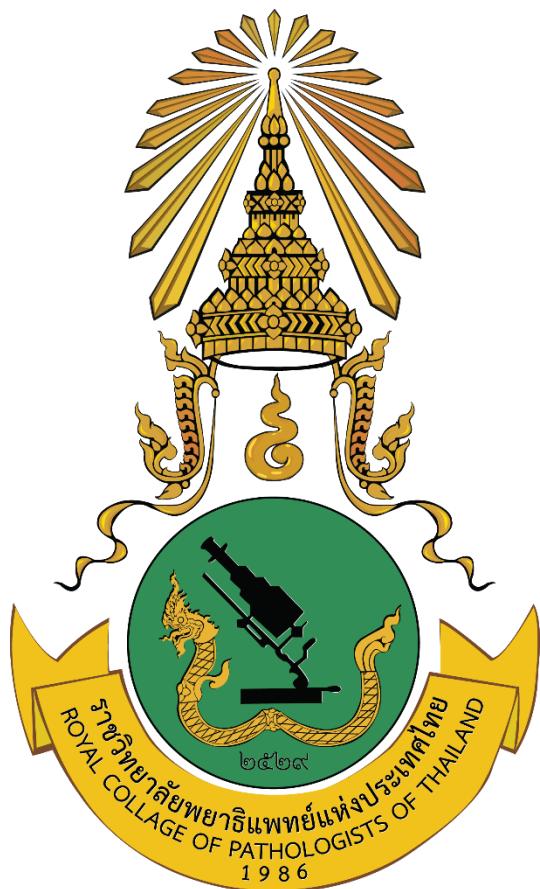


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ABOUT THE JOURNAL

Aims and Scope

Asian Archives of Pathology (AAP) is an open access, peer-reviewed journal. The journal was first published in 2002 under the Thai name “วารสารราชวิทยาลัยพยาธิแพทย์แห่งประเทศไทย” and English name “Journal of the Royal College of Pathologists of Thailand”. The journal is a publication for workers in all disciplines of pathology and forensic medicine. In the first 3 years (volumes), the journal was published every 4 months. Until 2005, the journal has changed its name to be “Asian Archives of Pathology: The Official Journal of the Royal College of Pathologists of Thailand”, published quarterly to expand the collaboration among people in the fields of pathology and forensic medicine in the Asia-Pacific regions and the Western countries.

The full articles of the journal are appeared in either Thai or English. However, the abstracts of all Thai articles are published in both Thai and English languages. The journal features letters to the editor, original articles, review articles, case reports, case illustrations, and technical notes. Diagnostic and research areas covered consist of (1) **Anatomical Pathology** (including cellular pathology, cytopathology, haematopathology, histopathology, immunopathology, and surgical pathology); (2) **Clinical Pathology (Laboratory Medicine)** [including blood banking and transfusion medicine, clinical chemistry (chemical pathology or clinical biochemistry), clinical immunology, clinical microbiology, clinical toxicology, cytogenetics, parasitology, and point-of-care testing]; (3) **Forensic Medicine (Legal Medicine or Medical Jurisprudence)** (including forensic science and forensic pathology); (4) **Molecular Medicine** (including molecular genetics, molecular oncology, and molecular pathology); (5) **Pathobiology**; and (6) **Pathophysiology**.

All issues of our journal have been printed in hard copy since the beginning. Around the late 2014, we developed our website (www.asianarchpath.com) in order to increase our visibility. We would like to acknowledge that our journal has been sponsored by the Royal College of Pathologists of Thailand. We have the policy to disseminate the verified scientific knowledge to the public on a non-profit basis. Hence, we have not charged the authors whose manuscripts have been submitted or accepted for publication in our journal.

On the other hand, if any authors request a printed copy of the journal issue containing the articles, each of the copied journals costs 450 bahts for Thai authors and 30 United States dollars (USD) for international authors.

Publication Frequency

Four issues per year

Disclaimer

The Royal College of Pathologists of Thailand and Editorial Board cannot be held responsible for errors or any consequences arising from the use of information contained in Asian Archives of Pathology. It should also be noted that the views and opinions expressed in this journal do not necessarily reflect those of The Royal College of Pathologists of Thailand and Editorial Board.

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ORIGINAL ARTICLE

Affecting Mortality Factor In COVID-19 Death At Home

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Abstract

Background: The COVID-19 pandemic has had a significant impact on global health systems. Thailand has experienced multiple waves of outbreaks that have resulted in a significant loss of lives. The country's healthcare infrastructure has been under immense pressure, with hospitals struggling to cope with the patient surge.

Objective: A recent study investigated the characteristics and factors associated with death-at-home cases during the pandemic, shedding light on key determinants of mortality outside medical facilities.

Materials and Methods: Descriptive study from the data we collected from the questionnaire in case we perform a scene investigation at their house

Result: The study of 74 individuals showed that fatalities were most common among the elderly and smoking habits were prevalent. Most cases were unvaccinated despite vaccine availability. Underlying diseases such as hypertension and diabetes were common. Delays in diagnosis were prevalent. These findings emphasize the complex interplay of demographic characteristics, vaccination status, and underlying health conditions in COVID-19 mortality.

Conclusions: Our study found that male gender, old age, and lack of immunization were the main characteristics associated with at-home deaths. However, there may be other

demographic factors that also play a role, which require further research to explore and understand.

Keywords: COVID-19, Vaccination, Death at home

Introduction

Since the first outbreak of coronavirus disease 2019 (COVID-19) in Wuhan, China, in December 2019, the disease rapidly became a global pandemic, with more than 600 million cases having been confirmed and more than 6 million fatalities. On January 12, 2020, Thailand reported the first COVID-19 case, a Chinese woman traveling from Wuhan, China. It was the first country to register a case outside China. On January 31, a local taxi driver was suspected to have been infected by a Chinese tourist he picked up, making this the first case of human-to-human virus transmission within Thailand. On February 29, 2020, Thailand reported the first known COVID-19-related death – a 35-year-old man who was admitted to hospital with dengue hemorrhagic fever and later found to be infected with COVID-19. He died after receiving treatment for more than a month. The infection spread more widely in Thailand and abroad until the World Health Organization declared the coronavirus outbreak a global pandemic on March 11, 2021.

Multiple waves of COVID-19 outbreaks hit Thailand. The third wave of infections happened with the Delta variant, which spreads more easily and causes increased hospitalizations ⁽¹⁾. At that time, available vaccines were less effective against the Delta variant, leading to a rapid rise in cases to more than 20,000 daily in early August 2021. The surge in infections has caused a severe shortage of hospital beds and staff, making it difficult to provide adequate care to patients. Sadly, many people died at home without receiving medical treatment.

The Forensic Division, Pathology Department, Faculty of Medicine Ramathibodi Hospital oversees the medical examination of unknown causes of death on the scene. Likewise, death-at-home from COVID-19 without any treatment is classified as an unknown cause of death. In Thailand, the number of death-at-home cases reached more than 100 cases on December 31, 2021, with various probabilities, including sex, age, underlying disease, and other factors. Therefore, this study aimed to determine the probabilities of any factor related to death- at-home cases.

Materials and Methods

Study design and setting

This descriptive study was conducted between May and December 2021 at the Forensic Medicine division of the pathology department, Faculty of Medicine Ramathibodi Hospital, Mahidol University.

Informed consent was obtained from the descendants' proxy. Ethical considerations were approved by the ethical committee of the Faculty of Medicine Ramathibodi Hospital, Mahidol University (MURA 2023/680)

Study objective

The objective of this study is to investigate mortality factors that contribute to death in COVID-19 patients who pass away at home.

Study population (descendants)

The study included 119 medicolegal descendants whose cause of death was COVID-19 infection confirmed by Reverse Transcription Quantitative Polymerase Chain Reaction. All cases were passed away at home and were performed scene investigation by two doctors from the Forensic Division, Pathology Department, Faculty of Medicine Ramathibodi Hospital

To ensure the accuracy of the study, exclusion criteria were applied, Non-Thai and non-relative descendants were excluded due to a lack of available health data and history. As a result, only 74 descendants were included in this study.

Reverse Transcription Quantitative Polymerase Chain Reaction

The reverse transcription PCR testing is conducted at the Virology Laboratory of Ramathibodi Hospital, certified with ISO15189 by the Department of Medical Sciences, Ministry of Public Health. The testing process proceeds as follows:

Samples are treated with a buffer (bioMérieux, Marcy-l'Étoile, France) to inhibit the activity of SARS-CoV-2. Subsequently, RNA extraction is performed from 200 μ L of the samples using the Zybio nucleic acid extraction kit (HuaYuan Technology Innovation Park, Guangdong, China) and an automated nucleic acid extraction system according to the manufacturer's instructions. For COVID-19 diagnosis, the Virology Laboratory of Ramathibodi Hospital uses the Bioperfectus COVID-19 Coronavirus Real-Time PCR Kit from Jiangsu Bioperfectus Technologies Co., Ltd.

For SARS-CoV-2 detection, primers and probes specific to the Open Reading Frame 11ab (ORF11ab) and nucleocapsid (N) gene of SARS-CoV-2 are used. After RNA extraction from the nasal swab samples, they are reverse transcribed into DNA and subjected to real-time PCR amplification. The target DNA quantity is monitored in each amplification cycle. If the cycle threshold (Ct) values are equal to or greater than 40, the result is considered negative,

indicating no detection of the SARS-CoV-2 virus. If the Ct values are less than 40, the numerical Ct values will be reported and calculated statistically for the Ct N gene.¹²⁴

Statistical analysis

Descriptive statistics were used in this study. Continuous data were shown with means and standard deviations, and categorical data were shown with numbers and percentages.

Results

Our observations reveal that the highest number of cases occurred in July (39 cases), followed by August (15 cases), in accordance with the country's trend, as displayed in Figure 1. Out of the 74 descendants under study, 48 were men (64.86%), and their mean age was 65.5 ± 14.27 years. The age range was between 25 years and 96 years, and most of the fatalities occurred in the geriatric population (those aged 60 years and above). Among the descendants, smoking information is available for 26 individuals, where 4 of them are active smokers, 7 used to smoke in the past, and 15 have never smoked before, as seen in Table 1.

