood culture test were included in the intervention (sepsis) group while healthy patients were classified as controls. Their MNV were assessed using the following hematological analyzers – LH750, LH780 and Dx800 Beckman Coulters (Fullerton, CA).

The included studies are heterogeneous based on nationality, analyzer used, and age-group. This necessitates a random effects model in pooling the sensitivity and specificity of the included studies. However, subgroup analysis cannot be made, because there is only one study in the following theoretical subgroups: DxH800 analyzer and Neonates.

Risk of bias within studies

The risk of bias is serious because the included studies utilized a case-control design, and the index test cut-off used was optimized. The summary of the risk of bias is shown in Tables 2 and 3, and it was determined to have a serious risk of selection bias.

Synthesis of results for the summary ROC analysis

The forest plot of the meta-analysis is seen in Figure 2. The calculated summary performance estimates and their corresponding 95% CIs are as follows: Sensitivity = 0.82 [0.71, 0.89], Specificity = 0.78 [0.68, 0.86], Positive LR = 3.7 [2.6, 5.4], Negative LR = 0.23 [0.15, 0.37] and Diagnostic Odds Ratio = 16 (9, 30). High heterogeneity was observed for the first two estimates – Q=33.78 (p<0.01) and Q=44.09 (p<0.01) respectively. To explain this, metaregression was made. It was found that the proportion of heterogeneity likely due to threshold effect was 27%.

The summary ROC curve is displayed along with the observed study data in Figure 3. The dashed line around the summary point estimate represents the 95% confidence region. The area

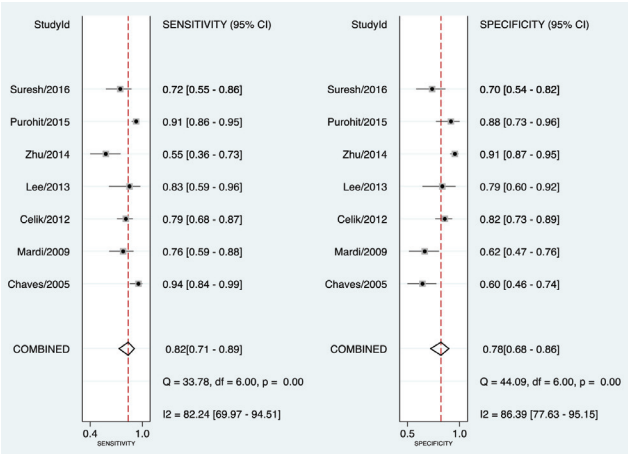

Figure 2. Forest plot showing study-specific and mean sensitivity and specificity with corresponding heterogeneity statistics.

Table 4. Evaluation of the quality of evidence using GRADE

Outcome	No. of studies (No. of patients)	Study Design	Factors that may decrease quality of evidence					Test accuracy QoE
			Risk of bias	Indirectness	Inconsistency	Imprecision	Publication bias (Effect size)	
True positives False negatives	7 studies (443 patients)	case-control type accuracy study	serious[a,b]	not serious	serious[c]	not serious	none (strong association)	⊕⊕⊕◯ MODERATE
True negatives False positives	7 studies (501 patients)	case-control type accuracy study	serious[a,b]	not serious	serious[c]	not serious	none (strong association)	⊕⊕⊕◯ MODERATE

a. Patient selection affected the risk of bias due to case-control study design used in the studies.
b. Index test affected the risk of bias due to optimization of cut-off.
c. High between study heterogeneity.

under the curve (AUROC), serves as a global measure of test performance. The AUROC is the average TPR over the entire range of FPR values. The calculated AUROC was 0.87 [0.83-0.89] with high heterogeneity, Q=27.54 (p<0.001).

The Deek's Funnel Plot Asymmetry Test was conducted to explore possible publication bias. The studies appear to cluster around the regression line (Figure 4). The calculated p-value was also less than alpha=0.05 which suggests a low likelihood of publication bias.

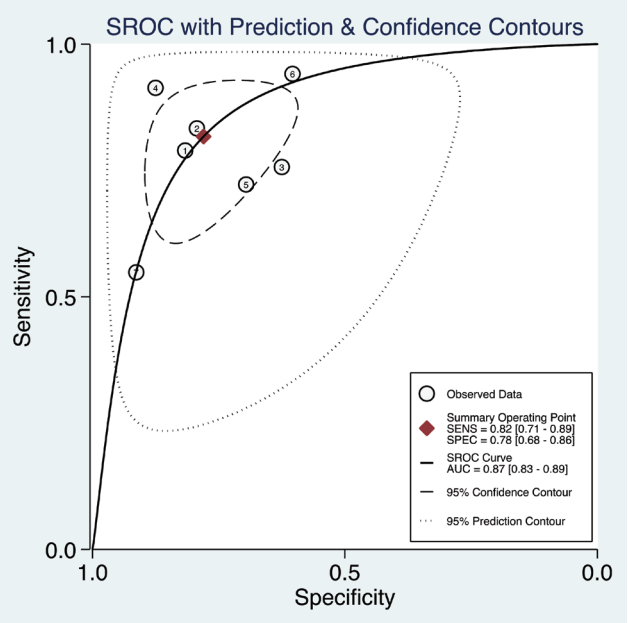

Figure 3. Summary ROC curve with confidence and prediction regions around mean operating sensitivity and specificity point.

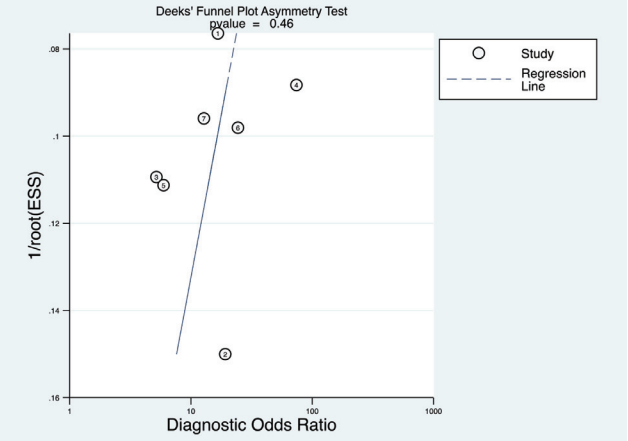

Figure 4. Deek's Funnel Plot Asymmetry Test.

DISCUSSION

Summary of evidence

MNV is not routinely reported in complete blood count. Most clinicians rely on the result of blood culture test, which could take at least 24-48 hours. This meta-analysis explores the accuracy of MNV in detecting sepsis. The mean sensitivity and specificity were 0.82 [0.71, 0.89] and 0.78 [0.68, 0.86] respectively. This means 82% of those with sepsis have elevated MNVs above the cut-off; and, 78% of those without sepsis have MNVs lower than the cut-off. The Likelihood Ratio Positive is computed to be 3.72 [2.57, 5.37]. This means that an elevated MNV above cut-off increases the probability of presence of sepsis by approximately 30%. The Likelihood Ratio Negative is computed to be 0.23 [0.15, 0.37]. This means that an MNV below the cut-off decreases the probability of presence of sepsis by approximately 30%.

However, high heterogeneity was observed for both parameters [Q=33.78 (p<0.01) and Q=44.09 (p<0.01)] and it was found that 27% of the variability is due to the variability in threshold (cut-off value). The mean cut-off value from the studies is 153.15 fL [149.1315, 157.1685]. Because of the heterogeneity due to the cut-off values, two cut-off points should be used to determine elevated and non-elevated MNV. Elevated MNV is values above 157 fL to be used to rule in sepsis; and an MNV below 150 fL is to be used to rule out sepsis. Indeterminate results within 150 to 157 fL will require blood culture testing to proceed.

The calculated AUROC was 0.87 [0.83-0.89]. This suggests moderate to high test accuracy. However, there is high heterogeneity, Q=27.54 (p<0.001) observed. This heterogeneity may be due to several factors such as age group, model of analyzer used, and nationality. The accuracy of the use of MNV in diagnosis sepsis may vary depending on age group, and nationality of patient, as well as model of analyzer used.

The quality of evidence was evaluated to be moderate (Table 4).

Limitations

The limitations of this study include: 1. no sub-group analysis was made because there is no sufficient number of studies for each subgroup; 2. a large proportion of heterogeneity was unaccounted for the Summary ROC Analysis; and, 3. included studies were limited to published studies. A subgroup analysis may account for the other proportions of heterogeneity. It may be due to the model of analyzer used, or variation in the cut-off points with regards to age group and nationality of patients. Since this study is limited to published studies, there might be unpublished studies that may fill up the subgroups needed to do a subgroup analysis.

CONCLUSION

In conclusion, MNV is a potential indicator for sepsis because of moderate to high test accuracy relative to blood culture. GRADE evaluation of quality of evidence, however, shows that there is a moderate level of the quality of evidence because despite the large effect size, the studies included were case-control, and there is high heterogeneity between studies. The authors recommend that subgroup analysis must be performed after attaining more studies per group, to explain the large proportion of unexplained heterogeneity.

AUTHOR DISCLOSURE

The authors declared no conflicts of interest.

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None.

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OPEN ACCESS – ORIGINAL ARTICLE

Serotype Distribution and Antimicrobial Resistance of *Streptococcus pneumoniae* in the Philippines, 2004-2011*

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ABSTRACT

Objective. Scarcity of data on the serotype composition and antibiotic resistance of invasive pneumococci from developing countries has been noted.[13] We describe in this study the serogroup distribution and antimicrobial resistance patterns of *Streptococcus pneumoniae* in the Philippines from 2004-2011.

Methodology. *S. pneumoniae* isolated from patients with invasive pneumococcal disease (IPD) were referred to the Antimicrobial Resistance Surveillance Reference Laboratory from 2004 to 2011. Typing of isolates was done through slide agglutination and antimicrobial susceptibility was determined following CLSI methods.

Results. The penicillin-resistant meningitis isolates were of serotypes 1, 5 and 14 which are covered by PCV10 and 13. The erythromycin resistant isolates were serotype 9 while cotrimoxazole resistant isolates were serotypes 1, 5, 6, 12 and 14. Forty-one percent of the cotrimoxazole resistant isolates are covered by PCV7, and 88% are covered by both PCV10 and PCV13. Levofloxacin resistant isolates were of serotypes 5 and 23 with PCV7 coverage of 50% and PCV10 and PCV13 coverages of 100%.

Conclusions. *S. pneumoniae* serotypes causing IPD in the country is largely similar to the dominant IPD serotypes worldwide. The serotype distribution in the Philippines remained stable from 2004 to 2011 and antimicrobial resistance among the isolates remained low. The serotypes of antibiotic resistant *S. pneumoniae* in this study were not similar with known serotype resistance profiles in other Asian countries. With the inclusion of PCV in the free national immunization program of the country beginning 2013, continued surveillance of prevailing pneumococcal serotypes should be done to monitor any shift in the prevalence of PCV associated serotypes to guide disease control measures including control of emergence of resistant pneumococcal isolates.

Key words: Streptococcus pneumoniae, antimicrobial resistance, serotype, ARSP

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INTRODUCTION

Streptococcus pneumoniae (S. pneumoniae) remains a significant pathogen causing morbidity and mortality in the Southeast Asian region. A World Health Organization (WHO) 2008 bulletin indicated that the 5 of the top 10 countries with biggest numbers of pneumonia cases, namely India, China, Indonesia, Vietnam and the Philippines, are from the geographic region of the Southeast Asia.[1]

Invasive pneumococcal disease (IPD), where *S. pneumoniae* are isolated from a normally sterile site, most frequently affects children less than 2 years old, adults at least 65 years old and immunocompromised individuals. It is associated with 25% morbidity and 6% mortality rates among children and a case fatality rate of 6-24% in the same population.[2,3] Mortality rates are much higher in young children in developing countries (10-40%), likely due to poorer access to healthcare and co-morbidities.[4] Currently, there are 93 known pneumococcal serotypes exhibiting a wide range of epidemiological profiles but only 20 of these account for over 80% IPD cases globally. Control measures against IPD through immunization had been directed largely against these most common serotypes causing IPD.

Vaccination has proven to be a successful intervention against infection by *S. pneumoniae*. The WHO in 2010 reports a reduction in the incidence of pneumococcal pneumonia in children less than 2 years old by about 30% since the introduction

of the pneumococcal vaccine.[5] However, it has been observed that the reduction of infection due to vaccine serotypes following immunization has been accompanied by an increase in infection due to non-vaccine serotypes.[6-10]

Pneumococcal conjugate vaccine 7 (PCV7), which covers *S. pneumoniae* serotypes 4, 6B, 9V, 14, 18C, 19F and 23F was introduced in the Philippines in 2006.[11,12]

It has been noted that there is scarcity of data on the serotype composition and antibiotic resistance of invasive pneumococcal strains from developing countries.[13] We describe in this study the serogroup distribution and antimicrobial resistance patterns of *S. pneumoniae* in the Philippines from 2004-2011. Each serogroup varies in prevalence, age group infected, geographical distribution, and antimicrobial resistance patterns. Knowledge of circulating serogroups and their antimicrobial susceptibility profiles is important for the development of effective vaccine strategies and will ensure a sustainable monitoring program on the effect of immunization on disease control.[14,15]

METHODOLOGY

The isolates

S. pneumoniae isolates from sterile body fluids were collected through the Philippine Department of Health - Antimicrobial Resistance Surveillance Program (DOH-ARSP) from January 1, 2004 to December 31, 2011. The DOH-ARSP is a sentinel based surveillance which receives isolates from 22 sentinel sites strategically distributed throughout the Philippines.[16-19] *S. pneumoniae* isolates from ARSP that were stored in skimmed milk and kept frozen at -80°C, and/or lyophilized were revived for re-identification. Identity of isolates were confirmed through optochin disk test and/or by bile solubility test.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing for penicillin, erythromycin, cotrimoxazole, ceftriaxone and levofloxacin was done following the Kirby Bauer Method using Clinical and Laboratory Standards Institute (CLSI) recommended antimicrobial disks and e-tests. Results were interpreted using appropriate breakpoints as defined by CLSI standards.[20]

Typing

Pneumococcal isolates were divided into serogroups and serotypes through slide agglutination following the Denka Seiken Slide Agglutination Method.[21,22] Serogrouping through slide agglutination test has a 95.7% overall agreement with the gold standard Quellung reaction. Due to unavailability of factor sera, no further typing was done within serogroups which have several serotypes.

RESULTS

A total of 195 isolates of *S. pneumoniae* were collected from patients with IPD during the 8-year study (Table 1). The age range of the patients was 0-90 years with a mean age of 29 years and median age of 20 years. There were more males (64%) than females among the patients. Of the 195 isolates, 63% (123) were from blood samples, 11% (21) were from cerebral spinal fluid and 26% (51) were from pleural fluid. Many of the isolates (39%) were isolated from patients of the 18-64 years age group, followed by 0-6 years old (32%), 6-18 years old (15%) and 65 years and older (14%).

Table 1. Frequency of *Streptococcus pneumoniae* serotypes (Philippines, 2004-2011, n=195)

Serotypes	2004	2005	2006	2007	2008	2009	2010	2011	Total
4		1				2	3	6	12
6		1	1	1		1	7	4	15
9		1					1	1	3
14							5	1	6
18						1	3		5
19						1			1
23		1	1			1	4	1	8
1	3	7	2	1	4	6	15	13	51
5	3	3	1	2	9	3	8	5	34
7					1		1		2
3		1						6	7
2				1	2	2	1	1	7
12			1			2	2	2	7
15					1		1	1	3
17					1				1
20				3				2	5
22			1				1	2	4
33				1					1
16						1	1	1	2
24				1					1
25				1		2		1	4
28							1		1
29						2		1	3
31							1		1
34							1		1
38	1						1		2
39							1		1
Non-Serotypable						3	1	3	7
Total	7	15	7	11	18	27	59	51	195

Legend:
PCV7 Serotypes: 4, 6B, 9V, 14, 18C, 19F, 23F
PCV10 Serotypes: PCV7 + 1, 5, 7F
PCV13 Serotypes: PCV10 + 3, 6A, 19A
PPV23 Serotypes: 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20, 22F, 23F, 33F

Over the 8 year study period, among 195 isolates, 96% (188) were serotyped while 4% (7) could not be typed. There were 27 serotypes identified with the most frequently occurring being serotypes 1 (26.15%), 5 (17.44%), 6 (7.69%), 4 (6.15%), 23 (4.10%), 3 (3.59%), 2 (3.59%) and 12 (3.59%) (Table 1). These 8 serotypes composed 72.3% of the total isolates.

Among patients under the age of 5, the most common serotype were 1(13%) & 6(18%) while among patients 6-17 years and 18-64 years, serotype 1(23%, 43%) and 5(35%, 23%) were most prevalent. Among the elderly (age more than 65 years), the most frequently occurring were serotype 1(14%), 3(17%), 4(14%) and 12(14%).

Coverage of currently available pneumococcal vaccines of the serogroups identified in this study were as follows: PCV7 - 25.6%, PCV10 - 70.3% and PCV13 - 73.8%.

Antibiotic resistance of the pneumococcal isolates were low (Figure 1). The overall penicillin resistance were at 0% and 2.5% using the non-meningitis and meningitis CLSI breakpoints, respectively. Erythromycin resistance was 0.51%, cotrimoxazole and levofloxacin resistance were at 8.20% and 1.03%, respectively. All of the isolates were observed to be susceptible to ceftriaxone. Two percent of the isolated *S. pneumoniae* was observed to be multi-drug resistant (3/195).