Figure 1: Daily new death cases

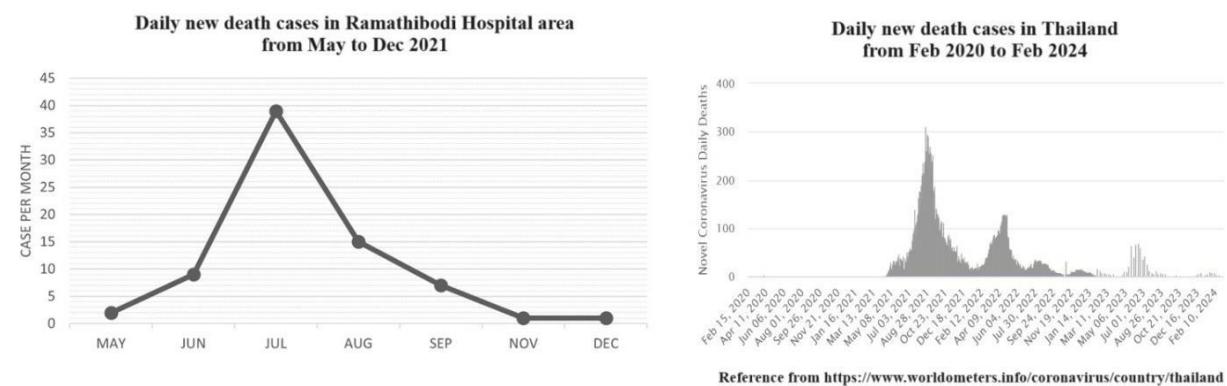


Table 1: Demographic data from the questionnaire

<i>Characteristics</i>	<i>Frequency</i>	<i>Percentage</i>
Sex		
Male	48	64.86
Female	26	35.14
Age in years ^a		
0-30 years	3	4.05
30-59 years	26	35.14
60-89 years	43	58.11
≥ 90 years	2	2.70
Vaccination		
Not yet	50	67.57
AstraZeneca vaccine 1 dose	7	9.46
AstraZeneca vaccine 2 doses	2	2.70
N/A	15	20.27
Smoking history		
Never smoke	15	20.27
Used to smoke	7	9.46
Active smoker	4	5.41
N/A	48	64.86

^a Mean age = 65.55 ± 14.27

Abbreviation: N/A, not applicable

According to our data suggests that a large majority of the cases, amounting to 84.75%, have not yet received the COVID-19 vaccine. The vaccination campaign in Thailand began on February 28, 2021, and so far, vaccination data is available for 79.73% of the total cases, which amounts to 59 cases. Out of these 59 cases, only 9 have received the vaccine; all are the AstraZeneca vaccine, with 7 of them having received a single dose and 2 having received two doses. The day after vaccination before death varies from 9 to 72 days, as seen in Table 2.

Table 2: Vaccination demographic

<i>Sex</i>	<i>Age</i>	<i>Dose of vaccination</i>	<i>Day after vaccination before death</i>	<i>Underlying disease</i>	<i>Smoking history</i>
Male	58	1 dose	48 days		Used to smoke
Male	61	1 dose	10 days	HT, DM	N/A
Male	61	1 dose	15 days	N/A DM	N/A
Male	68	1 dose	9 days	No	Never
Male	71	1 dose	9 days	HT	Never
Male	79	2 doses	72 days	HT, CKD	N/A
Female	59	1 dose	16 days	N/A DM	N/A
Female	74	2 doses	21 days	HT, DM	Never
Female	85	1 dose	9 days		Never

Abbreviation: HT, Hypertension, DM, Diabetes Mellitus, CKD, Chronic kidney disease, N/A, not applicable

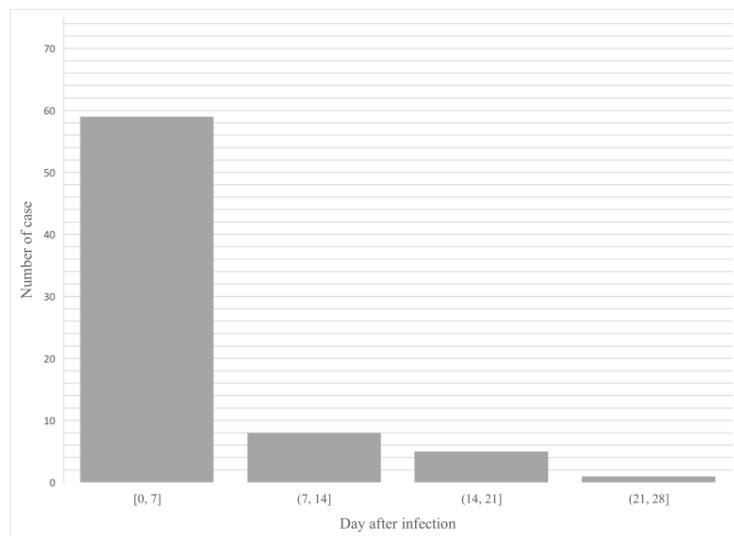
Additionally, 28 descendants have underlying diseases, including hypertension, diabetes mellitus, dyslipidemia, chronic kidney disease, chronic obstructive pulmonary disease, asthma, cardiovascular disease, stroke, lung cancer, colon cancer, and obesity, and mostly have multiple underlying diseases, as seen in Table 3.

Table 3: Underlying disease information

<i>Characteristics</i>	<i>Frequency</i>	<i>Percentage</i>
Underlying disease		
No	5	6.76
Yes	28	37.84
Asthma	1	1.35
COPD	1	1.35
Cardiovascular disease	2	2.70
Hypertension	9	12.16
Hypertension and Diabetes Mellitus	4	5.41
Hypertension, Diabetes Mellitus, and Dyslipidemia	2	2.70
Hypertension and Chronic Kidney Disease	1	1.35
Hypertension and Cardiovascular disease	1	1.35
Hypertension and COPD	1	1.35
Diabetes Mellitus	2	2.70
Stroke	1	1.35
Obesity	1	1.35
Lung cancer	1	1.35
Colon cancer	1	1.35
N/A	41	55.40

Abbreviation: COPD, Chronic obstructive pulmonary disease

Furthermore, we possess comprehensive information regarding the commencement of the viral infection, as well as the duration of diagnosis leading up to the unfortunate demise of the individual. It is noteworthy that 58.11% (43 descendants) were not diagnosed until after they had passed away and the forensic team performed the scene investigation. The rest of the individuals were undergoing a home isolation program when they were diagnosed. The length of diagnosis before death varied widely, ranging from as little as one day to a maximum of twenty-six days, as depicted in Figure 2.

Figure 2: Day after infection

Discussion

This study is a retrospective study of the death-at-home case of COVID-19 infection in the third wave of an outbreak in Thailand in the restricted area under the responsibility of The Forensic Division, Pathology Department, Faculty of Medicine Ramathibodi Hospital. We gathered all the information from the questionnaire, which contains demographic information, including sex, age, underlying disease, smoking habit, and underlying disease, but it is mostly incomplete. This is because most descendants live alone due to issues like relationship problems and their socioeconomic status. As a result, we won't have access to important information like symptoms, underlying diseases, smoking habits, or prior treatment before death. We will interpret the data based on the information we have completed for each case.

According to the data presented in Table 1, it is evident that a significant proportion of COVID-19 cases are concentrated among males, which is consistent with findings from other studies⁽²⁾. Although global data suggests a more equitable distribution of cases among genders⁽³⁾, males exhibit higher rates of intensive care admissions and fatalities⁽⁴⁾. Prior data from China also indicates that men tend to develop more severe cases based on clinical severity classification. It is important to consider the potential impact of social contexts, healthcare and social support systems, and cultural norms on the varying impact of COVID-19 on men and women, despite the biological differences.

Age is a significant factor in our observations, where descendants' ages range from 25 to 96 years, with an average age of 65.5 ± 14.27 years. Interestingly, more than 60% of the descendants are over 60 years old, consistent with prior research. This trend can be attributed to multiple underlying diseases that become more prevalent in older individuals, making them more susceptible to infections and severe illnesses leading to death. As previous studies have shown, the risk of severe illness and mortality tends to increase with age⁽⁵⁾.

Researchers are still investigating the connection between smoking and COVID-19 outcomes. Although only a small number of individuals in the studies are active smokers, it is important to consider the impact of smoking history on the severity of the disease. Understanding the relationship between smoking and COVID-19 susceptibility, progression, and mortality could help in crafting targeted health messages and cessation interventions.

It is important to acknowledge that individuals with pre-existing conditions are at a greater risk of severe illness or death due to COVID-19. Therefore, understanding the impact of pre-existing conditions on the disease progression and outcome of COVID-19 is crucial for identifying and managing the risks associated with the disease. This will help develop preventive measures and provide appropriate clinical management to these high-risk patients.

Our latest research is examining the vaccination status of descendants, specifically looking at their immunization status. We collected information from 59 out of 74 descendants, and unfortunately, only nine of them have received one or two vaccine doses. It's important to note that Thailand's vaccination program began on February 28, 2021. Previous research showed that trained immunity is induced after immunization for three months ⁽⁶⁾, but all the cases we studied received immunization in less than three months, with a maximum of 72 days. This means that their bodies may not have produced enough immunity to protect themselves, so they can be considered non-vaccinated.

Numerous studies have shown the benefits of vaccination in reducing symptoms and the severity of the disease. However, several factors are preventing descendants from receiving the vaccine. Some are still questioning its effectiveness, and others need more information or have received misinformation ⁽⁷⁾. Additionally, some individuals are willing to accept the vaccine but face accessibility issues with the immunization system, even though they live in Bangkok.

In conclusion, there are the main characteristics that we found in our dying at-home case are male, old age, and no immunization, which is the same as other prior studies; however, there are many demographic factors that may be associated with mortality, but we lack information to interpret, which suggest for future research to solve.

Limitation

The study may still have some limitations; for instance, the number of cases involved is small and limited. The collected data from the questionnaire are incomplete in some parts in many cases, such as missing data about underlying disease or history of smoking, which may contribute to affecting mortality factor.

Acknowledgments

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Conflicts of Interests: The authors declare no conflict of interest.

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ORIGINAL ARTICLE

Coronary Artery Stenosis: Post-mortem CT Angiography with air contrast injection VS. Autopsy

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Abstract

Background: The global rise in coronary artery disease fatalities prompts increased use of Postmortem Computed Tomography Angiography (PMCTA). Yet, its expense and potential for histological inaccuracies remain concerns. Substituting contrast agents with air injections has been explored but lacks a thorough comparison with conventional autopsy.

Objective: To investigate and compare the results of assessments of coronary artery stenosis in deceased individuals between PMCTA with air injection and conventional autopsy.

Material and methods: This diagnostic study examined 139 coronary arteries from 47 deceased individuals at Department of Forensic Medicine, Chulalongkorn University. Air injection into the left common carotid artery was followed by Postmortem Computed Tomography (PMCT) scans. Subsequently, coronary artery stenosis levels were measured and compared with conventional autopsy findings.

Results: PMCTA with air injection effectively diagnoses severe coronary artery stenosis in deceased individuals, boasting sensitivity and specificity levels of 97.22% and 94.17%, respectively, compared to conventional post-mortem dissection.

Conclusions: The assessment of coronary artery stenosis in deceased individuals using PMCTA with air injection instead of radiographic contrast agents has the potential to diagnose severe

coronary artery stenosis (critical stenosis). This study may serve as a foundation for the development of an effective diagnostic tool for identifying coronary artery stenosis.

Keywords: Post-mortem imaging, Postmortem computed tomography angiography (PMCTA), Negative contrast, Coronary artery stenosis

Introduction

Cardiovascular diseases, especially coronary artery disease, have become a leading global cause of mortality, accounting for approximately 42.1% of deaths attributed to cardiovascular diseases ⁽¹⁾. Presently, the standard procedure for assessing coronary artery stenosis is traditional autopsy and histological finding ⁽²⁾, which inherently involve a degree of subjectivity and necessitate significant internal organ destruction. In situations where a second autopsy is required, it may introduce potential interpretation discrepancies. Additionally, this approach may conflict with certain religious beliefs, causing distress to the deceased's relatives ⁽³⁾.

Currently, Postmortem imaging (PMI) such as PMCT has been utilized for forensic purposes. Nevertheless, in PMCT, detecting a coronary occlusion is impossible without visualizing the vascular lumen. Only calcifications, which are the radiological features most closely associated with atherosclerosis, can be observed in PMCT. Due to this reason, Postmortem Imaging (PMI) such as PMCT has been utilized in forensic medicine. However, these methods cannot detect coronary stenosis because they cannot visualize the lumen of the blood vessels. They may only detect calcifications, which are radiological features closely associated with atherosclerosis. Consequently, Postmortem Coronary Artery Calcium Scoring (CACS) or the Agatston method has been developed, which evaluates direct calcium quantification. Nonetheless, studies from Australia have found that approximately one-third of patients who died from acute CAD had a CACS value of zero. This event occurs due to the nature of the tissue in the coronary plaque in these cases, which typically consists of fibrous and cellular material together but lacks calcium within the blood vessels ⁽⁴⁾.

As a response to the limitations mentioned above, several forensic institutes have incorporated PMCTA to screening the coronary artery stenosis ⁽⁵⁻⁶⁾. However, this method is not without drawbacks, as radiographic contrast agents are costly and can lead to interpretation errors in histology ⁽⁷⁻⁸⁾.

Considering these limitations, recent research efforts have sought to utilize room air injection into the arteries of deceased individuals as an alternative to radiographic contrast agents. This approach has demonstrated the capability to significantly improve the clarity of computed tomography images within the arteries ^(7, 9).

Nevertheless, to date, no comparative studies have been conducted to assess the outcomes of post-mortem coronary artery assessment using PMCTA with air injection versus conventional autopsy.

With this context in mind, the research team endeavors to conduct a comparative analysis between the method utilizing computed tomography with air injection and conventional autopsy for post-mortem coronary artery stenosis assessment.

Materials and Methods

Research Design, Population and Sample

This research endeavors to explore the comparison in assessing the coronary arteries of deceased individuals between PMCTA with air as a substitute for radiopaque contrast material and conventional autopsy. The study employs a diagnostic design, determining the necessary sample size for sensitivity and specificity using the "Sample size requirements for sensitivity and specificity" formula ⁽¹⁰⁾. The study assumes expected values of 0.90 for sensitivity, 0.85 for specificity, 0.10 for precision, a prevalence rate of 0.25, and a 95% confidence interval. The calculated sample size is found to be 139 coronary arteries.

The study sample comprises deceased individuals whose initial recorded cause of death from crime scene investigation is classified as "undetermined cause of death." These cases underwent PMCTA with air injection and autopsy at the Department of Forensic Medicine, Faculty of Medicine, Chulalongkorn University, Thailand, during the period spanning May 2023 to September 2023, totaling 47 cases. Exclusion criteria were applied as defined:

- Decomposed bodies
- Severely charred bodies
- Skeletonized bodies
- Bodies with severe neck trauma
- Bodies with severe chest trauma
- Bodies that have undergone cardiac surgical procedures such as stent placement or coronary artery bypass surgery

Data Collection & Ethics

The research team prepared the necessary documentation to request consent for the utilization of corpses in research activities and to coordinate the acquisition of post-mortem examination data from the Faculty of Medicine, Chulalongkorn University. Each cadaver brought in for this research study was approved with consent by the forensic doctor authorized to conduct autopsies. This data was collected from May 2023 to September 2023 and included information regarding the causes of death, postmortem computed tomography images, and autopsy reports.

The Research Ethics Committee of the Faculty of Medicine, Chulalongkorn University, undertook the evaluation of the research ethics, following the established international ethical research standards as per the Institutional Review Board (IRB). This evaluation resulted in the granting of an exemption for ethical review, documented under IRB number 0341/66, and Certificate of Exemption number 032/2023.

Method

Corpses sourced from the Department of Forensic Medicine, Chulalongkorn University, which met the defined selection criteria, underwent both an external examination and a standardized PMCT imaging process, respectively. This process was carried out using the GE Revolution EVO CT scanner, configured with specific machine settings, including a rotation time of 0.8 seconds, tube voltage set at 120 kV, tube current at 200 mA, slice collimation of 1x64x0.625 mm, a pitch value of 0.98, and a total scan time of 16.9 seconds. The acquired PMCT images were then reviewed and analyzed utilizing the GE Healthcare Media Viewer software.

Following this, the corpse was prepared for the cardiac air infusion procedure, commencing with a longitudinal incision approximately 4.0 centimeters in length, positioned at the anterior midline of the neck. This incision was made to access the left common carotid artery. Then, a ligature was employed to secure the catheter in place, facilitating further procedural steps (Figure 1 (a)).

Subsequently, cut the left common carotid artery beneath the ligature point, positioned perpendicular to the artery, ensuring that the artery's lumen remains undamaged. Following this, a 14 Fr Foley catheter was gently inserted into the artery, reaching a depth of approximately 10.0 to 15.0 centimeters or until resistance was encountered ⁽⁹⁾. The aim was to position the distal end of the catheter at the opening of the coronary ostia, located above the heart's aortic valve. Subsequently, 30 milliliters of water were injected into the balloon of the Foley catheter (Figure 1 (b)), securing it in the desired position and preventing retrograde flow of air during the subsequent air injection process.

Once these preparatory steps were completed, the corpse was repositioned within the CT scanner to confirm and verify the catheter's placement before commencing the actual air injection procedure.

Once the catheter was appropriately positioned, the next step involved the injection of air into the coronary artery. An automated oil-free air pump, the "PUMPKIN PTT-DS9007" model with a 7-liter capacity, was employed for this purpose. Air was injected into the artery at a pressure of 10 psi (68947.5 pascal) for a duration of 2 minutes ^(9, 11) (Figure 1 (c)). This was achieved through the catheter placed on the left side of the neck. After the completion of the air injection, the corpse underwent another round of PMCT imaging before proceeding to the subsequent standard autopsy.



Figure 1: The process of injecting air into the coronary arteries.

The assessment of coronary artery stenosis was conducted using PMCT with air as the contrast agent (Figure 2 (a)-(c)), visualized with the GE Healthcare Media Viewer program. The process began by identifying the most stenosed segment of the coronary arteries (Figure 2(c)). Next, we measured the cross-sectional area using semiautomatic lumen contour detection algorithms for coronary PMCTA (Figure 2(b)). Following that, we assessed the stenosis percentage by comparing the minimal luminal area at the site of maximal stenosis with the normal reference area proximal or distal to the stenosed segment, using the equation below (12).

- % Area stenosis = $[1 - \text{minimum lumen area} / (\text{normal proximal or distal reference vessel area})] \times 100$

In each corpse specimen, the degree of stenosis was evaluated in all three major coronary artery branches, namely the left anterior descending artery (LAD), the left circumflex artery (LCx), and the right coronary artery (RCA). Subsequently, the data was collected and categorized into two groups: one group with coronary artery stenosis greater than 70% (critical stenosis) and the other group with stenosis less than 70% (non-critical stenosis) (15).