The penicillin-resistant meningitis isolates were of serotypes 1, 5 and 14 (Table 2). These serotypes are all covered by PCV10 and 13 but not by PCV7 which does not cover for serotypes 1 and 5. The erythromycin resistant isolates were serotype 9 while cotrimoxazole resistant isolates were serotypes 1, 5, 6, 12 and 14. Forty-one percent of the cotrimoxazole resistant isolates are covered by PCV7, 88% are covered by PCV10 and PCV13 and 6% were non-vaccine serotype. Levofloxacin resistant isolates were of serotypes 5 and 23. PCV7 coverage of levofloxacin resistant isolates were 50% and PCV10 and PCV13 coverages were both 100%.

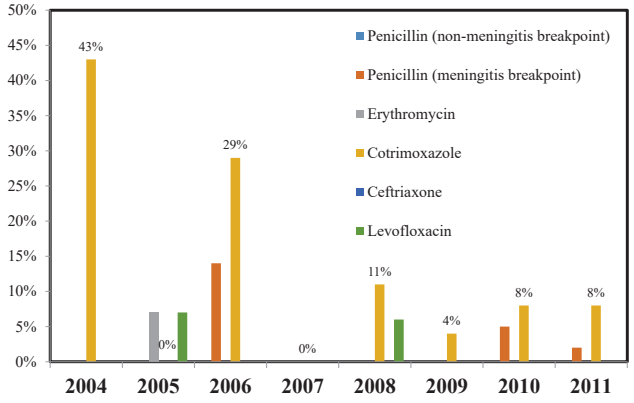

Figure 1. Resistance profile of *S. pneumoniae* isolates (Philippines, 2004-2011, n=195).

Table 2. Serotypes of antibiotic resistant *Streptococcus pneumoniae* (Philippines, 2004-2011)

Antibiotic Resistance among *Streptococcus pneumoniae* isolates	Serotype
Penicillin resistance	1, 5, 14
Erythromycin resistance	9
Cotrimoxazole resistance	1, 5, 6, 12, 14
Levofloxacin resistance	5, 23

DISCUSSION

Serotype distribution

The Philippines have been identified by the United Nations Children's Fund and World Health Organization to be among the 15 countries that contributes the most to the total childhood pneumonia cases worldwide registering childhood mortality rates of 37.8/100,000 in year 2000.[3] It is recognized that pneumococcal serotypes varies in prevalence, age group infected, geographical distribution, and antimicrobial resistance patterns. There is thus a need for country-specific knowledge of the predominant pneumococcal serotypes and their distribution. The Philippines, however, have paucity of data to provide knowledge on *S. pneumoniae* prevalence and distribution which may be used to evaluate the effect of disease prevention measures.[23-24] The present study provides an estimate of the serotype distribution and antimicrobial resistance of *S. pneumoniae* causing invasive pneumococcal disease in the Philippines over an 8 year period (2004 to 2011).

The dominant serotypes associated with IPD worldwide include 14, 4, 1, 6A, 6B, 3, 8, 7F, 23F, 18C, 19F and 9V. The serotype profile seen in the present study is largely similar to the dominant IPD serotypes worldwide with the most common serotypes in this study being 1, 5, 6, 4, 23, 3, 2 and 12 (Table 1). Of the 27 serotypes identified, we report here the identification of 10 serotypes – 3, 16, 17, 24, 25, 29, 31, 34, 38 and 39 – which have not been reported in previous local studies.[23,25] It was noted that two nonPCV serotypes - serotypes 1 and 5 - were seen to be present yearly throughout the 8 year study period in contrast with the other serotypes which occurred sporadically over the 8-year study period. Serotype 1 were isolated yearly from age group 19-64 years and were seen to occur sporadically in the rest of the age groups. Serotype 5 were consistently seen from age groups 6-18 years and 19-64 years throughout the 8 year period in the study. This observation provides support for the current local adult immunization recommendations of giving both PCV13 and polysaccharide vaccine to adults.[26]

Phongsamart et al. reports that in Thailand, the pneumococcal serotypes associated with invasive pneuomococcal pneumonia (IPD) among children under 5 years of age were 6B, 6a, 9V, 14, 19A, 19F, and 23F.[14] In a study done by Capeding et al. at a tertiary care center in the Philippines from 2000 to 2005, serogroup 6 was also isolated from children with IPD admitted in a tertiary hospital, and together with serotypes 18 and 14, were responsible for 50% of the admitted IPD cases in children.[23] In our study, serotype 6 was likewise the most common serotype isolated from patients under the age of 5 (18%). Serotype 1 which was not observed from 2000-2005 in a local study was also fairly more common (13%) in the present study.

The use of PCV7 has been reported to be successful in combating IPD cases. It is recognized that even the non-vaccinated individuals may benefit from the vaccine due to the decreased circulation of the pathogen as a result of the reduction in nasopharyngeal carriage of pneumococci among those who received the vaccine.[27] There are reports, however, of increasing prevalence rate of nonPCV7 associated serotype infections following introduction of PCV7.[9,27-29] According to Feiken et. al., factors that contribute to the rising cases of nonPCV7- associated serotypes include 1) secular trends in serotype prevalence occur over time, 2) changes in antibiotic use 3) characteristics

of surveillance systems and 4) changes in susceptibility of the population to pneumococcal diseases.[30]

In the present study, it was observed that the prevalence of serotypes covered by PCV7 remained stable from 27% in 2005 to 27% in 2011. Consequently, the observed prevalence of nonPCV7 serotypes likewise remained stable at 73% in 2011 which is the same nonPCV7 rate in 2004. The absence of a decrease in the prevalence of PCV7 serotypes even after the introduction of PCV7 in the country in 2006 may reflect poor coverage of PCV7 among susceptible population. Administration of PCV7 was introduced in the country in 2006 through private institutions. It was not, however, included in the mandatory free national immunization program of the government during the period of the study. Though PCV7 was available in the private institutions in the Philippines by year 2006, the vaccine is largely considered to be expensive in a generally poor and developing third-world country. Beginning the year 2013, PCV has since been included in the free national immunization program of the country. As we anticipate better PCV immunization coverage among the susceptible age groups, continued surveillance of prevailing pneumococcal serotypes may be done henceforth to monitor any shift in the prevalence of PCV associated serotypes to guide disease control measures.

Antimicrobial Resistance

The levels of antimicrobial resistance of *S. pneumoniae* isolates in many Asian countries are among the highest in the world during the early part of this century. Isolates from Vietnam showed the highest prevalence of penicillin resistance at 71.4%; and erythromycin resistance at 92.1%. Isolates from Hong Kong showed the highest rate of ciprofloxacin resistance at 11.8%. Resistance to penicillin ranged from 38.6% among isolates from Taiwan to 71.4% among isolates from Vietnam. Erythromycin resistance was from 73.9% (China) to 92.1 % (Vietnam) while resistance to ciprofloxacin was from 6.5% (Korea) to 11.8% (Hong Kong). Data from the Philippines showed that 18.2% of the isolates were resistant to erythromycin while 9.1% were ciprofloxacin resistant.[32] Statistics from the Philippines' Antimicrobial Resistance Surveillance Program (ARSP) progress reports also showed resistance rates of pneumococci to cotrimoxazole to be higher (from 14% in 2006 to 15% in 2011) relative to other antibiotics. ARSP annual progress reports showed that resistance rates to penicillin, and erythromycin among all pneumococcal isolates have remained low at <4%, and 2% respectively from 2007 to 2011.[17-19,31-32] The current study shows that antimicrobial resistance among IPD isolates remained low in the country over the study period (Figure 1). Resistance to cotrimoxazole was seen to decrease from 43% in 2004 to 6% in 2011 while resistance to penicillin, erythromycin and levofloxacin remained less than 5% in the later years of the study (2009-2011).

Serotype and Antimicrobial Resistance

Certain pneumococcal serotypes have been identified to be associated with specific drug resistance among pneumococcal isolates. Data would show that most penicillin-resistant and macrolide-resistant isolates are derived from five serotypes (6B, 9V, 14, 19F, 23F), all of which are covered by PCV7.[4] In a study among 555 pneumococcal isolates from 10 Asian countries, it was observed that penicillin resistance was most common among serotypes 19F and 23F while erythromycin resistance was more commonly seen among serotypes 19F, 23F, 14, 6B and 6A.[33] An earlier study among pneumococcal isolates received at the Pneumococcal Reference Laboratory in Spain from January 1990 to December 1996 showed similar results with serotypes 6, 9, 14, 19 and 23 being associated with penicillin resistance. From the same set of isolates, it was observed that the difference in penicillin resistant rates is mainly due to differences in the prevalence of pneumococci belonging to the aforementioned serotypes.[15]

Contrary to data from other Asian countries, there were relatively few serogroups 19 and 23 seen in the present study and none of them were observed to be resistant to penicillin. Penicillin resistance was mostly seen among serotypes 1, 5 and 14. Half (3 out of 6) of the serotype 14 isolates exhibited penicillin resistance with 2 of the isolates coming from children less than 1 year. Penicillin resistance among serotype 1 was at 2% (1 out of 51) and was at 6% (2 out of 34) among serotype 5. It was noted that 67% (4 out of 6) of penicillin resistant serotypes (1 serotype 1, 1 serotype 14, and 2 serotype 5) came from age groups 6-18 years and 19-64 years. Prevention of infection from pneumococcal isolates serotypes 1, 5 and 14 that is conferred by both PCV10 and 13 will appear therefore to help ensure continued low resistance to penicillin among pneumococcal isolates in the country.

Eight percent of the total isolates in this study were found to be resistant to cotrimoxazole. Cotrimoxazole resistance was seen to decrease through the eight year study period from 43% in 2004 to 6% in 2011. This reduction may reflect the shift from the use of cotrimoxazole as first line antibiotics to beta-lactam antibiotics during the study period. Cotrimoxazole resistant isolates were of serotypes 1, 5, 6, 12 and 14 with serotype 5 making up 44% of all isolates that were cotrimoxazole resistant and serogroup 6 accounting for 38%. It was noted that there was only 1 isolate of serotype 14, the serotype associated with cotrimoxazole resistance in Asia,[6] which was found to be cotrimoxazole resistant in the present study.

It was observed that 21% (7 out of 34) of all serotype 5 isolates in the study were resistant to cotrimoxazole while 40% (6 out of 15) of serotype 6 were cotrimoxazole resistant. Through the eight year study period, the percentage of serotype 5 that were cotrimoxazole resistant decreased from 100% (3 out of 3) in 2004 to 0% (0 out of 5) in 2011. In contrast, the percentage of cotrimoxazole resistance among serotype 6 increased from zero in 2005 to 50% (2 out of 4) in 2011. Cotrimoxazole resistance in the later 3 years of the study was mainly due to serotype 6. It was also noted that 56% (9 out of 16) of cotrimoxazole resistant serotypes were seen among patients less than 5 years old and the rest were seen in the 6-17 years and 8-64 age groups. No cotrimoxazole resistant serotypes were seen among the >65 age groups. These observations would favor the introduction of PCV to help reduce not only the incidence of IPD among the susceptible age group but to also reduce cotrimoxazole resistance among pneumococcal isolates in the country.

Though it is not clear why particular pneumococcal serogroups have a higher probability of containing specific resistance genes, it is acknowledged that there are evidences of strong associations between resistance patterns and serotype.[34] However, an exchange of the gene encoding capsular serotype can happen between pneumococcal strains through transformation. It is thus possible that highly resistant clones may become members of highly invasive serotypes which are currently not associated with drug resistance. Information from continued surveillance of

resistant serotypes to guide immunization policies will therefore be very useful in the control of IPD as well as in the control of antimicrobial resistance among pneumococcal isolates.

To be noted in this study is the high infection rate observed in males which can be due to more exposure to socio-economically related risk factors, such as alcohol, smoking and labor in a polluted environment.[35] This can also be factor in the high isolation rate of *S. pneumoniae* from the 18-59 year age group as this is generally considered as the working age group in the Philippines thus exposing the males more to the risk factors. Also, during the study period, the vaccination against *S. pneumoniae* was recommended primarily among target children or those included in 0-5 year old age group.

CONCLUSION

The present study showed that *Streptococcus pneumoniae* serogroup/serotype profile causing IPD in the country is largely similar to the dominant IPD serogroup/serotypes worldwide. The most common serogroups/serotypes causing IPD in this study includes 1, 5, 6, 4, 23, 3, 2 and 12. The serotype distribution of *S. pneumoniae* in the Philippines remained stable from 2004 to 2011 and the antimicrobial resistance among the isolates remained low. The serogroups/serotypes of antibiotic resistant *Streptococcus pneumoniae* in this study were not similar with known serotype resistance profiles in other Asian countries. With the inclusion of PCV in the national free national immunization program of the country beginning 2013, better PCV immunization coverage among the susceptible age groups is expected. Continued surveillance of prevailing pneumococcal serotypes should be done henceforth to monitor any shift in the prevalence of PCV associated serotypes to guide disease control measures including control of emergence of resistant pneumococcal isolates.

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AUTHOR DISCLOSURE

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A Case Report and Literature Review of Fetus in Fetu – A Rare Aberration of Embryogenesis in a 22-month-old Infant*

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ABSTRACT

Fetus in fetu (FIF) is an uncommon pathology resulting from an abnormal embryogenesis occurring in a diamnionic, monochorionic twinning during pregnancy. It is so rare that there is only one underdocumented case reported in the Philippines. We are faced with a curious case of a 22-month old male child who presented with a gradually enlarging abdomen, diagnosed as FIF as confirmed by radiologic studies. After undergoing the necessary laboratory and radiologic work-ups, the patient was stabilized and eventually cleared for surgery. He underwent exploratory laparotomy with excision of FIF, from which a fetoid structure was recovered. Thorough gross and further radiologic evaluation of the recovered fetoid structure reveals findings that fulfilled the diagnostic criteria of a FIF.

Key words : fetus-in-fetu, teratoma, intra-abdominal mass, fetoid

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INTRODUCTION

The term "fetus in fetu" (FIF) was first used by Johann Friedrich Meckel during the late 18th century.[1] Subsequently, Willis described it as a rare condition where a malformed parasitic twin resides in the body of its host, usually in the host's abdominal cavity.[2] The incidence is about 1 in 500,000 births and only about 100 cases of FIF have been reported to date.[3] In the Philippines, only one case of FIF was reported in August of 2007.[4] The majority of FIF cases presents as a retroperitoneal mass[5-7] while others are seen in the sacrum and sacrococcygeal area[5,6] and rarely within the cranium.[8] Other reported cases show FIF existing within a teratoma.[6]

The condition represents an aberration of monozygotic diamniotic twinning where the unequal division of the totipotent inner cell mass of the developing blastocyst leads to the inclusion of a smaller cell mass within a maturing sibling embryo. It is supposed to be a highly differentiated form of teratoma.[9]

However, in view of the fact that body parts can be identified within it, there is a tendency to consider this condition as being distinct from a teratoma. It has been suggested that if spinal elements are absent, the lesion is a teratoma, whereas if they are present the tumor can be considered to be a FIF.[10]

This rare phenomenon, being the second reported and the first well-documented incident of FIF in the Philippines, as well as the importance of differentiating it from a teratoma, makes it a reportable case for which it is now presented.

CASE

This is a case of a 22-month-old infant who presented with a gradually enlarging abdomen, which started at the age of 3 months. An initial ultrasound of the whole abdomen was done at 4 months of age, revealing a thick-walled, cystic mass at the right upper abdomen, suspected to be hepatic in origin. The age of the patient and the initial ultrasound findings lead to an initial consideration of Hepatoblastoma. The patient was then lost to follow-up. However, due to a progressively enlarging abdomen, the patient and his mother came back and sought consult with a private physician

wherein a second whole abdominal ultrasound was requested and revealed FIF.

The patent was subsequently admitted. Pertinent laboratory tests such as Beta HCG, CEA and AFP levels are all within normal range.

Thorough physical examination reveals a distended abdomen with an abdominal girth of 54 cm (Normal: 50 cm).

A firm abdominal mass was palpated at the right upper quadrant, hence, a CT Scan was requested. The scan reveals a huge complex intraabdominal mass with septations, calcifications, long bones and axial skeleton, highly considering Fetus in Fetu (Figure 1). After being cleared, the patient underwent exploratory laparotomy with incidental appendectomy and excision of FIF.

Intraoperative findings reveal a large retroperitoneal mass measuring 9.4x10.7x9 cm attached to the mesentery along with its feeding vessels. The mass was excised and opened, revealing a thick-walled cyst containing a fetoid structure measuring 750 grams, entirely covered by thick vernix caseosa. The vernix was wiped off, revealing that the specimen is entirely covered with skin, and shows several structures resembling poorly-formed body parts.

The fetoid structure consists of a partially-formed head lacking a well-formed cranium and brain (anencephalic) (Figure 2). It measures 21 cm in circumference and is partially covered with fine black hair. There is a partially-formed structure resembling the right eye, a small mid-line protrusion resembling a nose and a wide slit-like opening which are presumed to be the optic placodes, nasal placode and the unfused brachial apparatus respectively. A few teeth are noted under the nasal placode. The trunk measures 30 cm in circumference and shows a tan, sac-like structure, measuring 3x3x2.3 cm, resembling an omphalocoele protruding out of the chest. The chest and the trunk were opened and the sac was noted to be an extension of the peritoneal cavity which contains a coiled tubular structure measuring 7 cm long, resembling a primitive gut. At the center of the pelvic area is a structure measuring 4 cm long and 7 cm in circumference, with a rounded tip showing a meatal opening, resembling a poorly-formed penis (phallus). The meatal opening was cannulated into the urethra and revealed absence of continuation into the peritoneal cavity. Bilateral limb buds resembling upper and lower extremities with absence of well-formed digits are seen. The posterocaudal area shows a gluteal cleavage with imperforate anus. Further dissection reveals presence of a fused vertebral column housing an underdeveloped spinal cord. Abundant adipose tissues, as well as cartilaginous and bony tissues are likewise seen in other areas.

Pertinent histologic findings confirm that the thick-walled cyst and the external surface of the fetoid structure are composed of skin tissue consisting of keratinized stratified squamous epithelium and adnexal structures such as sweat glands, sebaceous glands and hair follicles. The right optic placode are lined by eroded corneal epithelium with scattered melanocytes and occasional acinar-like structures resembling lacrimal glands. The primitive gut is lined by intestinal epithelium. The urethra is lined by urothelial cells. The spinal reveal a central canal lined by pseudostratified cuboidal to columnar epithelium. The surrounding gray matter is poorly delineated and contains several multipolar motor neurons, scattered microglia and a few lymphocytes. The white matter contains a few poorly formed axons. Mature adipose tissues, cartilage and bone are likewise seen. There are no immature components seen in the sections examined.

Post operatively, the patient had an uneventful course in the wards and was discharged as soon as he was convalescent. Follow-up appointments at the Outpatient Department was done and revealed that the patient has improved appetite and has gained weight.

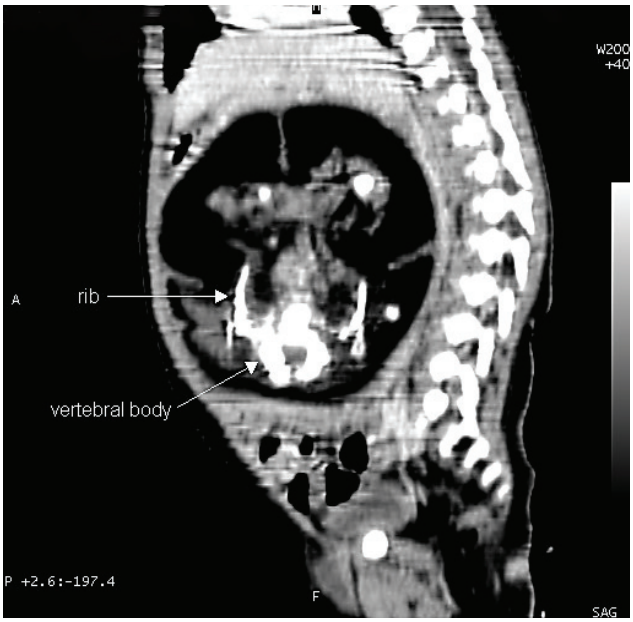

Figure 1. Computed tomography, sagittal view, depicting a huge, complex and septated intrabdominal mass with opacities representing the vertebral body and rib.

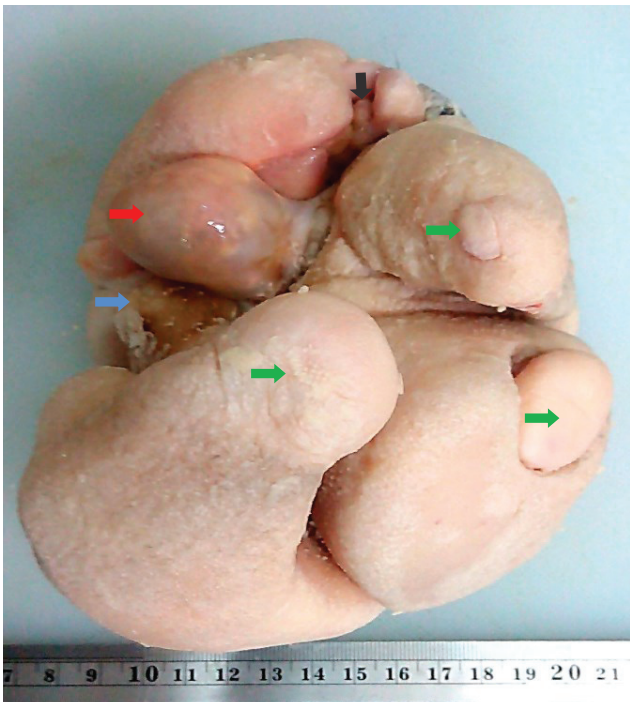

Figure 2. A fetoid structure showing a midline, unfused slit-like structure (black arrow) presumed to be the brachial apparatus; three out of four limb buds (green arrows); the inferior surface of the phallus (blue arrow); and a sac-like structure (red arrow) containing the primitive gut.

DISCUSSION

Fetus in fetu is defined by the presence of one or more of the following characteristics: (1) it is enclosed within a distinct sac; (2) it is partially or completely covered by normal skin; (3) it has grossly recognizable anatomic parts; and (4) it is attached to the autosite by a pedicle containing a few relatively large blood vessels. On the other hand, teratoma consists of a chaotic mixture of tissues, which are also found within the body of the autosite, but it has the following characteristics: (1) it is enclosed in connective tissue; (2) it is broadly attached to the surrounding tissue; and (3) it is capable of independent growth or malignant behavior.[11]

Our case fulfilled the diagnostic criteria of fetus in fetu on sonography and pathologic examination. On pre-operative CT scan, there was an anencephalic acardiac mass with identifiable ribs, extremities and spine within an intraabdominal cystic mass (Figure 1). Autopsy findings revealed a solid mass with immature upper extremities and spine inside a fluid-filled sac. Microscopic evaluation of the solid mass had the general tissue characteristics of Fetus in Fetu, with right optic placode, primitive gut, underdeveloped spinal cord and urethra.

This case had an initial presentation of progressive abdominal enlargement of the right upper quadrant in a 22-month-old infant. The major presenting complaint is a palpable abdominal mass, predominantly in upper abdomen.[12] Few reports describe antenatal diagnosis of FIF. Preoperative diagnosis can be made on plain radiographs and CT scan/MRI. The presence of vertebrae, long bones, bones of hands and feet etc are the common radiological findings. Visualization of a non-homogenous mass with bones especially vertebrae is considered pathognomonic of FIF. Failure to visualize vertebrae however does not rule out possibility of FIF. The other frequent differential is teratoma.[13-15]

The fetus is always anencephalic, the vertebral column and the limbs are present in the fetus in fetu in almost all cases (91% and 82.5%, respectively).[16] The lower limbs are more developed than the upper limbs. Fetus in fetu was rarely found in the central nervous system, gastrointestinal tract, vessels, or the genitourinary tract; however, it was found in 55.8%, 45%, 40% and 26.5% of cases, respectively. It was rarer still to find fetus in fetu in the lungs, adrenal glands, pancreas, spleen, and lymph nodes. The heart was very rarely found in fetu.17 Patient in this case was male, anencephalic and acardic with a right optic placode, primitive gut, underdeveloped spinal cord and urethra. There is no definite data that states the number of FIF with phallus, however, two cases reported its presence.

Serum AFP and BHCG may be normal in most cases or occasionally elevated.[18] One case reveals elevation of both tests. In this patient, both are within normal range.

FIF is usually overlooked in the differential diagnosis of a newborn abdominal calcification. Clinically, FIF can be differentiated from teratoma by the presence of vertebral bodies and limbs. The presence of vertebral bodies not only means that the FIF passed the primary stage of gastrulation, but also may reflect its derivation from a primitive streak. The formation of the primitive streak normally starts during the 3rd week, together with gastrulation that will lead to the notochord formation and subsequently to the vertebral column and segmental axis. Therefore, FIF likely arises from a zygote at a primitive-streak stage and fetoid mass develops to a certain degree in a manner similar to normal fetal development.[19]

In our patient, pathologic examination showed vertebral column within the mass, further supporting the diagnosis of FIF.[20]

The recommended treatment for FIF is surgical excision. Because the final diagnosis of FIF is not made until pathological analysis, all parts of the mass should be removed to prevent malignant recurrence.

Although the prognosis for FIF is more favorable than for cystic teratoma, the presence of immature elements nevertheless indicates the need for close clinical and radiological follow up.

CONCLUSION

In conclusion, FIF is a rare and interesting entity that typically presents as an abdominal mass in infancy or early childhood. It can be diagnosed in the preoperative period through imaging techniques. Complete excision of the mass is curative and confirmatory. Though a rare entity, it should be kept in mind as a differential diagnosis for any abdominal mass in infancy and early childhood and must be differentiated from teratoma, since teratomas have a possibility to develop malignancy.

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AUTHOR DISCLOSURE

The authors declared no conflicts of interest.

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OPEN ACCESS – CASE REPORT

Langerhans Cell Histiocytosis of the External Auditory Canal in an Adult Patient with Myelodysplastic Syndrome: A Case Report

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ABSTRACT

A 68-year-old female with myelodysplastic syndrome presented with a 1-year history of gradually enlarging bilateral external auditory canal masses without temporal bone involvement. Material from the bilateral external auditory masses showed intraepidermal and dermal proliferation of cells exhibiting ovoid grooved or folded nuclei, fine chromatin and moderate amount of eosinophilic cytoplasm. The neoplastic cells are strongly and diffusely positive for CD1a and Langerin (CD207). A diagnosis of Langerhans cell histiocytosis was made and the patient administered with topical steroids. The patients' response to topical corticosteroid administration was less than favorable.

Key words: Langerhans cell histiocytosis, external auditory canal, adult, myelodysplastic syndrome

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INTRODUCTION

Langerhans cells (LC) represent a subtype of bone marrow derived dendritic antigen-presenting cells that normally reside in the skin, lymph nodes and mucosal lining of various organs.[1] Langerhans cell histiocytosis (LCH) is a rare disease characterized by the clonal proliferation of pathologic cells with the characteristics of Langerhans cells.[2] Patients may present with localized disease involving only a single organ system - most commonly bone, skin or lymph nodes or they may have multiple organ involvement already at the time of diagnosis.[3] Although they are typically encountered in children, adult onset is uncommon and its association with myelodyspalstic syndrome rarer still. Thus, this unusual presentation may often times present diagnostic difficulty among clinicians and practicing pathologists.

CASE

A 68-year-old female, non-smoker, presented with a 1-year history of intermittent ear pain associated with gradually enlarging bilateral external auditory canal masses resulting in progressive hearing loss. Physical examination showed non-tender, fixed, cream-colored external auditory canal masses obscuring direct visualization of the tympanic membrane bilaterally. Minimal yellowish discharge was also noted from the left ear (Figure 1). The patient was apyrexial, no skin lesions and she had no neurological deficits. Past medical history was generally unremarkable except for a splenectomy for myelodysplastic syndrome.

Temporal bone computed tomography (CT) scan showed soft tissue densities within the external auditory canals without definite evidence of bone erosion. Cholesteatoma or keratosis obturans was a consideration at this time.

Intraoperatively, the external auditory canals were filled with friable soft tissue not extending beyond the meatus. The tympanic membranes were not visible and no evidence of cholesteatoma formation was appreciated. Excision biopsy was performed on the left external auditory canal mass and and incision biopsy on the right with concomitant submission of the material for histopathology.

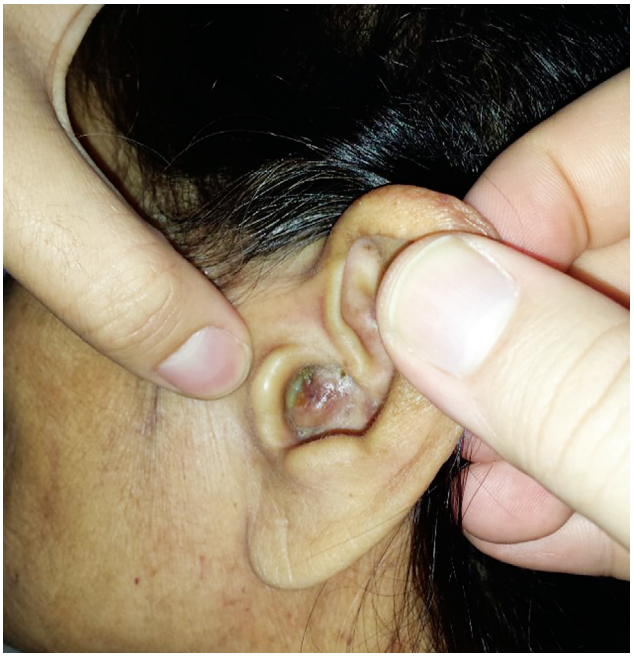

Figure 1. C.M. is a 68-year-old female with a chief complaint of intermittent ear pain secondary to gradually enlarging bilateral ear masses and is shown here with complete obstruction of the left external auditory canal with minimal purulent discharge.

Material from the bilateral external auditory masses showed intraepidermal and dermal proliferation of mononuclear cells. These cells have round to ovoid grooved or folded nuclei, fine chromatin and moderate amount of eosinophilic cytoplasm. These were admixed with abundant eosinophils. The neoplastic cells are strongly and diffusely positive for CD1a and Langerin (CD207) (Figure 2).

The external auditory canals of the patient were then applied with intertulle impregnated with Bethamethasone, Clotrimazole and Gentamicin sulfate (Triderm) ointment. No evidence of systemic disease was appreciated after blood and imaging analysis. However, regrowth within the external auditory canal was noted within merely three weeks after application of the topical steroid. Patient was then given the option for radiotherapy or intralesional steroid injection using Dexamethasone but the patient refused further management.

DISCUSSION

Langerhans cells (LC) are bone marrow derived dendritic antigen-presenting cells that normally reside in the skin, lymph nodes and mucosal lining of various organs.[1] Langerhans cell histiocytosis (LCH) is a clonal proliferation of activated Langerhans cells (LCs) occurring as an isolated lesion or as part of a systemic multifocal proliferation.[2] LCH is more commonly encountered in children and as such, most of the available information about its clinical features, pathogenesis, treatment and prognosis are derived from the pediatric perspective.[3]

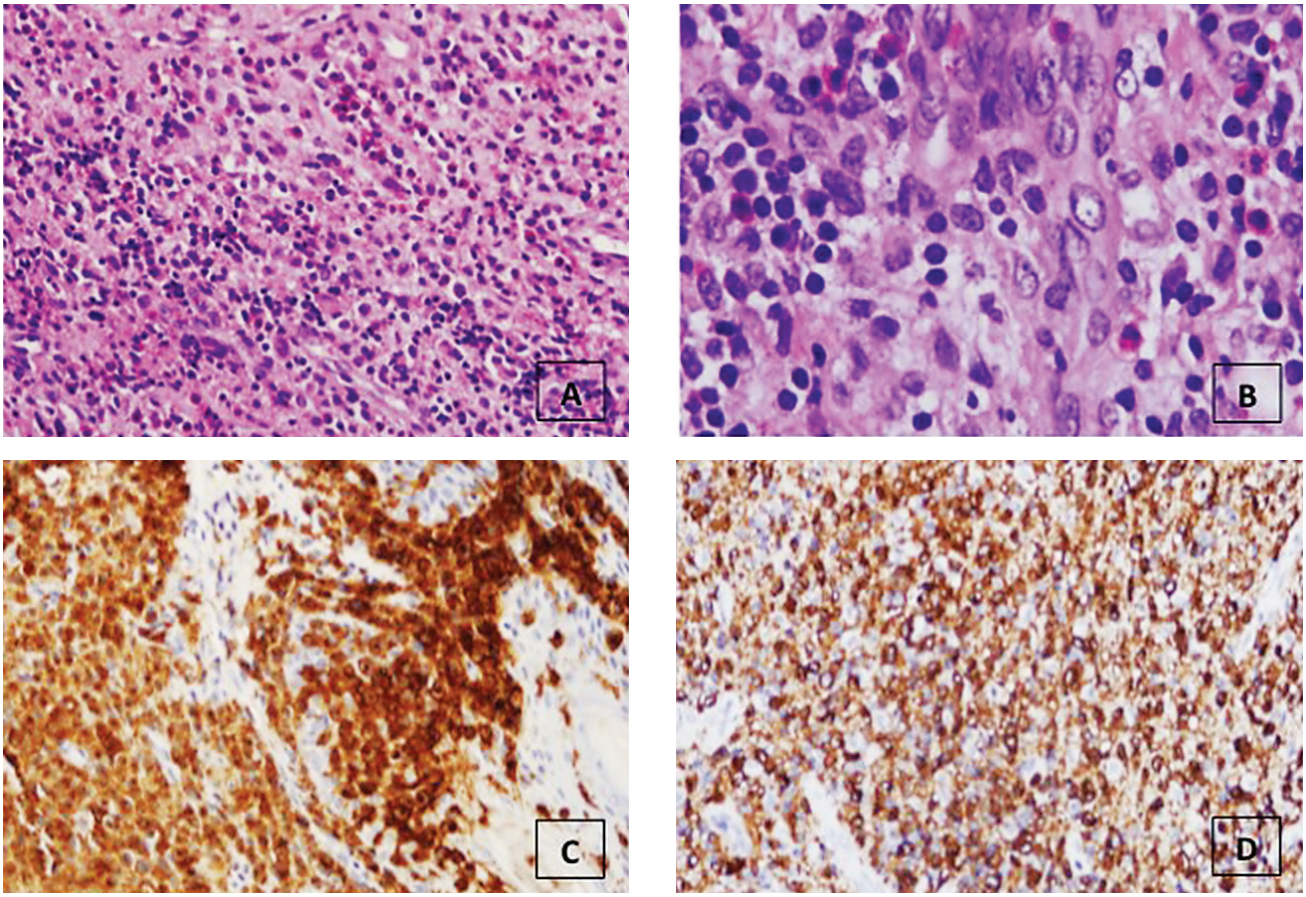

Figure 2. (A) The inflammatory cell infiltrates are comprised of a mixed population of polymorphonuclear leukocytes, plasma cells, lymphocytes and predominated by eosinophils (hematoxylin and eosin, 10x). **(B)** Cellular proliferation of cells with indentations of the nuclear membrane, nuclei with vesicular chromatin, inconspicuous centrally located nucleoli and moderate amount of eosinophilic cytoplasm (hematoxylin and eosin, 40x). Langerhans cells are strongly and diffusely immunoreactive for **(C)** CD1a and **(D)** Langerin (CD207).