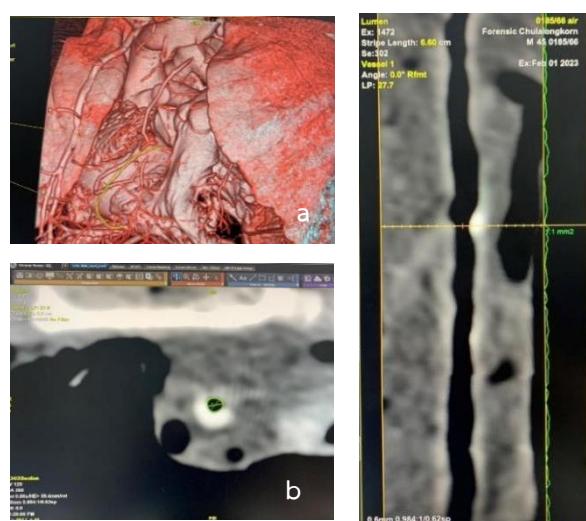


Figure 2: The assessment of coronary artery stenosis using PMCTA with air as the contrast agent

Next, the assessment of coronary artery stenosis was conducted using the autopsy sectioning method. The process started from the base of each of the three coronary artery branches. It is noted that the forensic doctors authorized to conduct standard autopsies and assess coronary stenosis in all cases had at least 10 years of experience in this field. Perpendicular cross sectioning was made until the distal end reached a size where further sectioning was not possible. The total number of cuts typically ranged from approximately 12 to 28 cross-sections of 3 to 5 millimeters per coronary artery^(2, 13). Subsequently, the degree of stenosis in each coronary artery was assessed by examining the inner cross-sectional area of the most constricted segment (Figure 3), following the reference table titled "Visual aid for quick assessment of coronary artery stenosis"⁽¹⁴⁾. The results were then expressed as a percentage. Data was collected and categorized as mentioned above⁽¹⁵⁾.

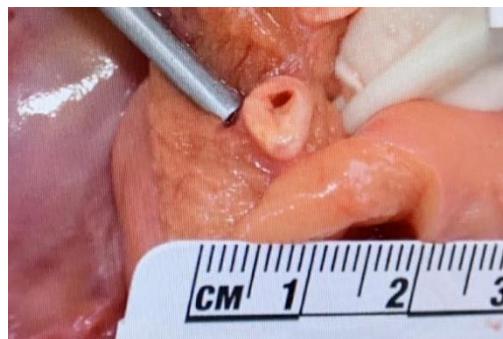


Figure 3: Cross-sectional area of the most constricted segment of coronary artery from gross autopsy. (This image is of the coronary artery, identical to that depicted in Figure 2.)

The assessment of coronary artery stenosis and data collection using the two methods were conducted at different time intervals, and data obtained from assessors were anonymized to reduce potential research bias.

Agreement measurement within this research involved evaluating intra-rater reliability by assessing coronary artery stenosis in a percentage format, repeating the measurement twice within the same artery at different time points. Subsequently, two sets of data were compared, totaling 15 coronary arteries, using the GE Healthcare Media Viewer program. Additionally, inter-rater reliability was evaluated between two researchers, both assessing coronary artery stenosis in a percentage format within the same arteries through the GE Healthcare Media Viewer program. This assessment included 15 cases. The data from both sets were then compared using intraclass correlation for analysis.

Statistical analysis

This study involves a comparative analysis of coronary artery assessment methods, specifically, PMCTA with air contrast and post-mortem dissection. The data obtained from

both methods is analyzed to determine key diagnostic metrics, including sensitivity, specificity, positive predictive value, and negative predictive value. The statistical analysis is performed using MedCalc for Windows, version 22.014 (MedCalc Software, Ostend, Belgium).

Results and Discussion

Demographic data

A total of 47 corpses were selected for analysis, comprising 43 males (91.49%) and 4 females (8.51%). In terms of ethnicity, 45 individuals were of Mongoloid origin (95.74%) and 2 were of Caucasoid origin (4.26%). The distribution of age data within the sample group followed a normal distribution (Figure 4). The mean age is 48.96 with a standard deviation of 10.914. The maximum age recorded is 71, and the minimum age is 25.

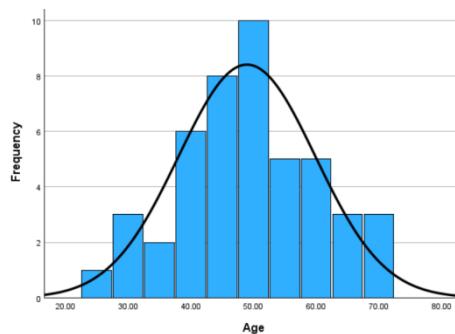


Figure 4: Age data is symmetrically distributed with no skew.

Each selected corpse underwent PMCT imaging before the standard autopsy process was carried out according to the protocols established by the Department of Forensic Medicine at Chulalongkorn University. The cause of death, as diagnosed by a medical professional, was determined based on the information obtained from both the autopsy and the analysis of PMCT images, as well as the deceased individuals' medical history. Throughout the autopsy procedure, causes of death were identified for 47 bodies. These causes included 16 cases of undetermined cause of death, 19 cases of coronary heart disease, 3 cases of intracranial hemorrhage, 2 cases of pulmonary disease, 5 cases of cardiomyopathy, 1 case of kidney disease, and 1 case of gastrointestinal bleeding.

Assessment of coronary artery stenosis

The assessment of coronary artery stenosis using PMCTA with air as a contrast agent, conducted by a third-year medical resident and a forensic medicine specialist, revealed critical stenosis in 41 arteries and non-critical stenosis in 98 arteries out of a total of 139 arteries

examined. Specifically, the left anterior descending artery exhibited critical stenosis in 20 arteries and non-critical stenosis in 27 arteries (totaling 47 arteries), the left circumflex artery showed critical stenosis in 8 arteries and non-critical stenosis in 38 arteries (totaling 46 arteries), and the right coronary artery displayed critical stenosis in 13 arteries and non-critical stenosis in 33 arteries (also totaling 46 arteries).

The assessment of coronary artery stenosis using autopsy technique, conducted by six specialists in forensic medicine, revealed critical stenosis in 36 arteries and non-critical stenosis in 103 arteries out of a total of 139 arteries examined. Specifically, the left anterior descending artery exhibited critical stenosis in 19 arteries and non-critical stenosis in 28 arteries (totaling 47 arteries), the left circumflex artery showed critical stenosis in 6 arteries and non-critical stenosis in 40 arteries (totaling 46 arteries), and the right coronary artery displayed critical stenosis in 11 arteries and non-critical stenosis in 35 arteries (also totaling 46 arteries).

Analyze the diagnostic value of assessing coronary artery stenosis in deceased individuals using PMCTA with air as a contrast agent in comparison with the results of the autopsy process.

From the assessment of coronary artery stenosis in a total of 139 coronary arteries from 47 corpses, the PMCTA with air as a contrast agent method identified 41 coronary arteries with critical stenosis. In contrast, the autopsy process identified 36 coronary arteries with critical stenosis. This research examined coronary arteries ranging from those that were normal without any signs of disease up to those with atherosclerosis, and it was found that 25.69% of the coronary arteries had atherosclerosis.

The comparative analysis of the two assessment methods revealed a sensitivity of 97.22%, specificity of 94.17%, positive predictive value of 84.76%, negative predictive value of 99.03%, accuracy of 94.94%, positive likelihood ratio of 16.69, and negative likelihood ratio of 0.03, as shown in Table 1.

Table 1. Demonstrating a comparison of the assessment results of coronary artery stenosis in deceased individuals between the PMCTA with air as a contrast agent method and the results of the autopsy process.

		Autopsy process		
		Critical stenosis	Non-critical stenosis	Total
PMCTA with air as a contrast agent method	Critical stenosis	35	6	41
	Non-critical stenosis	1	97	98
	Total	36	103	

Note: sensitivity = 97.22%, specificity = 94.17%, positive predictive value (PPV) = 84.76%, negative predictive value (NPV) = 99.03%

Agreement measurement

The results of the intra-rater reliability (Intra-rater Reliability) and the inter-rater reliability between both assessment methods (Inter-rater Reliability) yielded statistical values of intraclass correlation coefficient equal to 0.990 and 0.979, respectively.

Discussion

Currently, certain medical institutions utilize the combination of PMCT scanners alongside the intravascular injection of contrast agents to facilitate the screening and diagnosis of coronary artery atherosclerosis. However, it is noteworthy that such methods are associated with high costs and may require the reliance on specialized equipment for their implementation.

Through the findings of this study, it has been determined that the use of PMCTA in conjunction with the injection of air can effectively diagnose critical stenosis of the coronary arteries. These results align with the research conducted by Mansharan Kaur Chainchel Singh et al. (2020) which investigated the relationship between coronary stenosis assessment using PMCTA method compared to histological analysis. The findings showed a sensitivity of 61.5%, specificity of 91.7%, positive predictive value (PPV) of 40.0%, negative predictive value (NPV) of 96.4%, and a P value <0.001. These results suggest that there is a fair level of sensitivity and good specificity in postmortem diagnosing the severity of coronary artery stenosis ⁽²⁾. In

our research, the findings exhibit exceptionally high sensitivity and specificity (sensitivity = 97.22%, specificity = 94.17%) when compared to the conventional autopsy-based method. Furthermore, it is noteworthy that this procedure is cost-effective, easily accessible and involves straightforward steps.

The reason this research chose to use cross-sectional area for calculating the percentage of coronary artery stenosis instead of using the diameter is that irregular lumen shapes in blood vessels are often encountered. These irregular shapes could lead to a misrepresentation of the true narrowing of the lumen when assessing diameter in many instances.

Nevertheless, the method necessitates the proficiency and expertise of the operator throughout the entire process, from locating the left common carotid artery to accurately positioning the foley catheter and injecting air into the blood vessel. In the initial stages of this study, the researchers spent approximately one to two hours to complete the procedure for each corpse. However, as time progressed after the researchers had undergone training with a group of around ten corpses, they were able to expedite the procedure, taking approximately forty minutes for each corpse. Additionally, it was found that during the initial phase of the experiment, injecting air with excessive pressure or prolonging the injection period could lead to the occurrence of subcutaneous emphysema. This condition may also introduce air into various tissues, potentially complicating the interpretation of PMCT images.