Myelodysplastic syndrome (MDS) is a group of bone marrow disorders associated with dyplasia of myeloid elements that may present with cutaneous manifestations. Some of these cutaneous manifestations may be in the form of non-specific infections, leukocytoclastic vasculitis (i.e. erythema elevatum diutinum), neutrophilic dermatoses (as in Sweet's syndrome), pyoderma gangrenosum, and leukemia cutis. In recent years, cutaneous LCH have been documented in adults with MDS.[4] To date, this is only the third case of MDS associated with LCH in an adult with a novel presentation in the skin of the external auditory canal. Proposed associations between these two entities range from anomalous cytokine production with concomitant reactive histiocytic reaction to divergent differentiation of hematopoietic stem cells producing proliferations of plasmacytoid dendritic cells and langerhans cells.[5,6]

In 1987, the World Health Organization's Committee on Histiocytic/ Reticulum Cell Proliferations and the Working Group of the Histiocyte Society, established a criteria for the definitive diagnosis of Langerhans cell histiocytosis requiring the identification of Birbeck granules in lesional cells by electron microscopy or demonstration of CD1a antigen expression by immunohistochemistry.[7] Although the detection of Birbeck granules remains the gold standard, in most instances immunohistochemistry provides the basis for diagnosis because of its wide spread utilization in most clinical laboratories. However, it should be noted that CD1a expression is not entirely specific for LCH. CD1a immunoreactivity has been observed in other histiocytic proliferations such as sinus histiocytosis with massive lymphadenopathy, juvenile xanthogranuloma, and histiocytic sarcoma.[7-12] Langerin (CD207) offers an additional marker for the immunohistochemical identification of LCH. Previous studies using immunoelectron microscopy have shown preferential expression of Langerin within Birbeck granules.[13,14] This suggests that the detection of Langerin immunoreactivity may serve as a surrogate marker for the presence of Birbeck granules without the use of electron microscopy. Data from Dziegiel et al,[15] supports this premise. They observed a strong correlation between Langerin expression and the ultrastructural presence of Birbeck granules in cases of Langerhans cell histiocytosis. In this case, the lesional cells were strongly and diffusely immunoreactive with both CD1a and Langerin.

No standard therapeutic approach has yet been established for adult LCH. Management depends largely on the pattern of disease manifestations and the sites involved. In a large number of adult patients, the initial treatment decision included a 'wait-and-see' approach.[16] While this approach may be reasonable for patients with localized disease that do not impede normal function, a patient presenting with loss of hearing will not be amenable to waiting. The patient in this case was given topical steroids but recurrence was almost immediate. And further management could not be administered given the patients' refusal.

CONCLUSION

In conclusion, Langerhans Cell Histiocytosis should be included in the differential diagnosis of cutaneous eruptions in adult patients with Myelodysplastic Syndrome even in areas without previous documented predilection.

ETHICAL CONSIDERATION

The patient has been lost to follow-up from the social service department.

AUTHOR DISCLOSURE

The authors declared no conflicts of interest.

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Validation using Nucleic Acid Testing (NAT) on Blood Donor Samples Non-Reactive to Transfusion-Transmissible Viruses by Immunoassay (EIA/ChLIA)

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ABSTRACT

Transfusion-transmissible infections (HIV, HBV and HCV) remain a threat to public health specifically in assuring safe transfusion practices. This study aims to determine the ability of a blood service facility to accurately detect HIV, Hepatitis B and C and assess the need to include nucleic acid testing as part of the routine screening algorithm. Of the 3,233 samples from participants with screened sero-negative blood units, testing for HIV and Hepatitis C showed no discrepancies with EIA and NAT in all samples. Testing for Hepatitis B showed 12 (4.00%) samples which are reactive in both EIA and NAT, 3 (0.09%) samples were reactive with EIA only and 48 (1.48%) were detected for the presence of Hepatitis B Virus via NAT.

Key words: blood transfusions, human immunodeficiency virus, hepatitis, transfusion transmissible infections, nucleic acid test

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INTRODUCTION

Transfusion-transmissible infections (HIV, HBV and HCV) remain a threat to public health specifically in assuring safe transfusion practices. Although constant efforts are being exerted to eliminate possible transfusion of undetected infected blood products, there is still a certain level of residual risk that threatens blood safety.

Majority of blood service facilities in the Philippines are limited to the use of serological tests to detect antigen and antibodies which are less sensitive than the nucleic acid screening tests (NAT). A study by Lam et al., reported that upon retesting of 449 screened non-reactive blood units with nucleic acid testing, 0.45% were missed using serological tests alone.[1]

This study aims to determine the ability of a blood service facility to accurately detect HIV, Hepatitis B and C and assess the need to include nucleic acid testing as part of the routine screening algorithm.

METHODOLOGY

Participants

A total of 32 lead blood service facilities that had a monthly blood collection of more than 500 and participates in the TTI Serology External Quality Assessment Scheme was identified to participate in this study by the technical working group of the National Council for Blood Services (NCBS-TWG) through purposive sampling design. A department circular with written instructions and disclosure of the study design was disseminated to the identified facilities. Only 15 facilities from Luzon (60%), Visayas (26.67%) and Mindanao (13.33%) participated in the study.[2]

Sampling

Five percent of the monthly blood collection that have been tested non-reactive by serology tests and ready for transfusion were randomly selected by the participants and sent to the TTI-NRL for a period of 6 months.

Testing

Samples received were transferred to a sterile cryotubes and were tested using the Roche cobas s201 MPX v2.0 in pools of 6. NAT-negative results were reported and NAT-detected results were tested using the same platform in pools of 1. Results for pools of 1 were reported. The samples were then tested with Bio-Rad Genscreen™ ULTRA HIV Ag-Ab, Monolisa™ ULTRA HBsAg, Monolisa™ HCV Ag-Ab ULTRA using the Bio-Rad Evolis™ automated enzyme immunoassay (EIA) processor. Non-reactive results were reported and reactive results were retested using the modular method (Figure 1).

Limitations

Samples were sent in frozen aliquots by the participants following proper referral guidelines. Although detailed instructions were sent to participants in terms of preparation and transport of sample, blood cold chain practices of blood service facilities vary. Supplies for collection and referral were not provided by the TTI-NRL but were shouldered by the participants. The participants were instructed to randomly pick 5% of their sample for every test run for a period of 6 months, no specific instructions were given as to ensure that random sampling was properly done.

RESULTS AND DISCUSSION

Of the 3,233 samples from participants with screened sero-negative blood units, testing for HIV and Hepatitis C showed no discrepancies with EIA and NAT in all samples. Testing for Hepatitis B showed 12 (4.00%) samples which are reactive in both EIA and NAT, 3 (0.09%) samples were reactive with EIA only and 48 (1.48%) were detected for the presence of Hepatitis B Virus via NAT (Table 1).

Table 1. Hepatitis B testing results

	NAT (+)		NAT (-)		TOTAL	
	No.	%	No.	%	No.	%
EIA (+)	12	4	3	0.09	15	0.46
EIA (-)	48	1.48	3170	98.50	3218	99.54
TOTAL	60	1.85	3173	98.14	3233	100.00

The twelve Hepatitis B EIA and NAT reactive samples may have been either due to technical errors on the part of the testing facility or the platform used was not sensitive enough to detect the virus or viral mutants. A study by Scheiblauer et al., showed that some EIA assay kits missed HBsAg mutants and showed reduced sensitivity on certain genotypes.[3] The three Hepatitis B EIA reactive and NAT

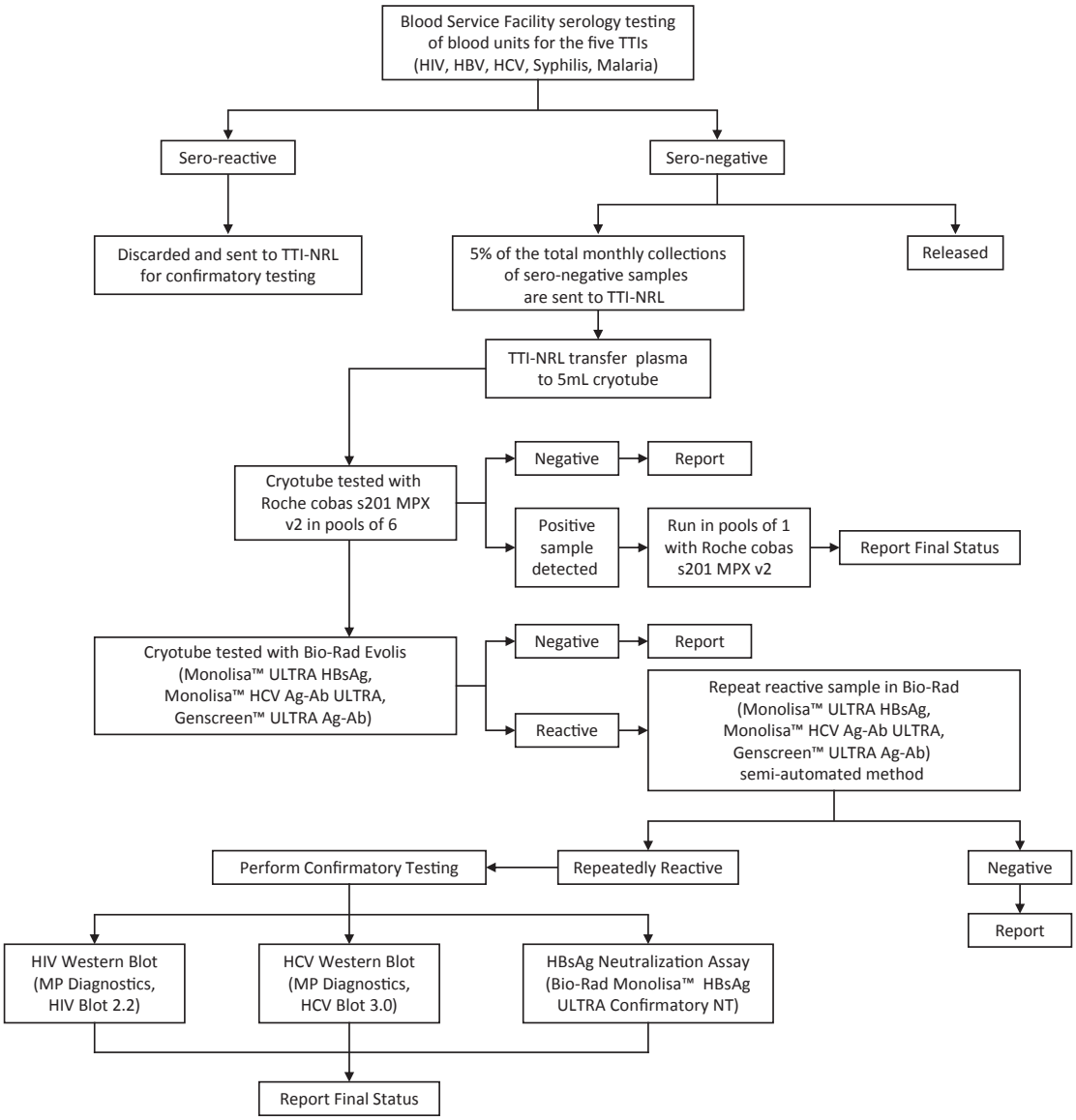

Figure 1. Testing process.

negative may be due to low viremia or the inactive carrier status of the donor.[4] The Hepatitis B discrepancy of 48 samples can be associated with the possibility of Hepatitis B viral mutants or occult blood infections (OBI) in which the presence of Hepatitis B Surface Antigen (HBsAg) in serological tests go undetected. This can be linked to the high prevalence of Hepatitis B infection in the country in which around 7.3 million Filipinos are chronically infected.[5]

CONCLUSION

NAT has been scientifically proven to reduce diagnostic window periods, and significantly decrease the residual risk of transfusion infection.[6] Blood donor testing in the Philippines is limited to the detection of HBsAg and in addition to serological testing, NAT can play a vital role in assuring that the risk of transfusion infected blood is significantly lowered specially in cases of OBI.[7] A study by Chigurapati et al., found that the combined yield (seronegative/NAT reactive) for HIV-1, HCV, and HBV was 4 out of the 8,000 samples tested (0.05%) and included only HBV.[8]

Discrepancies in the result of the BSF and the NRL may imply that quality systems in the BSF must be checked and validated.

RECOMMENDATIONS

All reactive results in the study will be subjected to further testing with HBV Monoplex RT PCR to replicate the results of Multiplex NAT and also for possible presence of anti-HBc and anti-HBs to determine cases of occult blood infection.

The use of NAT in the Philippines may be considered to be part of the national routine blood screening algorithm. Since NAT is highly expensive, a cost-benefit analysis for use of NAT may be done. Centralized testing on a national level may offer a more cost effective strategy as the high volume of units to be tested may impel suppliers to lower down costing. This approach however must be carefully planned since the geography of the country can be challenging and a feasibility study be conducted to ensure that hospitals requiring blood units are given stocks appropriately. Also, an alternative such as the addition of another serological marker (i.e. anti-HBc) may be considered.[9] Validation of screened non-reactive blood units may be part of the routine process in referrals as part of the quality assurance in the national blood program.

The NRL also recommends that all BSFs establish and practice strict Quality Assurance Activities in Blood Screening. Realizing the current limitation in testing, there is a need for a paradigm shift in assuring blood safety. The safety of the blood supply does not depend on the strength of the testing platform but on the quality of blood donors. Blood donor recruitment and donor selection should be strengthened among all blood service facilities.

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AUTHOR DISCLOSURE

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Acanthomatous Ameloblastoma

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Key words: ameloblastoma, acanthomatous, odontogenic

A 22-year-old female noticed a 1.0x1.0 cm gingival mass of one-year duration. Two months prior to consult, a panoramic radiograph was performed, revealing a defined unicystic, mixed radiopaque and radiolucent lesion between the premolars on the right hemi-mandible, causing displacement on the roots of the premolar without signs of resorption and not associated with any unerupted tooth (Figure 1). Physical examination revealed a swelling at the right mandibular premolar area (Figure 2). Enucleation with peripheral ostectomy was performed and the mass was submitted for histopathologic examination. The patient was advised follow-up.

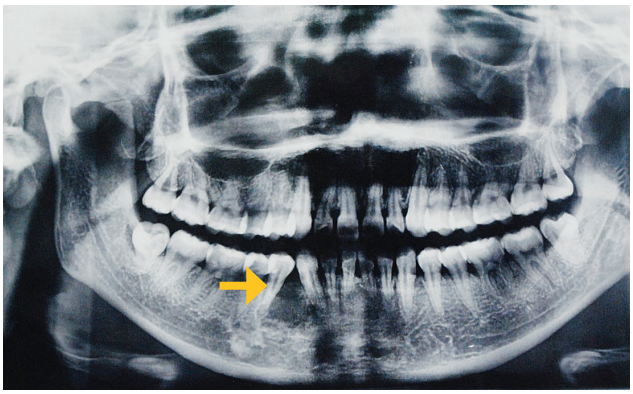

Figure 1. Radiographic appearance of the lesion.

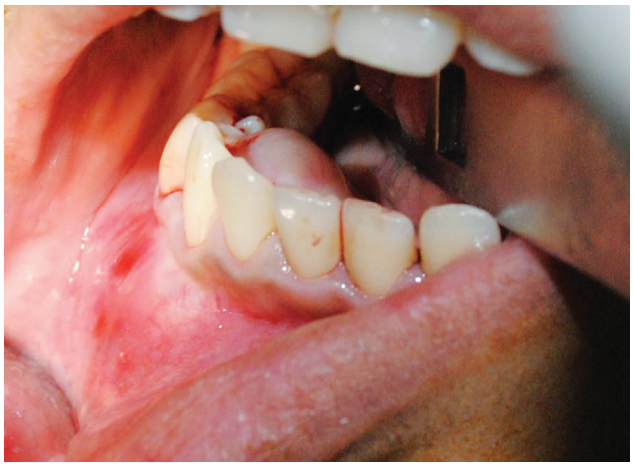

Figure 2. Clinical appearance of the lesion.

Histopathologic examination revealed a lesion composed of odontogenic epithelial islands with peripheral palisading columnar basal cells and central stellate reticulum. The basal cells have vacuolated cytoplasm with nuclei exhibiting reverse polarity (Figure 3). Microcysts and squamous differentiation were seen. (Figure 4). The morphologic features were consistent with an acanthomatous ameloblastoma.

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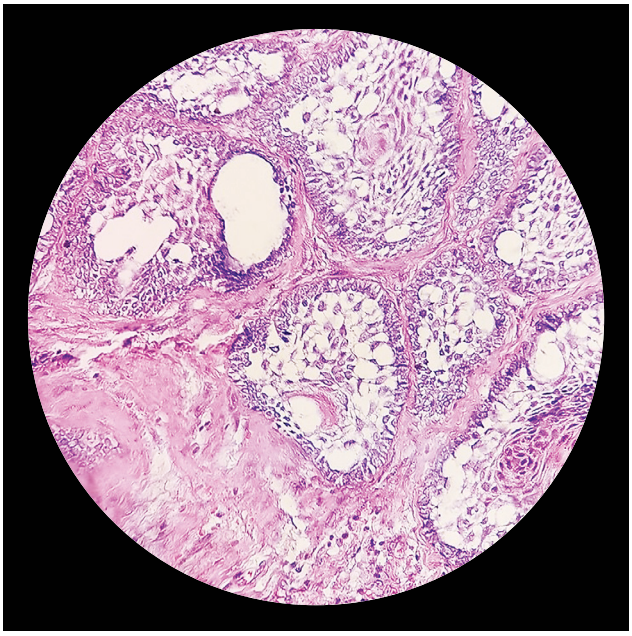

Figure 3. Odontogenic islands, central stellate reticulum palisading basal cells, and reverse polarity of the nuclei (H & E, 40x).

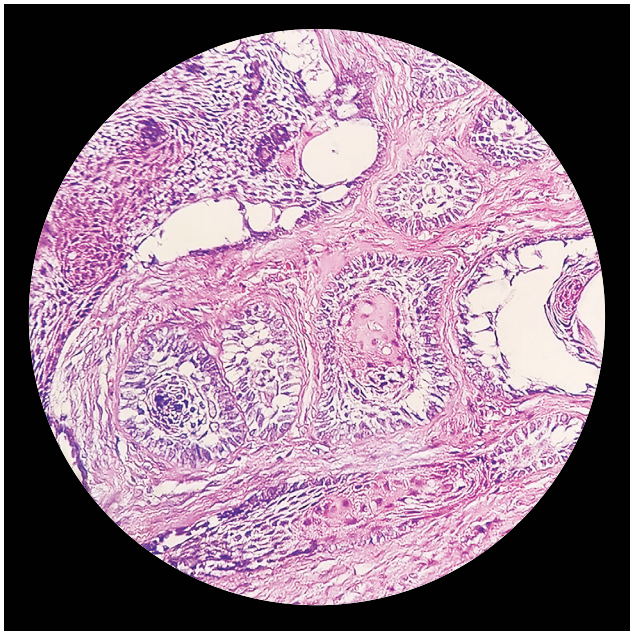

Figure 4. Microcyts and squamous differentiation (H & E, 40x).

Ameloblastoma is a benign odontogenic tumor that comprises about 1% of all oral tumors and 9-11% of odontogenic tumors.[1-4] Over 80% occur in the mandible and 20% in the maxilla.[3,5,6] It has no sex predilection, has a wide age range, and appears as a lytic expansile lesion radiographically.[2,5,7] Depending on the appearance of the central reticulum, the terms spindle cell, granular, basal cell and acanthomatous are used. The term acanthomatous ameloblastoma is used when the central stellate reticulum displays squamous differentiation.[8]

Ameloblastoma is placed under borderline (low-grade malignant) category, rather than benign, due to its aggressive properties and tendency to recur. Although rare, metastases have been documented.[5]

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APAME 2013 Tokyo

Asia Pacific Association of Medical Journal Editors Convention 2013
Tokyo, Japan
2 - 4 August 2013, Japan Medical Association (JMA) Auditorium

Tokyo Declaration on Research Integrity and Ethical Publication in Science and Medicine in the Asia Pacific Region

We, the participants in the Joint Meeting of the Asia Pacific Association of Medical Journal Editors (APAME), the Index Medicus for the South-East Asian Region (IMSEAR) and the Western Pacific Region Index Medicus (WPRIM) held in Tokyo from 2 to 4 August 2013:

CONSIDERING

That overwhelming data in science and medicine may differ in their reliability and the quality control is important for compiling scientific and health information;

That equitable circulation of scientific and health information is facilitated by fair collaboration among policy makers, researchers, and industry sectors including pharmaceuticals and publishers.

That APAME, IMSEAR, and WPRIM are important collaborative initiatives that can implement the global guidelines for publication and dissemination of scientific and medical knowledge in an equitable and ethical manner;

CONFIRM

Our commitment to endorse that scientific and medical knowledge is imperishable and should not be assessed evaluated by only economic or temporal considerations;

Our commitment to improve the quality and reliability of scientific and medical knowledge through the IMSEAR and WPRIM;

Our commitment to publish reliable and high quality information by education of researchers, implementation of fair review processes, and organization of networks through the APAME;

Our commitment to collaborate with publishers, academic or public libraries, and research bodies to achieve equitable and ethical publication and dissemination of scientific and medical knowledge;

COMMIT

Ourselves, to publishing reliable and high-quality information, thereby setting the ethical standard for our colleagues, editors, and librarians in the Region;

Our publishers, to disseminate scientific and medical knowledge fairly and impartially through digital library services including, but not limited to, IMSEAR, WPRIM, and Global Health Library;

Our organization, APAME, to build further networks, convening conferences, and organizing events to educate and empower editors, peer reviewers, and authors to achieve internationally acceptable, but regionally realistic, scholarly standards.

4 August 2013, Tokyo

MANILA DECLARATION ON THE AVAILABILITY AND USE OF HEALTH RESEARCH INFORMATION IN AND FOR LOW- AND MIDDLE-INCOME COUNTRIES IN THE ASIA PACIFIC REGION

We, the participants in the Joint Meeting of the Asia Pacific Association of Medical Journal editors (APAME), the Index Medicus of the South East Asia Region (IMSEAR), and the Western Pacific Region Index Medicus (WPRIM) held in Manila from 24 to 26 August 2015, in conjunction with the COHRED Global Forum on Research and Innovation for Health held in Manila from 24-27 August 2015, drawing on the Pre-Forum Discussions on HIFA from 20 July to 24 August 2015 "Meeting the information needs of researches and users of health research in low- and middle-income countries" available at http://www.hifa2015.org/meeting-the-information-needs-of-researchers-and-users-of-health-research-2/ and the BMJ Blogs 20 July 2015 "How can we improve the availability and use of health research in developing countries?" available at http://blogs.bmj.com/bmj/2015/07/20/how-can-we-improve-the-availability-and-use-of-health-research-in-developing-countries/:

CONSIDERING

That the WHO Constitution "enshrines the highest attainable standard of health as a fundamental right of every human being," and that "the right to health includes access to timely, acceptable, and affordable healthcare of appropriate quality in tandem with "the underlying determinants of health," including "access to health-related education and information;"

That increasing the availability of quality health research information is fundamental to the successful attainment of global health and progressive realization of the right to health; and that all healthcare stakeholders (individuals, researchers, providers, professionals, leaders and policymakers) need seamless access to peer-reviewed research and information that are relevant to their respective contexts, and presented in a language they can understand;

That despite a growing momentum towards free and open access to research literature, and important initiatives, such as HINARI Access to Research In Health Programme and IRIS Institutional Repository for Information Sharing, that have helped to improve the availability of research in low- and middle-income countries, there continue to be many challenges, limitations and exclusions that prevent health research information from becoming freely and openly available to those who need it;

That the Global Health Library (GHL), Index Medicus of the South East Asia Region (IMSEAR), Western Pacific Region Index Medicus (WPRIM), and Asia Pacific Association of Medical Journal Editors (APAME) are important collaborative initiatives that can promote and uphold the availability and use of health research information especially in and for low- and middle-income countries in the Asia Pacific Region;

CONFIRM

Our commitment to champion and advocate for the increased availability, accessibility and visibility of health research information from and to low- and middle-income developing countries through our Journals, our respective National Associations of Medical Editors, and APAME;

Our commitment to make research information freely and openly available in the right language to producers and users of health research in low- and middle-income countries through IMSEAR, WPRIM, the ASIA Pacific Medical Journal Articles Central Archives (APAMED Central) and other platforms;

Our commitment to improve availability, accessibility and interoperability of the different formats of health information suitable to different users in their respective contexts including through both conventional and alternative channels of research dissemination such as new and social media, mobile and disruptive technologies, blogging and microblogging tools and communities, and communities of practice;

CALL ON

Member States of and governments in the South East Asia and Western pacific Regions, in collaboration with stakeholders from the non-government and private sectors to formulate and implement policies and certification schemes such as the COHRED Fairness IndexTM (CFI) that promote free and open availability of health research information for both its producers and users, especially in low- and middle-income countries;

Stakeholders from the public and private sectors, national and international organizations, universities and academic societies, and discussion groups such as Healthcare Information for ALL (HIFA2015) to support IMSEAR, WPRIM, the GHL, APAMED Central, and develop Integrated Scholarly Information Systems and similar initiatives, in order to ensure the free, open and global accessibility of health research done in the South East Asia and Western Pacific Regions;

The Eastern Mediterranean Association of Medical Editors (EMAME), the Forum for African Medical Editors (FAME), the European Association of Science Editors (EASE), the World Association of Medical Editors (WAME), the International Committee of Medical Journal Editors (ICMJE), the Committee on Publication Ethics (COPE) and other editors' and publishers' associations to support APAME in implementing various activities, guidelines and practices that would improve the quality, availability and accessibility of scientific writing and publications in the Asia Pacific Region and the world;

Bibliographic, Citation and Full-Text Databases such as PubMed, Global Health Database (CAB Direct), the Directory of Open Access Journals (DOAJ), EMBASE, ScieELO Citation Index, Scopus, and the Web of Science to review their policies and processes for indexing Journals from low- and middle-income countries, as well as making health research information freely and openly available to users in these countries who cannot afford to pay for it.

COMMIT

Ourselves and our Journals to publishing innovative and solution-focused research in all healthcare and related fields such as health promotion, public health, medicine, nursing, dentistry, pharmacy, other health professions, health services and health systems, particularly health research applicable to low- and middle-income countries;

Ourselves and our publishers to disseminating scientific, healthcare and medical knowledge fairly and impartially by developing and using Bibliographic Indices, Citation Databases, Full-Text Databases and Open Data Systems including, but not limited to, such Regional Indexes of the Global Health Library as IMSEAR, WPRIM and APAMED Central;

Our organization, APAME, to building collaborative networks, convening meaningful conferences, and organizing participative events to educate and empower editors, peer reviewers, authors, librarians and publishers to achieve real impact, and not just impact factor, as we advance free and open access to health information and publication that improves global health-related quality of life.

26 August 2015, Manila

This declaration was launched at the 2015 Convention of the Asia Pacific Association of Medical Journal Editors (APAME) held in Manila from 24 to 26 August 2015. *www.wpro.who/int/apame apame@wpro.who.int*

ICMJE INTERNATIONAL COMMITTEE of MEDICAL JOURNAL EDITORS

PJP

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

Updated December 2016

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I. About the Recommendations

A. Purpose of the Recommendations

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

B. Who Should Use the Recommendations?

These recommendations are intended primarily for use by authors who might submit their work for publication to ICMJE member journals. Many non-ICMJE journals voluntarily use these recommendations (see www.icmje.org/journals.html). The ICMJE encourages that use but has no authority to monitor or enforce it. In all cases, authors should use these recommendations along with individual journals' instructions to authors. Authors should also consult guidelines for the reporting of specific study types (e.g., the CONSORT guidelines for the reporting of randomized trials); see http://equator-network.org.

Journals that follow these recommendations are encouraged to incorporate them into their instructions to

authors and to make explicit in those instructions that they follow ICMJE recommendations. Journals that wish to be identified on the ICMJE website as following these recommendations should notify the ICMJE secretariat via e-mail at icmje@acponline.org. Journals that in the past have requested such identification but who no longer follow ICMJE recommendations should use the same means to request removal from this list.

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

C. History of the Recommendations

The ICMJE has produced multiple editions of this document, previously known as the Uniform Requirements for Manuscripts Submitted to Biomedical Journals (URMs). The URM was first published in 1978 as a way of standardizing manuscript format and preparation across journals. Over the years, issues in publishing that went well beyond manuscript preparation arose, resulting in development of a number of Separate Statements on editorial policy. The entire Uniform Requirements document was revised in 1997; sections were updated in May 1999 and May 2000. In May 2001, the ICMJE revised the sections related to potential conflicts of interest. In 2003, the committee revised and reorganized the entire document and incorporated the Separate Statements into the text, and revised it again in 2010. Previous versions of this document can be found in the "Archives" section of www.icmje.org. Now renamed "Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals" (ICMJE Recommendations), the document was revised in 2013, 2014, 2015, and the current version in 2016.

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

A. Defining the Role of Authors and Contributors

1. Why Authorship Matters

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

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

2. Who Is an Author?

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

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

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

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

The individuals who conduct the work are responsible for identifying who meets these criteria and ideally should do so when planning the work, making modifications as appropriate as the work progresses. It is the collective responsibility of the authors, not the journal to which the work is submitted, to determine that all people named as authors meet all four criteria; it is not the role of journal editors to determine who qualifies or does not qualify for authorship or to arbitrate authorship conflicts. If agreement cannot be reached about who qualifies for authorship, the institution(s) where the work was performed, not the journal editor, should be asked to investigate. If authors request removal or addition of an author after manuscript submission or publication, journal editors should

seek an explanation and signed statement of agreement for the requested change from all listed authors and from the author to be removed or added.

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

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

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

3. Non-Author Contributors

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

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

B. Author Responsibilities—Conflicts of Interest

Public trust in the scientific process and the credibility of published articles depend in part on how transparently conflicts of interest are handled during the planning, implementation, writing, peer review, editing, and publication of scientific work.

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

Financial relationships (such as employment, consultancies, stock ownership or options, honoraria, patents, and paid expert testimony) are the most easily identifiable conflicts of interest and the most likely to undermine the credibility of the journal, the authors, and of science itself. However, conflicts can occur for other reasons, such as personal relationships or rivalries, academic competition, and intellectual beliefs. Authors should avoid entering in to agreements with study sponsors, both for-profit and nonprofit, that interfere with authors' access to all of the study's data or that interfere with their ability to analyze and interpret the data and to prepare and publish manuscripts independently when and where they choose.

1. Participants

All participants in the peer-review and publication process—not only authors but also peer reviewers, editors, and editorial board members of journals—must consider their conflicts of interest when fulfilling their roles in the process of article review and publication and must disclose all relationships that could be viewed as potential conflicts of interest.

a. Authors

When authors submit a manuscript of any type or format they are responsible for disclosing all financial and personal relationships that might bias or be seen to bias their work. The ICMJE has developed a Form for Disclosure of Conflicts of Interest to facilitate and standardize

authors' disclosures. ICMJE member journals require that authors use this form, and ICMJE encourages other journals to adopt it.

b. Peer Reviewers

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

c. Editors and Journal Staff

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

2. Reporting Conflicts of Interest

Articles should be published with statements or supporting documents, such as the ICMJE conflict of interest form, declaring:

– Authors' conflicts of interest; and

– Sources of support for the work, including sponsor names along with explanations of the role of those sources if any in study design; collection, analysis, and interpretation of data; writing of the report; the decision to submit the report for publication; or a statement declaring that the supporting source had no such involvement; and

– Whether the authors had access to the study data, with an explanation of the nature and extent of access, including whether access is on-going.

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

C. Responsibilities in the Submission and Peer-Review Process

1. Authors

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

a. Predatory Journals

A growing number of entities are advertising themselves as "medical journals" yet do not function as such ("predatory journals"). Authors have a responsibility to evaluate the integrity, history, practices and reputation of the journals to which they submit manuscripts. Further guidance is available at http://www.wame.org/about/principles-of-transparency-and-best-practice.

2. Journals

a. Confidentiality

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

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

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

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

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

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

vealed to the author or anyone else without the reviewers' expressed written permission.

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

b. Timeliness

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

c. Peer Review

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

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

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

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

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

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

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

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

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

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

d. Integrity

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

clusive findings may prevent unwarranted replication of effort or otherwise be valuable for other researchers considering similar work.

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

3. Peer Reviewers

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

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

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

Reviewers should declare their conflicts of interest and recuse themselves from the peer-review process if a conflict exists.

D. Journal Owners and Editorial Freedom

1. Journal Owners

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

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

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

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

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

2. Editorial Freedom

The ICMJE adopts the World Association of Medical Editors' definition of editorial freedom, which holds that editors-in-chief have full authority over the entire editorial content of their journal and the timing of publication of that content. Journal owners should not interfere in the evaluation, selection, scheduling, or editing of individual articles either directly or by creating an environment that strongly influences decisions. Editors should base editorial decisions on the validity of the work and its importance to the journal's readers, not on the commercial implications for the journal, and editors should be free to express critical but responsible views about all aspects of medicine without fear of retribution, even if these views conflict with the commercial goals of the publisher.