The research team anticipates that the 6 cases of false positive results arise from the CT machine's capability to assess detailed cross-sectional images of blood vessels surpassing the precision of conventional post-mortem dissections. On the contrary, 1 false negative result is hypothesized to result from inaccuracies in manually tracking of the coronary arteries. Another significant challenge in this research lies in the utilization of software for locating and assessing the stenosis of the coronary arteries. The use of such software is impeded by the fact that these programs are equipped with pre-existing operating systems capable of tracking the arteries and assessing their stenosis when conventional contrast agents are employed. However, in this study, air was utilized instead of conventional contrast agents, necessitating manual tracking and measurement of coronary artery stenosis by the research team. Consequently, this manual approach extended the time required for the procedure compared to the conventional use of contrast agents. The study and development of software for processing the lumen size of blood vessels after air injection in the future can help address the aforementioned issues.

Based on the results of the study regarding the comparison between the assessment of coronary artery stenosis in deceased individuals using PMCTA with air as a contrast agent and conventional autopsy, it is evident that the method yields exceptionally high sensitivity and specificity, with values of 97.22% and 94.17%, respectively. This implies the potential of utilizing the described method for diagnosing severe coronary artery stenosis. Therefore, the

research team suggests that the assessment of coronary artery stenosis in deceased individuals using PMCTA with air as a contrast agent may evolve into an efficient diagnostic tool for distinguishing coronary artery stenosis.

Conclusion

The assessment of coronary artery stenosis in deceased individuals using PMCTA with air as a contrast agent can diagnose severe coronary artery stenosis (critical stenosis) effectively, with remarkable sensitivity and specificity values of 97.22% and 94.17%, respectively when compared to conventional autopsy.

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Note: During the preparation of this work the author used chatGPT in order to improve language and readability. After using this tool, the author reviewed and edited the content as needed and takes full responsibility for the content of the publication.

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ORIGINAL ARTICLE

Correlation of Hounsfield unit of the Humerus, First lumbar spine, and Femur with Postmortem interval in Deceased body in Thailand

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Abstract

Background: Forensic pathologists are responsible for the assessment of postmortem interval (PMI) during crime scene investigation. Postmortem computed tomography (PMCT) is one of the instruments that can assist for estimating PMI via changes in internal organs.

Objective: To study the correlation between PMI in a decomposed body and the Hounsfield unit of the humerus, 1st lumbar spine, and femur on PMCT

Research method: The retrospective descriptive correlation study analyzed PMCT images of three specific bones in decomposed bodies to compare with PMI. The study included 90 decomposed bodies which were divided into 3 groups: 1 to 3 days, 3 to 5 days, and 5 to 7 days.

Results of the study: The Hounsfield unit values of bones exhibited a statistically significant reverse correlation with PMI. Notably, among the bones examined, the right humerus stands out with the highest correlation coefficient ($r = -0.613$).

Conclusion: The right humerus showed moderate to strong correlation with PMI while 1st lumbar spine and femur were moderately correlated. The statistically significant correlation observed among the Hounsfield unit values of the humerus, 1st lumbar spine, and femur to PMI suggests that the Hounsfield unit may offer a potential method for estimating the time of death.

Keywords: Postmortem interval, Postmortem computed tomography (PMCT), lumbar, femur, humerus

Introduction

Forensic pathologists are responsible for the assessment of PMI, which is determined using various techniques depending on the progression of decomposition. There are 4 stages of decomposition: fresh, early decomposition, advanced decomposition, and skeletonization. During fresh stage, PMI estimation is assessed by using rigor and livor mortis which are the stiffening of muscles and blood pooling after the cessation of blood circulation respectively. Rigor mortis typically begins after death and reaches its peak at around 6-12 hours PMI then it gradually diminishes and eventually disappears. However, in the stages of early decomposition, advanced decomposition, and skeletonization, the postmortem interval is mainly determined by using the alteration of the external appearances of the body instead. These observational changes may include greenish discoloration of the skin, the presence of venous marbling, the accumulation of gases, and the formation of vesicles ^[1-3]. The estimation according to those changes is subjective and mostly based on expert opinion. Therefore, standardizing the PMI estimation method via quantitative method is a priority. PMCT is one of the instruments that could assist forensic pathologists in estimating PMI. The measurement CT value or Hounsfield unit (HU) of cerebrospinal fluid ^[4-5], the changes of intrahepatic gas and intrarectal gas ^[6], the elevation of femoral head attenuation (correlation coefficient = 0.90, regression coefficient = 0.34) ^[7] and the liver settling ratio ^[2] have been studied and concluded that they possibly be used to estimate the PMI.

Decomposition is a continuous process that involves two mechanisms: autolysis and putrefaction ^[3]. Autolysis, the aseptic process of cellular disintegration through intracellular enzymes, occurs all over the body including bone marrow tissue but at a slower rate compared to the other ^[8]. Bone marrow tissue is protected by a strong and durable structure. Nonetheless, they still undergo some structural changes that could be analyzed after death, for instance, using radioisotope, amino acid, microstructure ^[9-11], and intraosseous gas pattern ^[12]. Therefore, we assume that postmortem changes in bone could be related to PMI. However, conducting a thorough examination of bones can be labor-intensive, and the utilization of PMCT can hasten the examination process. Accordingly, the objective of this

study is mainly focused on the correlation between the value of the Hounsfield unit from PMCT of the humerus, 1st lumbar spine, and femur with PMI.

Materials and Methods

Research Design, Population & Sample

A retrospective-descriptive correlation study was carried out within the Department of Forensic Medicine, Chulalongkorn University. The sample size was accomplished through the utilization of Pearson's correlation estimation formula, by using a correlation coefficient of 0.74, a margin of error of 0.1, along with 95% confidence interval setting, resulting in a required sample size of 90 samples ^[13].

The research study employed a sample comprising 90 Thai deceased individuals, all of whom were deceased for a period ranging from 1 to 7 days and underwent computed tomography (CT) scanning prior to autopsy. The data collection took place during March 2021 to August 2023. The selected subjects for this study fell within an age range of 25 to 65 years for females and 25 to 70 years for males ^[14-17]. Cases were excluded from the study ^[15] if the PMCT scans of the proximal humerus, proximal femur, or lumbar spine were incomplete, the presence of pathological conditions, fractures, or the existence of artificial bone implants in the specific bones that focused on this study.

Subsequently, the study samples were categorized into three distinctive groups based on external appearance criteria, as delineated in Diagram 1: individuals within the postmortem interval of 1 to 3 days, those within the interval of 3 to 5 days, and those within the interval of 5 to 7 days ^[2].

Data collection & Ethics

The data set utilized in this research encompassed comprehensive details of the deceased individuals, the cause of death, PMCT images, as well as photographic records taken during the autopsy procedures.

The Research Ethics Committee at the Faculty of Medicine, Chulalongkorn University, has issued a certificate of exemption from ethics review for this research. This exemption is based on the Institutional Review Board (IRB) number 0520/66, and it is documented as Certificate of Exemption number 046/2023.

Statistics

The Hounsfield unit of the 1st lumbar spine, head of humeri, and proximal femurs were measured. The analysis of the frequencies, minimum and maximum values, mean standard deviation, coefficient of variation, intraclass correlation, Wilcoxon signed rank test, Spearman

rank correlation coefficient, and the ordinal logistic regression model was performed by using IBM SPSS Statistics Subscription version 29.0.1.0 (2023).

Methods

Deceased individuals who had undergone post-mortem examinations were refrigerated at a temperature of 4 degrees Celsius for a maximum duration of 24 hours. These cadavers were subsequently CT scanned, utilizing the GE Revolution EVO model CT scanner. The CT machine's parameters were configured as follows: a rotation time of 0.8 seconds, 120 kV tube voltage, 200 mA maintaining of tube current, 1x64x0.625 mm slice collimation, pitch value of 0.98, and a scan duration of approximately 16.9 seconds. These CT scans were performed under an ambient temperature range of 20 to 25 degrees Celsius. Subsequently, the acquired CT images were visualized and analyzed using GE AW Server version 3.2 Ext. 4.0, before proceeding to the standard autopsy procedure.

The samples were selected after PMCT. PMI estimation was evaluated based on external appearances for each body with criteria shown in Table 1. ^[2]

Table 1 Characteristics of postmortem changes of the deceased

PMI	External appearance
18 - 24 hours	Greenish discoloration of abdomen and fixation of livor mortis
1 - 3 days	Greenish discoloration of head and body, venous marbling, vesicle formation, swelling of the face, bloating and skin slippage in few area
3 - 5 days	Generalized bloating of body, more greenish discoloration along extremities, more skin slippage, hair slippage
5 - 7 days	Partial skeletonization of the face

The measurement of the HU in each decomposed body at the proximal humerus, 1st lumbar spine, and femurs was done by the following steps:

- For the measurement of the proximal humerus bone, firstly positioning the axis of humerus in sagittal and coronal plane as shown in Fig. 1, then follow humerus in axial plane to mark the position of the humeral head by using lesser tubercle as anterior landmark. The region of interest (ROI) is the circular area containing bone marrow posterior to the superior edge of lesser tubercle, which lies in the axial plane of the humeral head ^[18].