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

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

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

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

E. Protection of Research Participants

When reporting research involving human data, authors should indicate whether the procedures followed have been assessed by the responsible review committee (institutional and national), or if no formal ethics committee is available, were in accordance with the Helsinki Declaration as revised in 2013 (www.wma.net/en/30publications/10policies/b3/index.html). If doubt exists whether the research was conducted in accordance with the Helsinki Declaration, the authors must explain the rationale for their approach and demonstrate that the institutional review body explicitly approved the doubtful aspects of the study. Approval by a responsible review committee does not preclude editors from forming their own judgment whether the conduct of the research was appropriate.

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

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

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

When reporting experiments on animals, authors should indicate whether institutional and national standards for the care and use of laboratory animals were followed. Further guidance on animal research ethics is available from the International Association of Veterinary Editors' Consensus Author Guidelines on Animal Ethics and Welfare (http://veteditors.org/ethicsconsensusguidelines.html).

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

A. Corrections, Retractions, Republications, and Version Control

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

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

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

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

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

B. Scientific Misconduct, Expressions of Concern, and Retraction

Scientific misconduct includes but is not necessarily limited to data fabrication; data falsification including deceptive manipulation of images; and plagiarism. Some people consider failure to publish the results of clinical trials

and other human studies a form of scientific misconduct. While each of these practices is problematic, they are not equivalent. Each situation requires individual assessment by relevant stakeholders. When scientific misconduct is alleged, or concerns are otherwise raised about the conduct or integrity of work described in submitted or published papers, the editor should initiate appropriate procedures detailed by such committees such as the Committee on Publication Ethics (COPE) (publicationethics.org/resources/flowcharts) and may choose to publish an expression of concern pending the outcomes of those procedures. If the procedures involve an investigation at the authors' institution, the editor should seek to discover the outcome of that investigation, notify readers of the outcome if appropriate, and if the investigation proves scientific misconduct, publish a retraction of the article. There may be circumstances in which no misconduct is proven, but an exchange of letters to the editor could be published to highlight matters of debate to readers.

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

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

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

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

C. Copyright

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

D. Overlapping Publications

1. Duplicate Submission

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

2. Duplicate and Prior Publication

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

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

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

This recommendation does not prevent a journal from considering a complete report that follows publication of a preliminary report, such as a letter to the editor, a preprint, or an abstract or poster displayed at a scientific meeting. It also does not prevent journals from considering a paper that has been presented at a scientific meeting but was not published in full, or that is being considered for publication in proceedings or similar format. Press reports of scheduled meetings are not usually regarded as breaches of

this rule, but they may be if additional data tables or figures enrich such reports. Authors should also consider how dissemination of their findings outside of scientific presentations at meetings may diminish the priority journal editors assign to their work.

In the event of a public health emergency (as defined by public health officials), information with immediate implications for public health should be disseminated without concern that this will preclude subsequent consideration for publication in a journal.

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

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

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

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

See COPE flowcharts for further guidance on handling duplicate publication.

3. Acceptable Secondary Publication

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

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

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

4. Manuscripts Based on the Same Database

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

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

Sometimes for large trials it is planned from the beginning to produce numerous separate publications regarding separate research questions but using the same original patient sample. In this case authors may use the original single trial registration number, if all the outcome parameters were defined in the original registration. If the authors registered several substudies as separate entries in, for example, clinicaltrials.gov, then the unique trial identifier should be given for the study in question, The main issue is transparency, so no matter what model is used it should be obvious for the reader.

E. Correspondence

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

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

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

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

F. Fees

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

G. Supplements, Theme Issues, and Special Series

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

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

H. Sponsorship or Partnership

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

I. Electronic Publishing

Most medical journals are now published in electronic as well as print versions, and some are published only in electronic form. Principles of print and electronic publish-

ing are identical, and the recommendations of this document apply equally to both. However, electronic publishing provides opportunities for versioning and raises issues about link stability and content preservation that are addressed here.

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

Electronic publishing allows linking to sites and resources beyond journals over which journal editors have no editorial control. For this reason, and because links to external sites could be perceived as implying endorsement of those sites, journals should be cautious about external linking. When a journal does link to an external site, it should state that it does not endorse or take responsibility or liability for any content, advertising, products, or other materials on the linked sites, and does not take responsibility for the sites' availability.

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

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

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

J. Advertising

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

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

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

K. Journals and the Media

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

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

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

- Editors can foster the orderly transmission of medical information from researchers, through peer-reviewed journals, to the public. This can be accomplished by an agreement with authors that they will not publicize their work while their manuscript is under consideration or awaiting publication and an agreement with the media that they will not release stories before publication of the original research in the journal, in return for which the journal will cooperate with them in preparing accurate stories by issuing, for example, a press release.
- Editors need to keep in mind that an embargo system works on the honor system—no formal enforcement or policing mechanism exists. The decision of a significant number of media outlets or biomedical journals not to respect the embargo system would lead to its rapid dissolution.
- Notwithstanding authors' belief in their work, very little medical research has such clear and urgently important clinical implications for the public's health that the news must be released before full publication in a journal.

When such exceptional circumstances occur, the appropriate authorities responsible for public health should decide whether to disseminate information to physicians and the media in advance and should be responsible for this decision. If the author and the appropriate authorities wish to have a manuscript considered by a particular journal, the editor should be consulted before any public release. If editors acknowledge the need for immediate release, they should waive their policies limiting prepublication publicity.

- Policies designed to limit prepublication publicity should not apply to accounts in the media of presentations at scientific meetings or to the abstracts from these meetings (see Duplicate Publication). Researchers who present their work at a scientific meeting should feel free to discuss their presentations with reporters but should be discouraged from offering more detail about their study than was presented in the talk, or should consider how giving such detail might diminish the priority journal editors assign to their work (see Duplicate Publication).
- When an article is close to being published, editors or journal staff should help the media prepare accurate reports by providing news releases, answering questions, supplying advance copies of the article, or referring reporters to appropriate experts. This assistance should be contingent on the media's cooperation in timing the release of a story to coincide with publication of the article.

L. Clinical Trial Registration

The ICMJE's clinical trial registration policy is detailed in a series of editorials (see Updates and Editorials [www.icmje.org/update.html] and FAQs [www.icmje.org/faq_clinical.html]).

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

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

The ICMJE accepts registration in any registry that is a primary register of the WHO International Clinical Trials Registry Platform (ICTRP) (www.who.int/ictrp/network/primary/en/index.html) or in ClinicalTrials.gov, which is a data provider to the WHO ICTRP. The ICMJE endorses these registries because they meet several criteria. They are accessible to the public at no charge, open to all prospective registrants, managed by a not-for-profit organization, have a mechanism to ensure the validity of the registration data, and are electronically searchable. An acceptable registry must include the minimum 20-item trial registration dataset (http://prsinfo.clinicaltrials.gov/trainTrainer/WHO-ICMJE-ClinTrialsgov-Cross-Ref.pdf or www.who.int/ictrp/network/trds/en/index.html) at the time of registration and before enrollment of the first participant. The ICMJE considers inadequate trial registrations missing any of the 20 data fields or those that have fields that contain uninformative information. Although not a required item, the ICMJE encourages authors to include a statement that indicates that the results have not yet been published in a peer-reviewed journal, and to update the registration with the full journal citation when the results are published.

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

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

The ICMJE encourages posting of clinical trial results in clinical trial registries but does not require it. The ICMJE will not consider as prior publication the posting of trial results in any registry that meets the above criteria if results are limited to a brief (500 word) structured abstract or tables (to include patients enrolled, key outcomes, and adverse events).

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

reporting or to other trials that they mention in the manuscript.

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

IV. Manuscript Preparation and Submission

A. Preparing a Manuscript for Submission to a Medical Journal

1. General Principles

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

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

2. Reporting Guidelines

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

3. Manuscript Sections

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

a. Title Page

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

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

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

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

Source(s) of support. These include grants, equipment, drugs, and/or other support that facilitated conduct of the work described in the article or the writing of the article itself.

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

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

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

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

b. Abstract

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

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

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

c. Introduction

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

d. Methods

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

The Methods section should include a statement indicating that the research was approved or exempted from the need for review by the responsible review committee (institutional or national). If no formal ethics committee is available, a statement indicating that the research was conducted according to the principles of the Declaration of Helsinki should be included.

i. Selection and Description of Participants

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

ii. Technical Information

Specify the study's main and secondary objectives—usually identified as primary and secondary outcomes. Identify methods, equipment (give the manufacturer's name and address in parentheses), and procedures in suffi-

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

iii. Statistics

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

e. Results

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

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

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

f. Discussion

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

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

g. References

i. General Considerations

Authors should provide direct references to original research sources whenever possible. References should not be used by authors, editors, or peer reviewers to promote self-interests. Although references to review articles can be an efficient way to guide readers to a body of literature, review articles do not always reflect original work accurately. On the other hand, extensive lists of references to original work on a topic can use excessive space. Fewer references to key original papers often serve as well as more exhaustive lists, particularly since references can now be added to the electronic version of published papers, and since electronic literature searching allows readers to retrieve published literature efficiently.

Do not use conference abstracts as references: they can be cited in the text, in parentheses, but not as page footnotes. References to papers accepted but not yet published should be designated as "in press" or "forthcoming." Information from manuscripts submitted but not accepted should be cited in the text as "unpublished observations" with written permission from the source.

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

Some but not all journals check the accuracy of all reference citations; thus, citation errors sometimes appear

in the published version of articles. To minimize such errors, references should be verified using either an electronic bibliographic source, such as PubMed, or print copies from original sources. Authors are responsible for checking that none of the references cite retracted articles except in the context of referring to the retraction. For articles published in journals indexed in MEDLINE, the ICMJE considers PubMed the authoritative source for information about retractions. Authors can identify retracted articles in MEDLINE by searching PubMed for "Retracted publication [pt]", where the term "pt" in square brackets stands for publication type, or by going directly to the PubMed's list of retracted publications (www.ncbi.nlm.nih.gov/pubmed ?term=retracted+publication+[pt]).

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

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

ii. Style and Format

References should follow the standards summarized in the NLM's International Committee of Medical Journal Editors (ICMJE) Recommendations for the Conduct, Reporting, Editing and Publication of Scholarly Work in Medical Journals: Sample References (www.nlm.nih.gov /bsd/uniform_requirements.html) webpage and detailed in the NLM's Citing Medicine, 2nd edition (www.ncbi.nlm .nih.gov/books/NBK7256/). These resources are regularly updated as new media develop, and currently include guidance for print documents; unpublished material; audio and visual media; material on CD-ROM, DVD, or disk; and material on the Internet.

h. Tables

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

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

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

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

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

i. Illustrations (Figures)

Digital images of manuscript illustrations should be submitted in a suitable format for print publication. Most submission systems have detailed instructions on the quality of images and check them after manuscript upload. For print submissions, figures should be either professionally drawn and photographed, or submitted as photographic-quality digital prints.

For radiological and other clinical and diagnostic images, as well as pictures of pathology specimens or photomicrographs, send high-resolution photographic image files. Before-and-after images should be taken with the same intensity, direction, and color of light. Since blots are used as primary evidence in many scientific articles, editors may require deposition of the original photographs of blots on the journal's website.

Although some journals redraw figures, many do not. Letters, numbers, and symbols on figures should therefore be clear and consistent throughout, and large enough to remain legible when the figure is reduced for publication. Figures should be made as self-explanatory as possible, since many will be used directly in slide presentations. Titles and detailed explanations belong in the legends—not on the illustrations themselves.

Photomicrographs should have internal scale markers. Symbols, arrows, or letters used in photomicrographs should contrast with the background. Explain the internal scale and identify the method of staining in photomicrographs.

Figures should be numbered consecutively according to the order in which they have been cited in the text. If a figure has been published previously, acknowledge the

original source and submit written permission from the copyright holder to reproduce it. Permission is required irrespective of authorship or publisher except for documents in the public domain.

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

j. Units of Measurement

Measurements of length, height, weight, and volume should be reported in metric units (meter, kilogram, or liter) or their decimal multiples.

Temperatures should be in degrees Celsius. Blood pressures should be in millimeters of mercury, unless other units are specifically required by the journal.

Journals vary in the units they use for reporting hematologic, clinical chemistry, and other measurements. Authors must consult the Information for Authors of the particular journal and should report laboratory information in both local and International System of Units (SI).

Editors may request that authors add alternative or non-SI units, since SI units are not universally used. Drug concentrations may be reported in either SI or mass units, but the alternative should be provided in parentheses where appropriate.

k. Abbreviations and Symbols

Use only standard abbreviations; use of nonstandard abbreviations can be confusing to readers. Avoid abbreviations in the title of the manuscript. The spelled-out abbreviation followed by the abbreviation in parenthesis should be used on first mention unless the abbreviation is a standard unit of measurement.

B. Sending the Manuscript to the Journal

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

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

A statement of financial or other relationships that might lead to a conflict of interest, if that information is not included in the manuscript itself or in an authors' form. See also Section II.B.

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

Contact information for the author responsible for communicating with other authors about revisions and final approval of the proofs, if that information is not included in the manuscript itself.

The letter or form should inform editors if concerns have been raised (e.g., via institutional and/or regulatory bodies) regarding the conduct of the research or if corrective action has been recommended. The letter or form should give any additional information that may be helpful to the editor, such as the type or format of article in the particular journal that the manuscript represents. If the manuscript has been submitted previously to another journal, it is helpful to include the previous editor's and reviewers' comments with the submitted manuscript, along with the authors' responses to those comments. Editors encourage authors to submit these previous communications. Doing so may expedite the review process and encourages transparency and sharing of expertise.

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

The manuscript must be accompanied by permission to reproduce previously published material, use previously published illustrations, report information about identifiable persons, or to acknowledge people for their contributions.

PHILIPPINE JOURNAL OF PATHOLOGY

Instructions to Authors

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

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

ARTICLE SECTIONS

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

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

Original articles

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

Reviews

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

Case Reports

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

Feature articles

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

Autopsy Vault

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

Images in Pathology

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

Brief Communications

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

Editorials

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

Letters to the Editor

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

Special Announcements

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

COVER LETTER

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

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

GENERAL FORMATTING GUIDELINES

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

SPECIFIC FORMATTING GUIDELINES

Title and Authors

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

Abstract

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

Keywords

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

Text

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

References

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

One to Six Authors

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

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

More than Six Authors

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

Authors Representing a Group

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

Book

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

World Wide Web

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

Tables

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

Figures and Graphs

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

Illustrations and Photographs

- Where appropriate, all illustrations/photographic images should be at least 800 x 600 dpi and submitted as image files (preferably as .png, .jpeg or .gif files).
- For photomicrographs, the stain used (e.g. H & E) and magnification (e.g. X400) should be included in the description.
- Computer-generated illustrations which are not suited for reproduction should be professionally redrawn or printed on good quality laser printers. Photocopies are not acceptable.
- All letterings for illustration should be of adequate size to be readable even after size reduction.
- Place explanatory notes and legends, as well as definitions of abbreviations used below the illustration/photograph.
- Up to a maximum of five (5) illustrations/ photographs are allowed.

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

EDITORIAL PROCESS (Figure 1)

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

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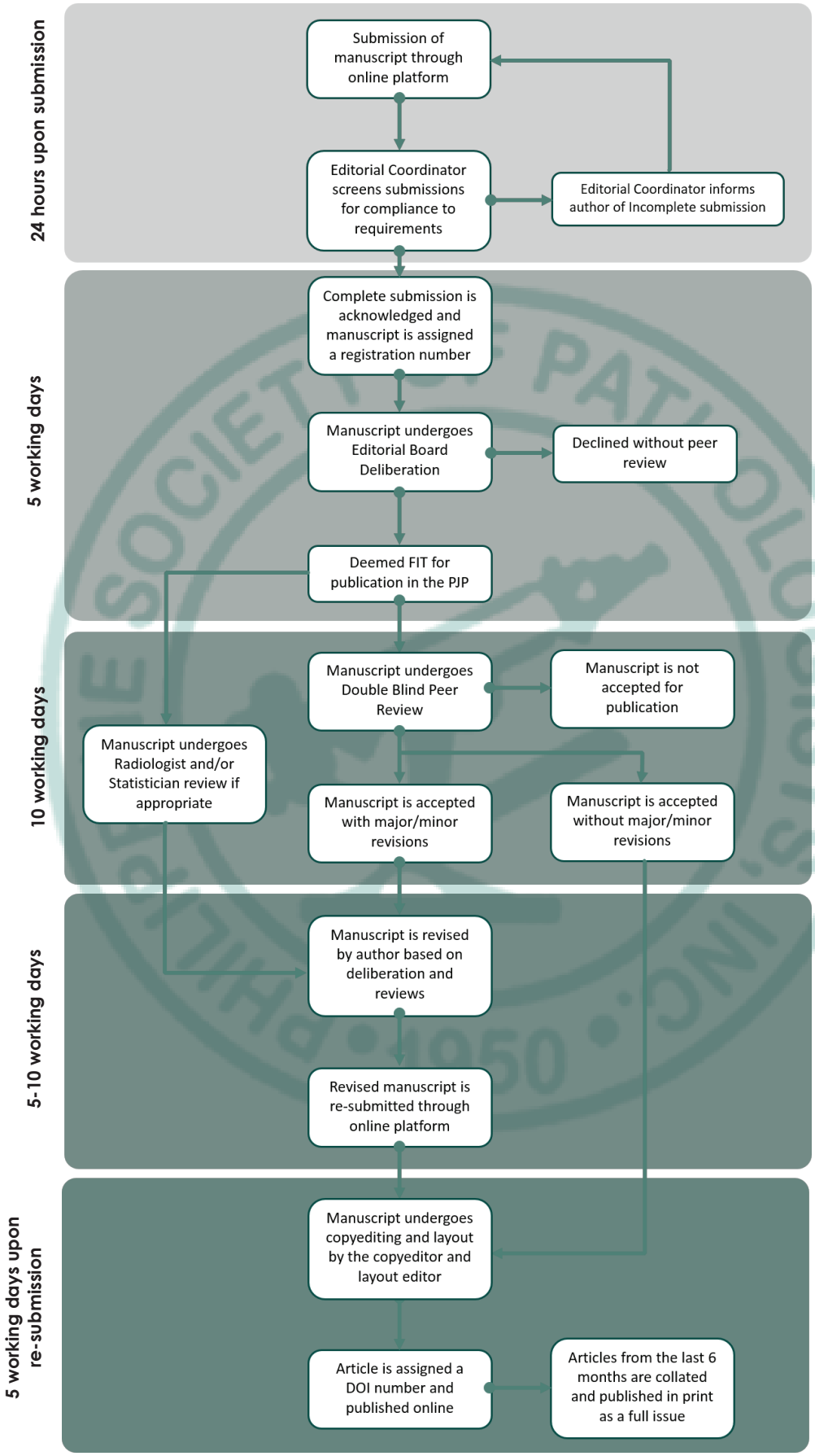

Figure 1. Editorial Process Flow.