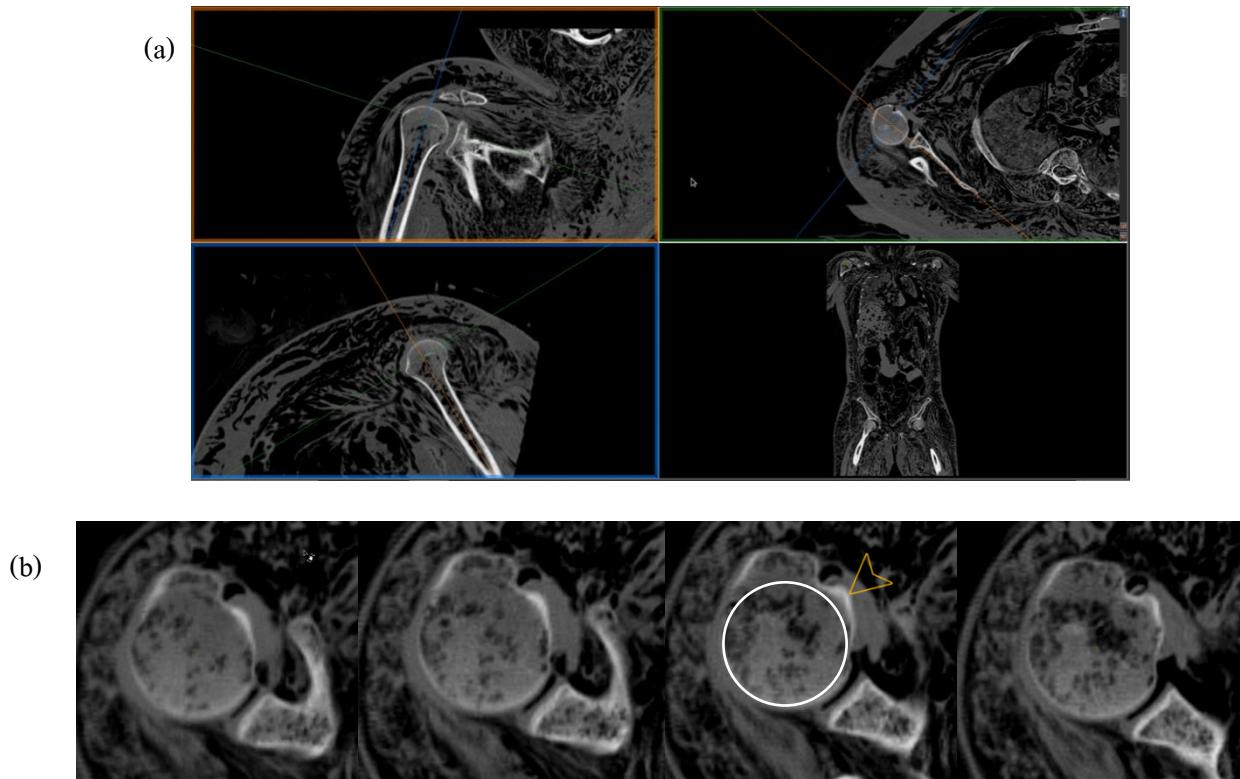


Fig. 1. Right humerus. (a) Demonstrate adjustment of right humeral axis before the measurement of HU. (b) Demonstrate selected axial plane of right humeral head. Yellow arrow shows the superior edge of lesser tubercle and white circle is the ROI of humeral head.

- The measurement of proximal femur was done as illustrated in Fig. 2 by positioning the axis of femur in coronal and sagittal plane. Then, follow the axial plane of femur and mark at the neck of femur. The ROI is located within the femoral head in the axial plane where the fovea capititis is no longer visible.

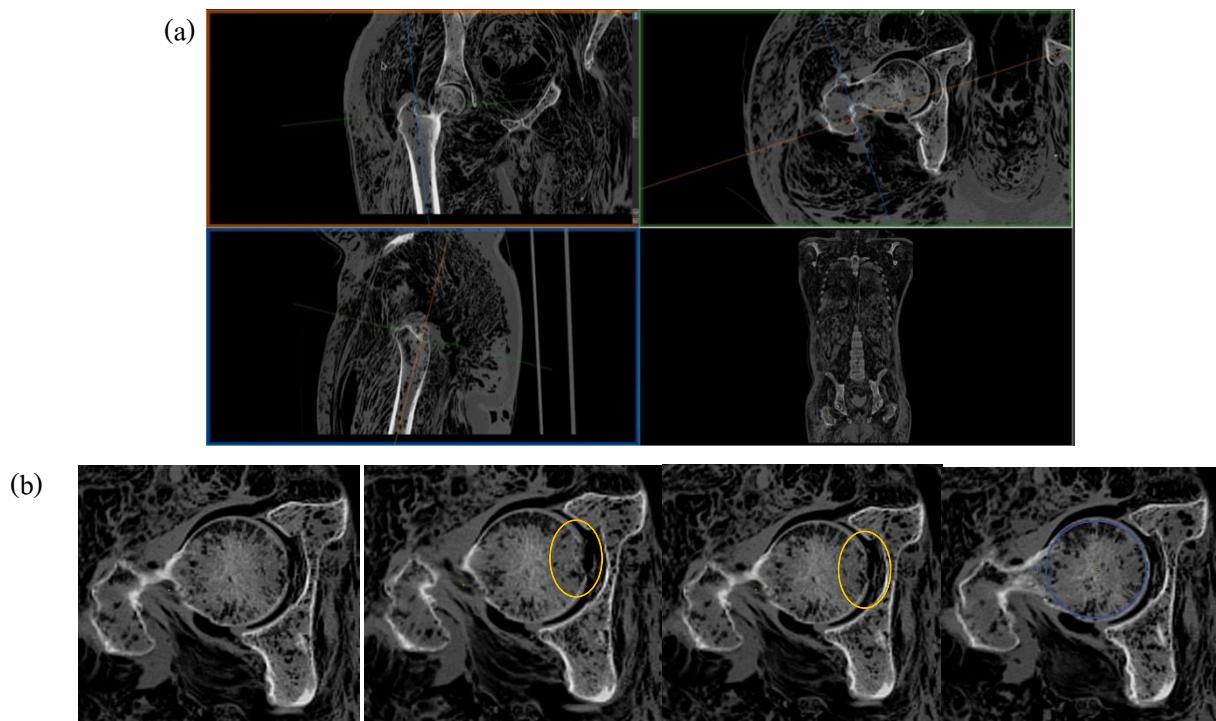


Fig. 2. Right femur (a) Demonstrate the adjustment of the right proximal femur axis before the measurement of HU. (b) Demonstrate the correct selected plane of the right femoral head. The yellow ellipse shows the existence of fovea capitis and the last picture on the right shows the plane which fovea capitis is no longer exists with blue circle marking around the ROI of the right femoral head.

- The 1st lumbar spine was viewed in the axial plane. The spinous process was used to adjust the anteroposterior dimension of the axial plane. Next step was adjusting the sagittal plane by using the midline and lower edge of the vertebral body. The final step was measuring the ROI depicted in Fig. 3 at the largest area of the 1st lumbar spine in the axial plane.

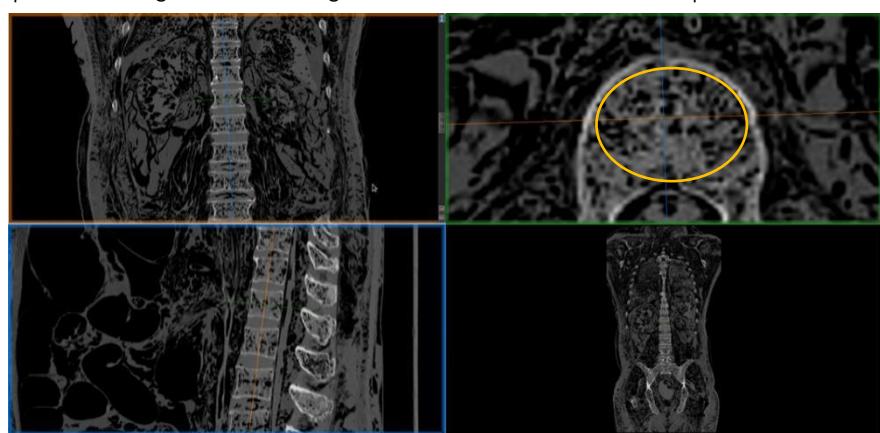


Fig. 3. Demonstrate the HU measurement of 1st lumbar spine. The yellow ellipse shows the ROI of 1st lumbar spine.

To be noted, the cortex of the bone is not involved in HU values measurement and intra-rater reliability was evaluated by the same procedure using 10 samples.

After the measurement of all bones was done. The collected HU values were statistically analyzed to determine the correlation with PMI.

Results

Demographic data

90 samples were included. 66 (73.3%) were male and 24 (26.7%) were female. The mean age was 51.24 ± 10.77 years (range from 27 to 70 years). The cause of death was categorized to natural disease including cardiovascular disease (32 cases), cerebrovascular disease (10 cases), upper gastrointestinal hemorrhage (3 cases), infection (3 cases), and senility (1 case), unnatural death (including hanging (7 cases), head injury (1 case), and carbon monoxide poisoning (3 cases) and undetermined death (30 cases).

The correlation between HU value and PMI

Table 2 shows the number of samples in each PMI category, HU from five interested bones and correlation coefficient with PMI.

Table 2. Number of samples, the HU values shown in median and range of minimum and maximum in each PMI group and Spearman correlation coefficient of each bone and PMI.

	PMI group			Correlation
	1-3 days	3-5 days	5-7 days	coefficient (r)
Number	34	50	6	
Right humerus	155.15 (-87.20 – 259.10)	-13.15 (-490.40 – 199.90)	-120.10 (-242.20 – 291.90)	-0.613*
Left humerus	142.70 (-120.90 – 270.30)	-46.20 (-373.10 – 237.00)	-43.25 (-463.50 – 102.30)	-0.543*
1 st lumbar spine	-18.20 (-463.40 – 215.60)	-183.35 (-460.90 – 185.70)	-351.30 (-649.10 – -51.80)	-0.518*
Right femur	240.25 (120.60 – 344.10)	122.05 (-99.50 – 420.00)	-20.15 (-185.30 – 234.30)	-0.500*
Left femur	256.85 (135.30 – 362.90)	112.70 (-218.40 – 460.80)	201.85 (-142.20 – 293.40)	-0.491*
Average of HU value from 5 sites	142.64 (27.10 – 245.96)	-3.00 (-255.06 – 182.02)	-42.77 (-287.70 – 95.00)	-0.685*

*p-value < 0.001, correlation is significant at the 0.01 level(2-tailed)

The HU values of each bone in the PMI group were shown in Fig. 4 which tend to decrease mostly as longer PMI.

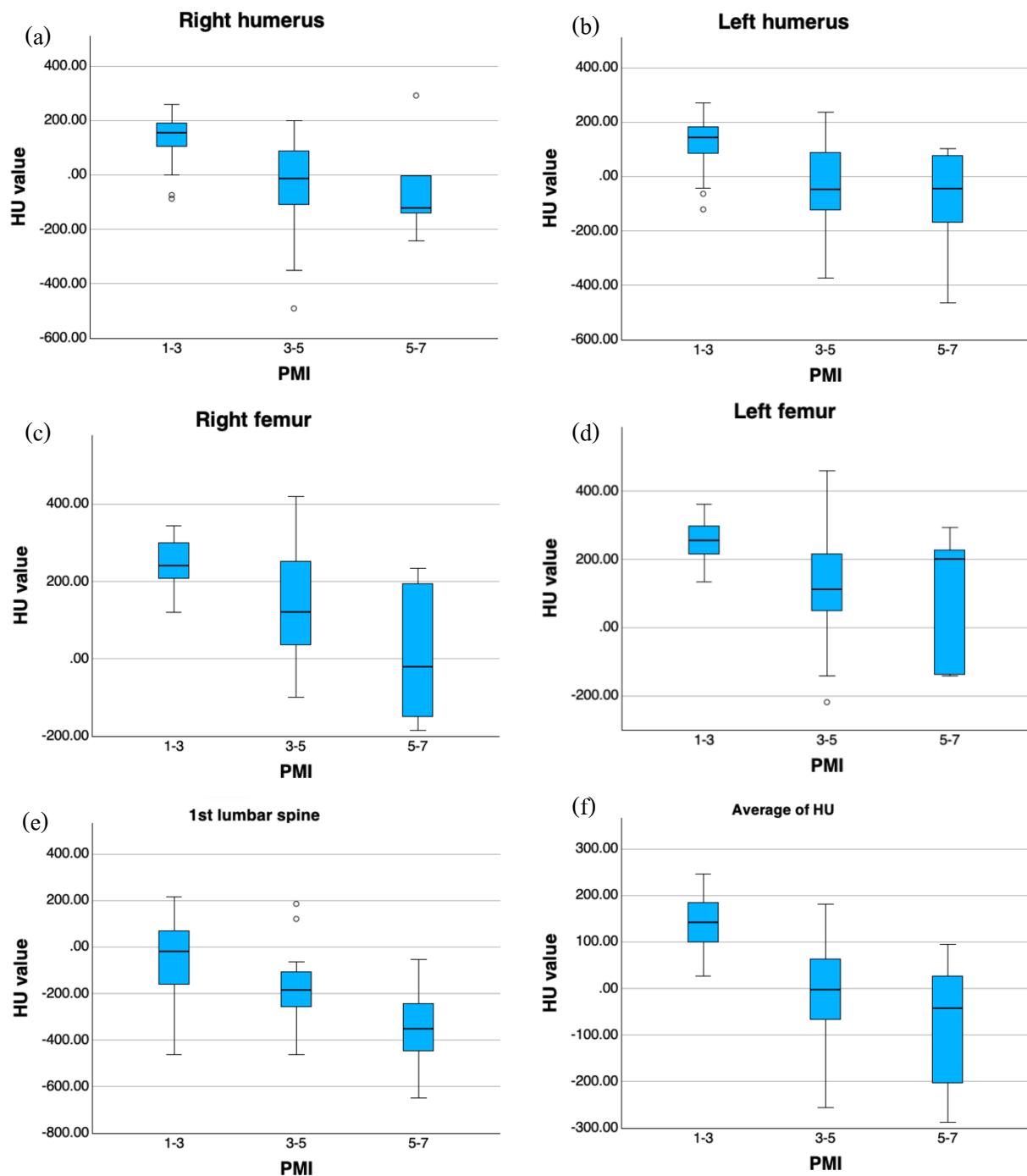


Fig. 4. Box-plot diagram of the HU value in each bone and PMI (days). (a) Right humerus (b) Left humerus (c) Right femur (d) Left femur (e) 1st lumbar spine (f) average of HU value of five sites.

The paired Wilcoxon test among both sides of humeri and femurs of 90 cases revealed no statistically significant difference ($p<0.05$).

The results showed the inversely proportional correlation between the two variables. The HU value of the average of five measurements showed the highest correlation with the postmortem interval ($r=-0.685$). Among individual bones, the right humerus had a better correlation with PMI ($r=-0.613$). The HU of each bone and the coefficient of variation were shown in Table 2.

Furthermore, a weak correlation between age and the average HU values from 5 sites was observed ($r = -0.226$, $p = 0.032$).

Agreement measurement

The outcomes of the intra-rater reliability test were in the range of 0.931 to 0.981.

Ordinal logistic regression

All bones, including the 1st lumbar spine, right proximal humerus, and right femur showed statistically significant in the prediction of PMI (with p -value <0.001, 0.38, and 0.008 respectively). The model fitting test indicates the significant difference between the coefficients of the equation and null model ($p < 0.001$)

Discussion

In the context of this study, the research objective was to examine the correlation between the postmortem interval (PMI) and the Hounsfield unit values of the humeri, the 1st lumbar spine, and the femurs. All deceased bodies in this study were found in Bangkok, Thailand and located in similar temperature. According to T. van Grinsven et al. ^[7], it was observed that the attenuation of the femoral head exhibited a statistically significant increase when correlated with the PMI. However, in contrast to this prior study, our study revealed an inverse correlation between the Hounsfield unit values of each bone and the postmortem interval. This difference could be attributed to local temperature, as well as a broader range of PMIs within the groups examined in our study. From our opinion, the Hounsfield unit measurements may initially elevate shortly after death due to livor mortis ^[7], followed by a subsequent decline at a later decomposed stage. Per the findings by Sapienza D. et al. ^[12], wider distribution of intraosseous gas is associated with longer PMI which corresponded with our findings. In one instance, a PMI falling within the range of 5 to 7 days displayed an average

HU value consistent with the 3 to 5 days PMI group due to the inhomogeneous decomposition between head and body.

There is a small difference between the 3 to 5 days group and the 5 to 7 days group within the average HU values among each PMI group, especially during the later stage of PMI, as shown in Fig. 4. One possible explanation for this phenomenon could be the structural integrity of the bones, which undergoes fewer changes during the later stages of the PMI and may result in the reduction of variability in HU values. This observation appeared to be similar to the findings in Erwan Le Garff et al. study ^[9] that noted the degradation of bone that tended to significantly increase within the initial two weeks following death.

This study also showed the more distinctive trabecular pattern which changes along with extended PMI, and it is probably due to a declination in bone marrow cellularity and inclination of gas-forming bacteria. This finding has also been described by Tattoli L. et al. ^[8] at the 1st lumbar vertebral body and explained to be from the colonization of gas-forming bacteria along vascular routes.

In 2020, Ford et al. ^[19] studied the HU value and volumetrics of the proximal femur and found an inverse correlation with age. Nevertheless, our study group showed a weak correlation between them. This could be the effect of decomposition more impact.

This retrospective study possesses several limitations. Firstly, all samples were from outside the hospital and the bodies already went to decomposed stage when forensic pathologists examined at the crime scene. Consequently, PMI could not be known certainly and had to be assessed by external appearance. In our study, the number of samples in the 5 to 7 days group is notably lower compared to other groups. Additionally, the absence of information on the pre-existing medical history of the samples could potentially influence Hounsfield unit values, which were a limitation of this study. Furthermore, distinguishing between the 3 to 5-day and 5 to 7-day postmortem interval groups could be challenging and potentially lead to an overlapping evaluation.

Another limitation of this study appeared to be the resolution of PMCT images that were restricted by routine PMCT protocol, which might result in difficulty in some landmark evaluation and measurement. However, the higher resolution PMCT for each specific bone that improves the overall quality of the imaging data requires longer time consumption and more steps compared to routine work.

Conclusion

The statistically significant correlation observed among the Hounsfield unit values of the humeri, 1st lumbar spine, and femurs in relation to the postmortem interval suggests that the measurement of Hounsfield unit values in the decomposed body may offer a new potential tool for estimating the PMI with higher precision and accuracy. To enhance the

accuracy of PMI estimation, further study should be performed and focused on additional measuring sites which will introduce a more comprehensive and precise PMI assessment. Lastly, the HU values are a measurable unit that can possibly be conducted by an advanced artificial intelligence in nearby future for the objective in reducing human errors.

Acknowledgments

Not applicable

Declarations of interest

The research team involved in this study did not receive any funding from external sources, and there are no reported conflicts of interest, whether financial or non-financial, associated with any individuals or organizations involved in this research.

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APPENDIX 1

INFORMATION FOR AUTHORS

All authors listed in a paper submitted to Asian Archives of Pathology (AAP) must have contributed substantially to the work. It is the corresponding author who takes responsibility for obtaining permission from all co-authors for the submission. When submitting the paper, the corresponding author is encouraged to indicate the specific contributions of all authors (the author statement, with signatures from all authors and percentage of each contribution can be accepted). Examples of contributions include: designed research, performed research, contributed vital new reagents or analytical tools, analysed data, and wrote the paper. An author may list more than one type of contribution, and more than one author may have contributed to the same aspect of the work.

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The submitted manuscripts will be reviewed by three members of the Editorial Board or three expert reviewers from different institutions. At the discretion of the Editorial Board, the manuscripts may be returned immediately without full review, if deemed not competitive or outside the realm of interests of the majority of the readership of the Journal. The decision (reject, invite revision, and accept) letter will be coming from the Editorial Board who has assumed responsibility for the manuscript’s review. The editor’s decision is based not just on technical merit of the work, but also on other factors such as the priority for publication and the relevance to the Journal’s general readership. All papers are judged in relation to other submissions currently under consideration.

Categories of Manuscripts

1. Letters to the Editor

The letters to the editor are the reactions to any papers published in AAP. These letters will be reviewed by the Editorial Board and sent to the authors of the original paper with an invitation to respond. Letters and eventual responses will be published together, when appropriate.

- *Word Count: 300 – 500 words (excluding references and figure or table legends)*
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- *References: Maximum of 10*
- *Figure or Table: Maximum of 1 (if needed)*

2. Original Articles

The original articles are the researches describing the novel understanding of anatomical pathology, clinical pathology (laboratory medicine), forensic medicine (legal medicine or medical jurisprudence), molecular medicine or pathobiology. Systematic reviews, meta-analyses and clinical trials are classified as articles. The articles should be clearly and concisely written in the well-organised form (see *Organisation of Manuscripts*): abstract; introduction; materials and methods; results; discussion; and conclusions. The manuscripts that have passed an initial screening by the Editorial Board will be reviewed by two or more experts in the field.