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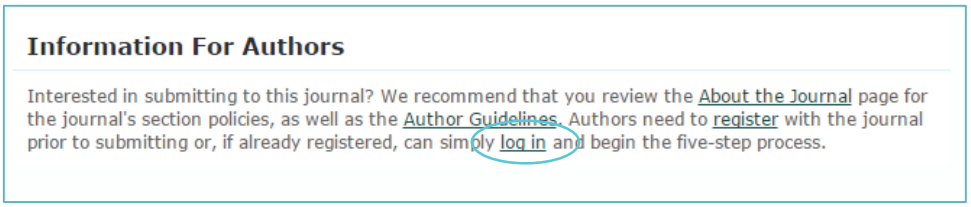
Select 'FOR AUTHORS'.



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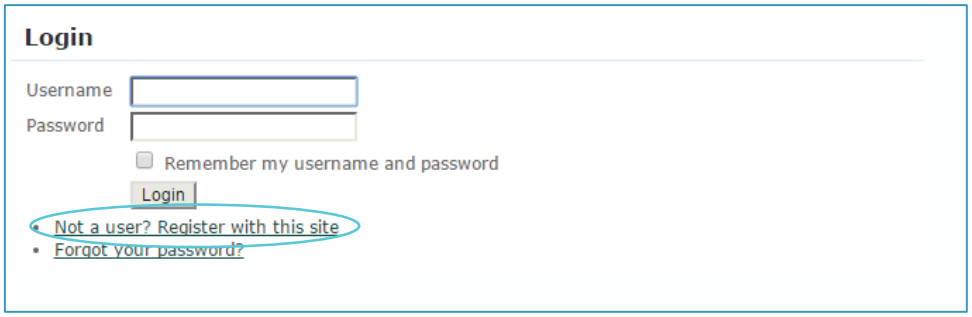

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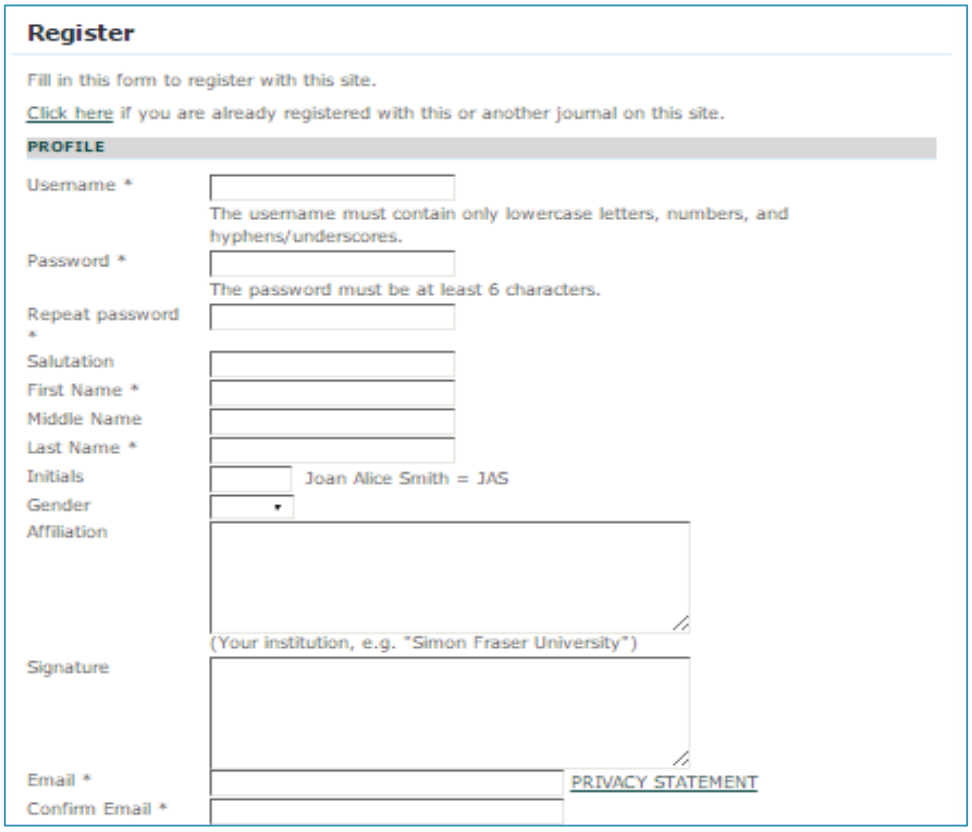

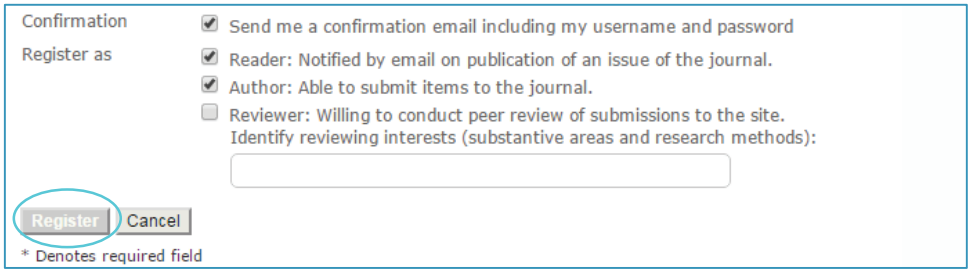

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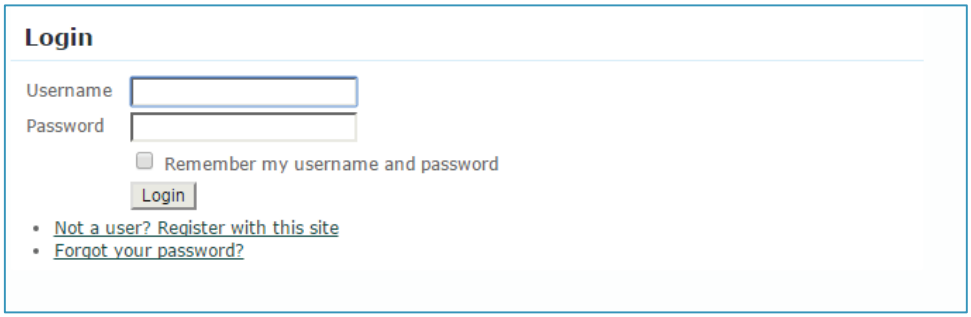

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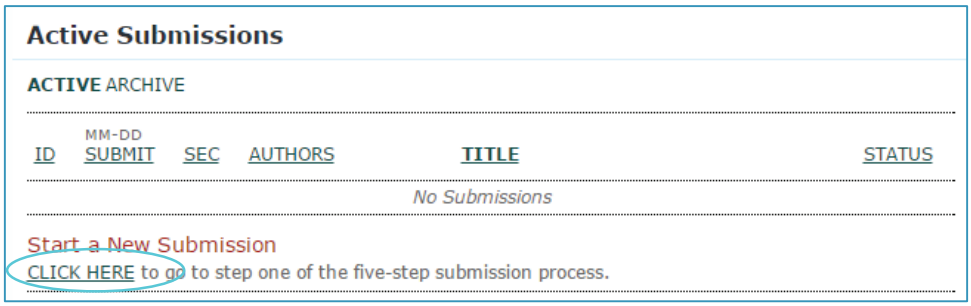

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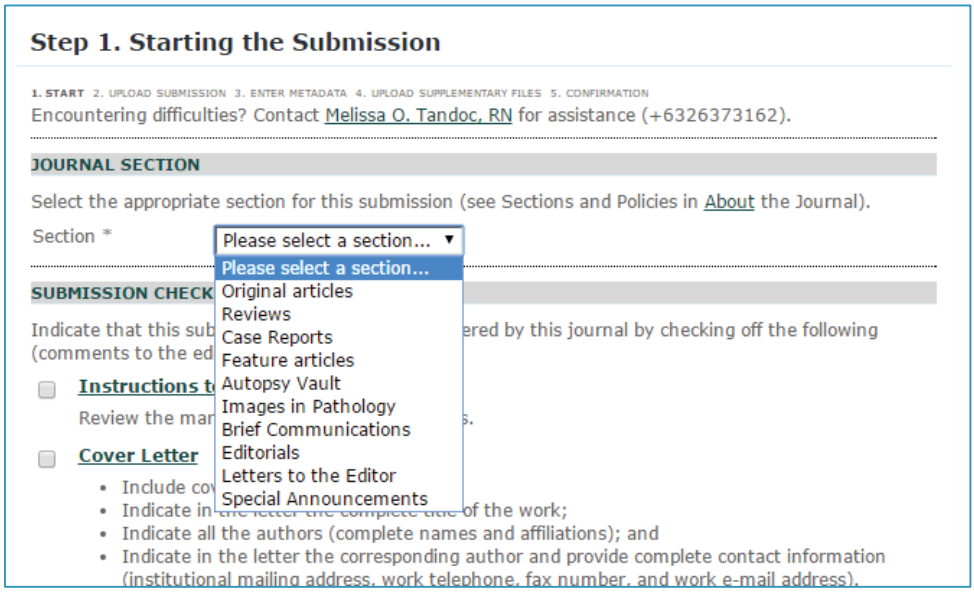

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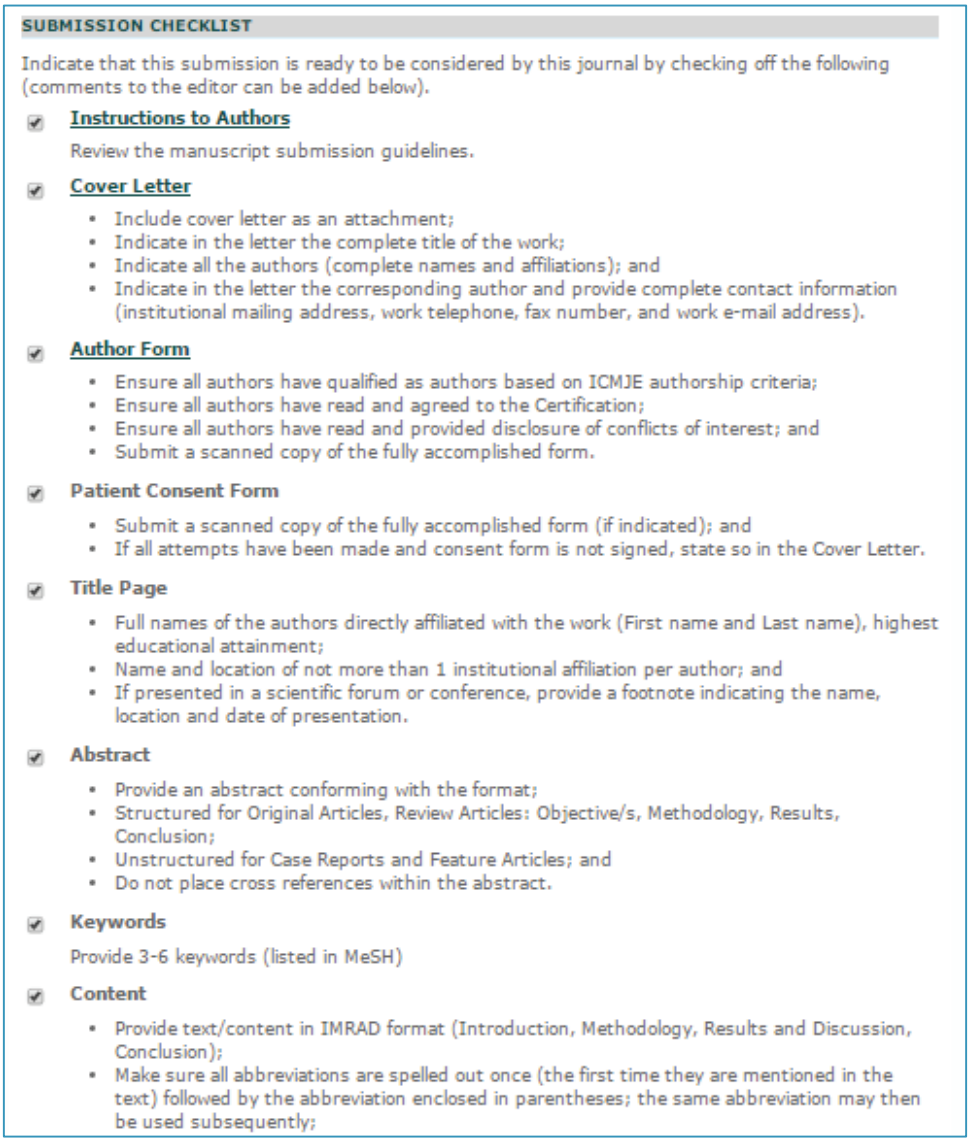

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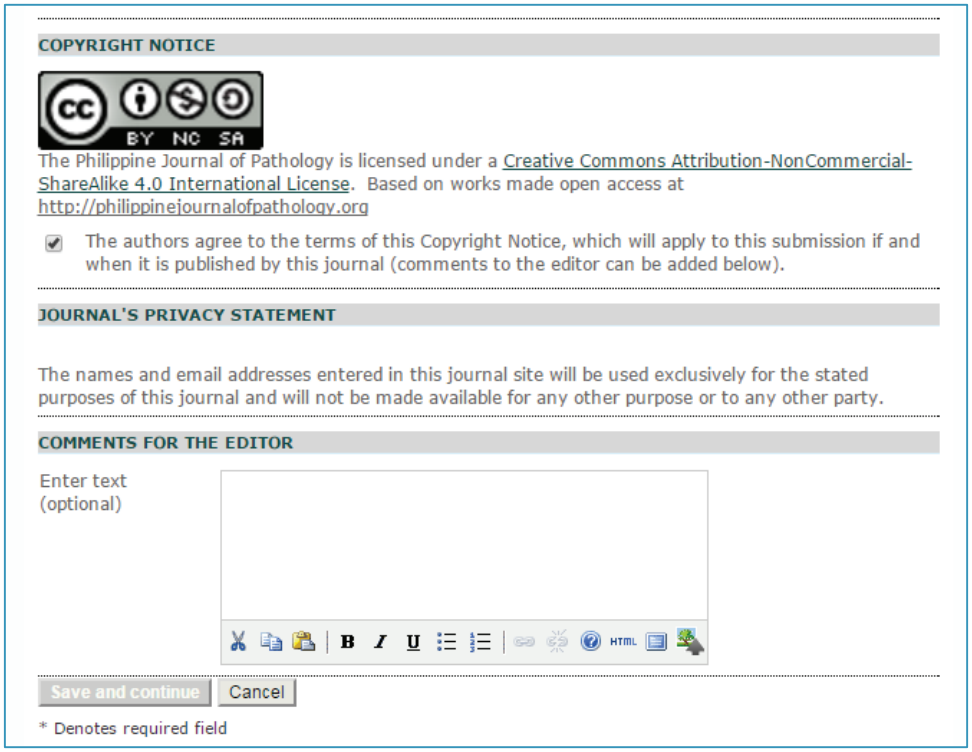

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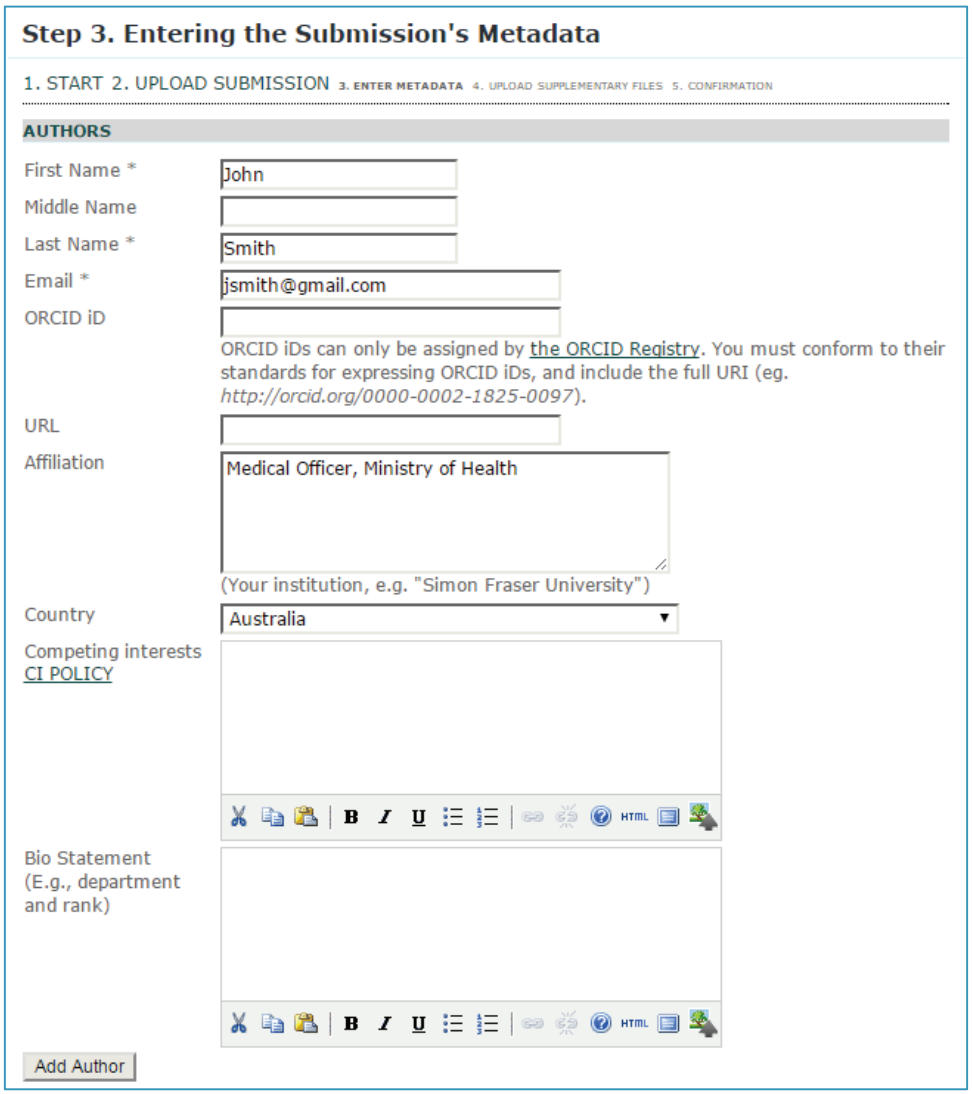

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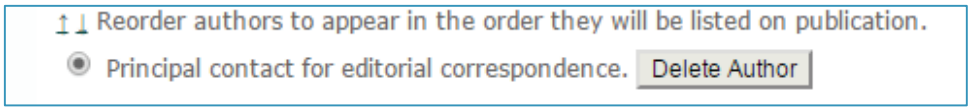

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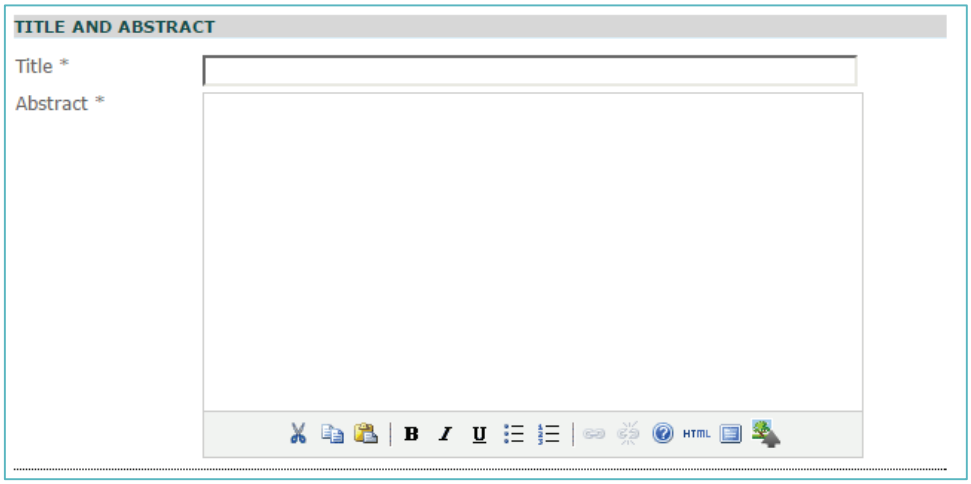

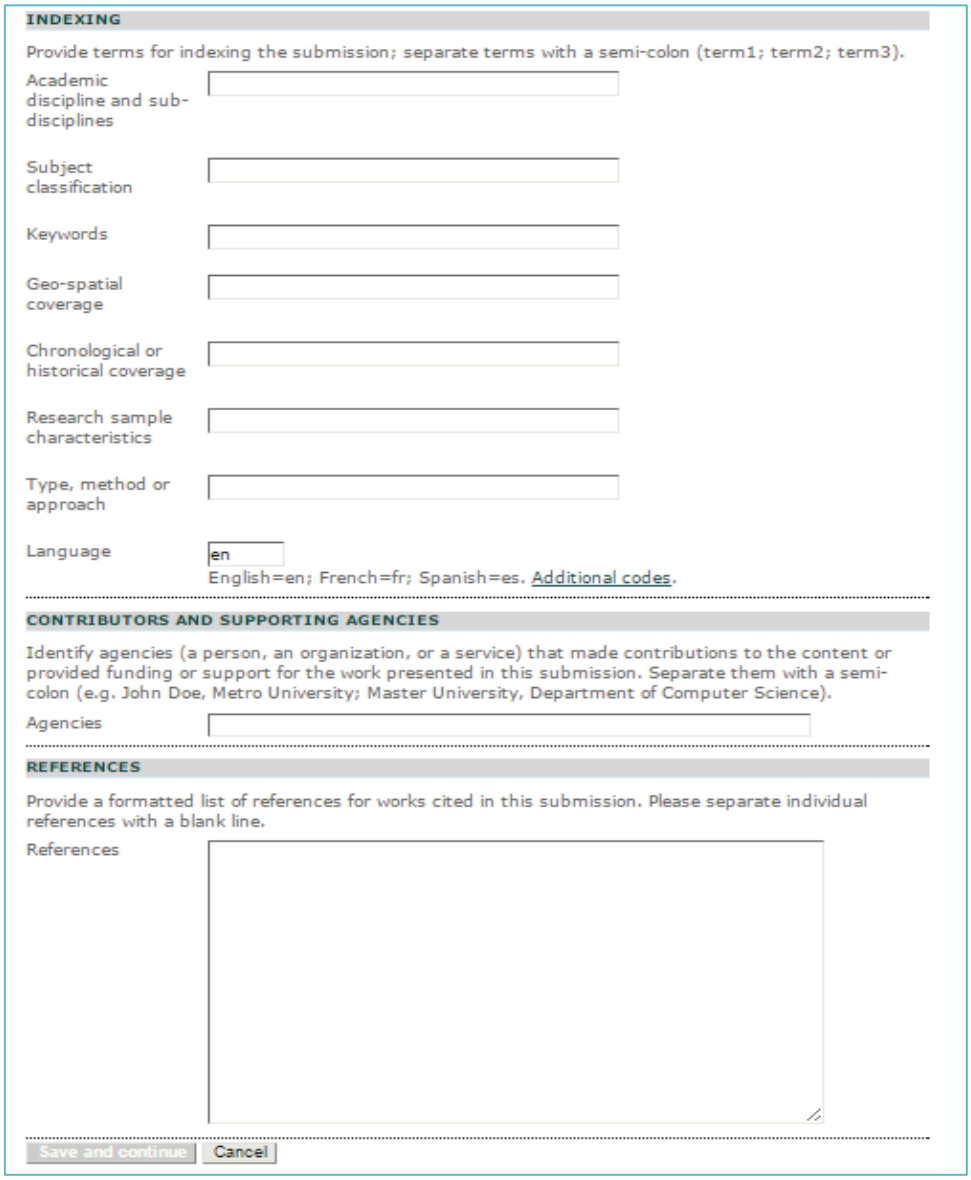

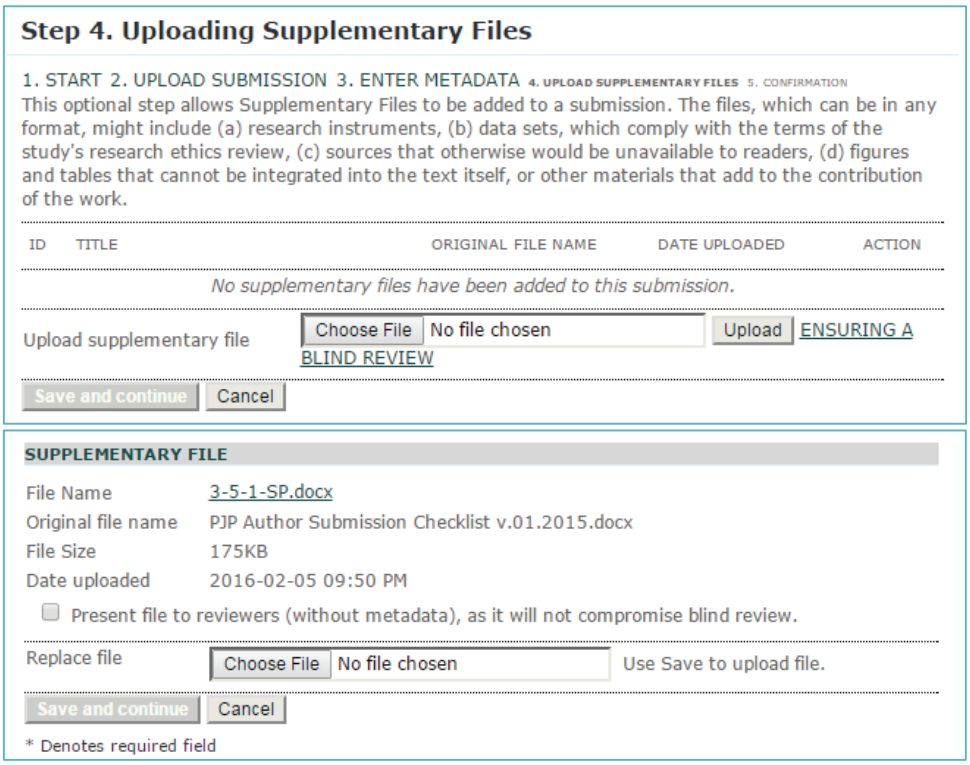

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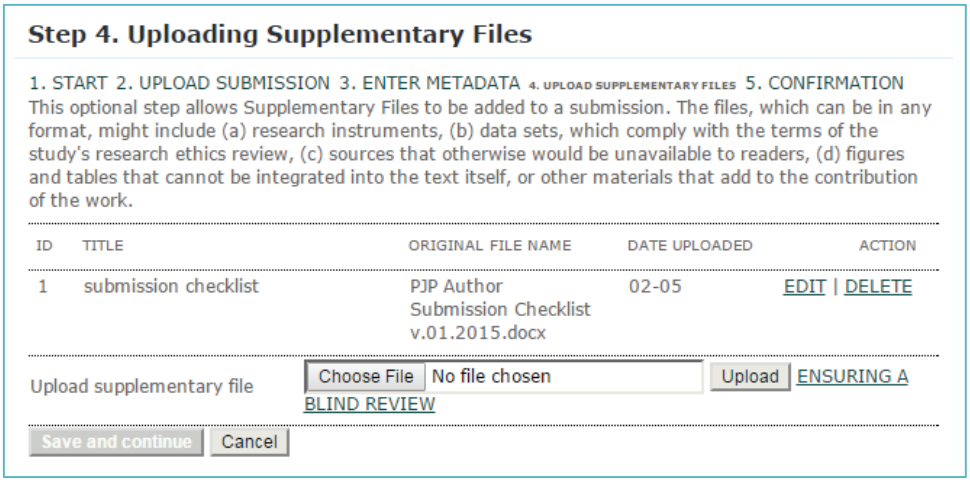

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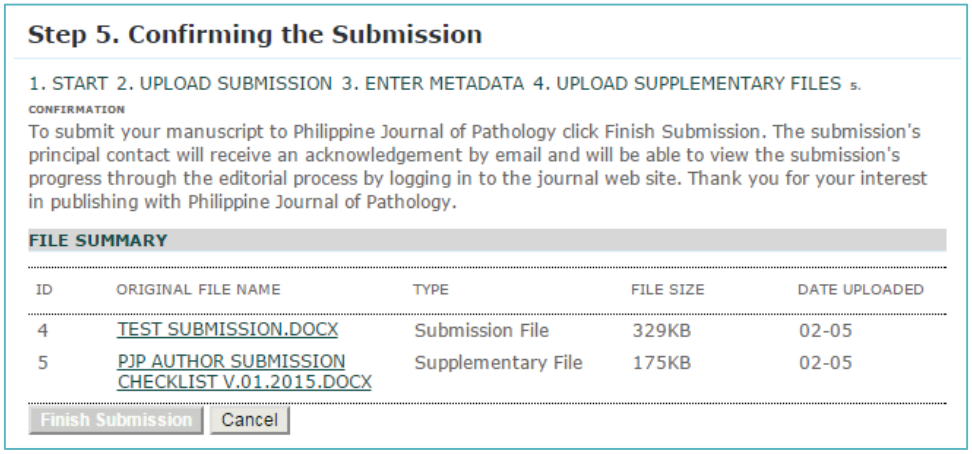

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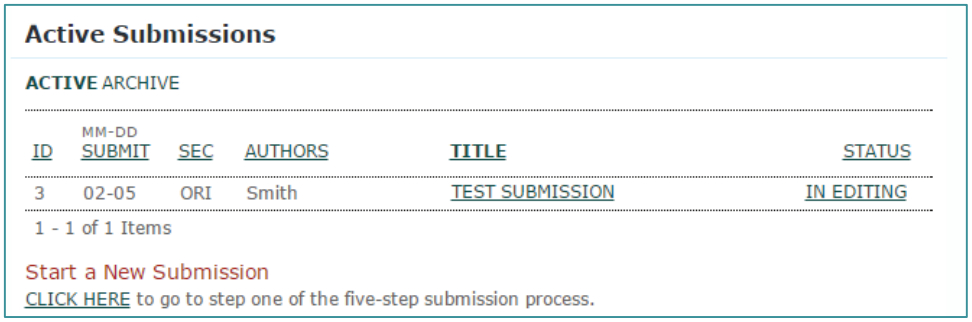

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3	02-05	ORI	Smith	TEST SUBMISSION	IN EDITING

1 - 1 of 1 Items

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>
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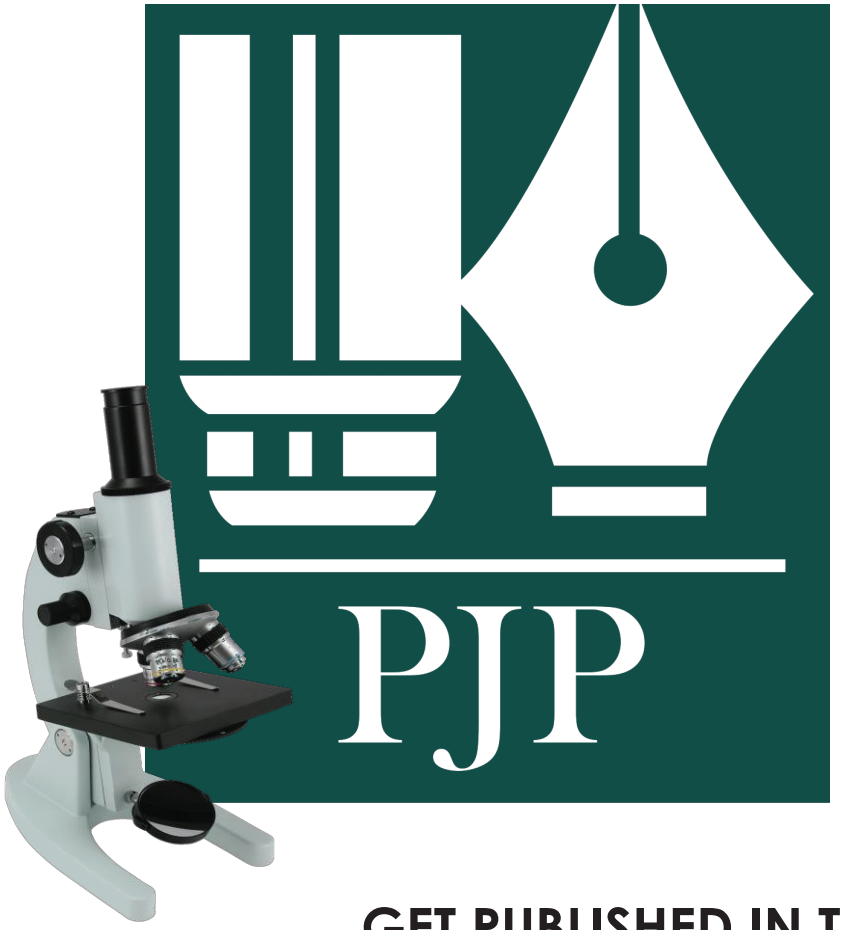

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