- Word Count: 3,000 – 5,000 words (excluding abstract, references, and figure or table legends)
- Structured Abstract (see *Organisation of Manuscripts*): 150 – 200 words
- References: Maximum of 150
- Figures or Tables: Maximum of 6

3. Review Articles

The review articles are generally invited by the Editor-in-Chief. They should focus on a topic of broad scientific interest and on recent advances. These articles are peer-reviewed before the final decision to accept or reject the manuscript for publication. Therefore, revisions may be required.

- Word Count: 3,000 – 5,000 words (excluding abstract, references, and figure or table legends)
- Unstructured Abstract: 150 – 200 words
- References: Maximum of 150
- Figures or Tables: Maximum of 4

4. Case Reports

AAP limits publication of case reports to those that are truly novel, unexpected or unusual, provide new information about anatomical pathology, clinical pathology (laboratory medicine) or forensic medicine (legal medicine or medical jurisprudence). In addition, they must have educational value for the aforementioned fields. The journal will not consider case reports describing preventive or therapeutic interventions, as these generally require stronger evidence. Case reports that involve a substantial literature review should be submitted as a review article. The submitted case reports will undergo the usual peer-reviewed process.

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- Unstructured Abstract: 150 – 200 words

- References: Maximum of 20
- Figures or Tables: Maximum of 4

5. Case Illustrations

Case illustrations are aimed to provide education to readers through multidisciplinary clinicopathological discussions of interesting cases. The manuscript consists of a clinical presentation or description, laboratory investigations, discussion, final diagnosis, and up to 5 take-home messages (learning points). Regarding continuous learning through self-assessment, each of the case illustrations will contain 3 – 5 multiple choice questions (MCQs) with 4 – 5 suggested answers for each question. These MCQs are placed after the final diagnosis and the correct answers should be revealed after the references. The questions and take-home messages (learning points) are included in the total word count. The manuscripts that have passed an initial screening by the Editorial Board will be reviewed by two experts in the field.

- Word Count: 1,000 – 2,000 words (excluding references and figure or table legends)
- Abstract: Not required
- References: Maximum of 10
- Figures: Maximum of 2
- Tables: Maximum of 5

6. Technical Notes

The technical notes are brief descriptions of scientific techniques used in the anatomical pathology, clinical pathology (laboratory medicine), forensic medicine (legal medicine or medical jurisprudence), molecular medicine or pathobiology. The submitted manuscripts are usually peer-reviewed.

- Word Count: Maximum of 1,000 words (excluding references and figure or table legends)
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- References: Maximum of 5
- Figures or Tables: Maximum of 2

Organisation of Manuscripts

1. General Format

The manuscripts written in English language are preferable. However, Thai papers are also acceptable, but their title pages, abstracts, and keywords must contain both Thai and English. These English and Thai manuscripts are prepared in A4-sized Microsoft Word documents with leaving 2.54-cm (1-inch) margins on all sides. All documents are required to be aligned left and double-spaced throughout the entire manuscript. The text should

be typed in 12-point regular Times New Roman font for English manuscript and 16-point regular TH SarabunPSK font for Thai manuscript.

The running titles of English and Thai manuscripts are placed in the top left-hand corner of each page. They cannot exceed 50 characters, including spaces between words and punctuation. For the header of English paper, the running title will be typed in all capital letters. The page number goes on the top right-hand corner.

Footnotes are not used in the manuscripts, but parenthetical statements within text are applied instead and sparingly. Abbreviations should be defined at first mention and thereafter used consistently throughout the article. The standard abbreviations for units of measure must be used in conjunction with numbers.

All studies that involve human subjects should not mention subjects' identifying information (e.g. initials) unless the information is essential for scientific purposes and the patients (or parents or guardians) give written informed consent for publication.

2. Title Page

The title page is the first page of the manuscripts and must contain the following:

- The title of the paper (not more than 150 characters, including spaces between words)
- The full names, institutional addresses, and email addresses for all authors (If authors regard it as essential to indicate that two or more co-authors are equal in status, they may be identified by an asterisk symbol with the caption "These authors contributed equally to this work" immediately under the address list.)
- The name, surname, full postal address, telephone number, facsimile number, and email address of the corresponding author who will take primary responsibility for communication with AAP.
- Conflict of interest statement (If there are no conflicts of interest for any author, the following statement should be inserted: "The authors declare that they have no conflicts of interest with the contents of this article.")

3. Abstract

A structured form of abstract is used in all Original Article manuscripts and must include the following separate sections:

- Background: The main context of the study
- Objective: The main purpose of the study
- Materials and Methods: How the study was performed
- Results: The main findings
- Conclusions: Brief summary and potential implications

- *Keywords:* 3 – 5 words or phrases (listed in alphabetical order) representing the main content of the article

4. Introduction

The Introduction section should clearly explain the background to the study, its aims, a summary of the existing literature and why this study was necessary or its contribution to the field.

5. Materials and Methods

The Materials and Methods section must be described in sufficient detail to allow the experiments or data collection to be reproduced by others. Common routine methods that have been published in detail elsewhere should not be described in detail. They need only be described in outline with an appropriate reference to a full description. Authors should provide the names of the manufacturers and their locations for any specifically named medical equipment and instruments, and all chemicals and drugs should be identified by their systematic and pharmaceutical names, and by their trivial and trade names if relevant, respectively. Calculations and the statistical methods employed must be described in this section.

All studies involving animal or human subjects must abide by the rules of the appropriate Internal Review Board and the tenets of the recently revised Helsinki protocol. Hence, the manuscripts must include the name of the ethics committee that approved the study and the committee's reference number if appropriate.

6. Results

The Results section should concisely describe the findings of the study including, if appropriate, results of statistical analysis which must be presented either in the text or as tables and figures. It should follow a logical sequence. However, the description of results should not simply repeat the data that appear in tables and figures and, likewise, the same data should not be displayed in both tables and figures. Any chemical equations, structural formulas or mathematical equations should be placed between successive lines of text. The authors do not discuss the results or draw any conclusions in this section.

7. Discussion

The Discussion section should focus on the interpretation and the significance of the findings against the background of existing knowledge. The discussion should not repeat information in the results. The authors will clearly identify any aspects that are novel. In addition, there is the relation between the results and other work in the area.

8. Conclusion

The Conclusion section should state clearly the main summaries and provide an explanation of the importance and relevance of the study reported. The author will also describe some indication of the direction future research should take.

9. Acknowledgements

The Acknowledgements section should be any brief notes of thanks to the following:

- *Funding sources*
- *A person who provided purely technical help or writing assistance*
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Thanks to anonymous reviewers are not allowed. If you do not have anyone to acknowledge, please write “Not applicable” in this section.

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- *Journal article*
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- *Books*
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11. Tables

The tables should be self-contained and complement, but without duplication, information contained in the text. They should be numbered consecutively in Arabic numerals (Table 1, Table 2, etc.). Each table should be presented on a separate page with a comprehensive but concise legend above the table. The tables should be double-spaced and vertical lines should not be used to separate the columns. The column headings should be brief, with units of measurement in parentheses. All abbreviations should be defined in footnotes. The tables and their legends and footnotes should be understandable without reference to the text. The authors should ensure that the data in the tables are consistent with those cited in the relevant places in the text, totals add up correctly, and percentages have been calculated correctly.

12. Figure Legends

The legends should be self-explanatory and typed on a separate page titled “Figure Legends”. They should incorporate definitions of any symbols used and all abbreviations and units of measurement should be explained so that the figures and their legends are understandable without reference to the text.

If the tables or figures have been published before, the authors must obtain written permission to reproduce the materials in both print and electronic formats from the copyright owner and submit them with the manuscripts. These also follow for quotes, illustrations, and other materials taken from previously published works not in the public domain. The original resources should be cited in the figure captions or table footnotes.

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All illustrations (line drawings and photographs) are classified as figures. The figures should be numbered consecutively in Arabic numerals (Figure 1, Figure 2, etc.). They are submitted electronically along with the manuscripts. These figures should be referred to specifically in the text of the papers but should not be embedded within the text. The following information must be stated to each microscopic image: staining method, magnification (especially for electron micrograph), and numerical aperture of the objective lens. The authors are encouraged to use digital images (at least 300 d.p.i.) in .jpg or .tif formats. The use of three-dimensional histograms is strongly discouraged when the addition of these histograms gives no extra information.

14. Components

14.1. Letters to the Editor

The Letter to the Editor manuscripts consist of the following order:

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- *Conclusions*
- *Acknowledgements*
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- *Table (s)*
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- *Acknowledgements*
- *References*
- *Table (s)*
- *Figure Legend (s)*
- *Figure (s)*

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- *Figure Legend (s)*
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- *Take-Home Messages (Learning Points)*
- *Acknowledgements*
- *References*
- *Correct Answers to MCQs*
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- *Figure Legend (s)*
- *Figure (s)*

14.6. Technical Notes

The Technical Note manuscripts consist of the following order:

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- *Introduction*
- *Main text*
- *Conclusions*
- *Acknowledgements*
- *References*
- *Table (s)*
- *Figure Legend (s)*
- *Figure (s)*

Proofreading

The authors of the accepted manuscripts will receive proofs and are responsible for proofreading and checking the entire article, including tables, figures, and references. These authors should correct only typesetting errors at this stage and may be charged for extensive alterations. Page proofs must be returned within 48 hours to avoid delays in publication.

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In many cases, the authors will be invited to make revisions to their manuscripts. The revised manuscripts must generally be received by the Editorial Board within 3 months of the date on the decision letter or they will be considered a new submission. An extension can sometimes be negotiated with the Editorial Board.

APPENDIX 2

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APPENDIX 5

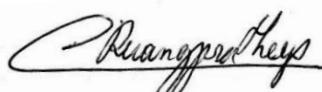
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