



RESEARCH ARTICLE

Post-mortem pulmonary tuberculosis: comparison of available diagnostic methods

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ABSTRACT

Tuberculosis (TB) caused by *Mycobacterium tuberculosis* remains a serious public health threat with the World Health Organisation (WHO) reporting 5.8 million cases and 1.3 million deaths in the year 2020 due to TB. TB can be diagnosed by imaging, histopathological and bacteriological methods with culture remaining the gold standard. This study was performed to look at the sensitivity and specificity of post-mortem computed tomography (PMCT) imaging when compared to culture in diagnosing pulmonary tuberculosis. This was a retrospective comparative study looking at post mortem cases where lung tissue samples sent for TB culture at Hospital Kuala Lumpur were compared against PMCT imaging. Exclusion criteria included contaminated samples, decomposed cases, immunocompromised subjects and those below 18 years of age. Subjects included 80 medico-legal autopsy cases at the National Institute of Forensic Medicine, Hospital Kuala Lumpur, Malaysia who had whole body PMCT done in accordance with the Institute's protocol and tissue samples sent for bacteriology culture for tuberculosis. PMCT findings were positively associated with acid-fast organisms in 23.5 out of 33 cases (71.2%). Our study also showed that PMCT had a sensitivity of 71.3% and specificity of 54.3% (95% CI: 39.5–68.4) in diagnosing TB based on the protocol set in this study. This study showed that there was relatively good agreement between radiological PMCT findings and bacterial culture, suggesting that radiological examination is a relatively reliable tool for preliminary screening and possible diagnosis of TB prior to a postmortem examination which would be beneficial in reducing the risk of transmission of TB to health workers during autopsy.

Keywords: Autopsy; pulmonary tuberculosis; post-mortem computed tomography; bacteriology culture; health workers.

INTRODUCTION

Tuberculosis (TB) today remains one of the world's most lethal infectious disease causing a serious public health threat and economic burden with escalating rates of drug resistance (Garg *et al.*, 2011; Ramos *et al.*, 2015). This disease is on the rise with the World Health Organization reporting 5.8 million cases with 1.3 million deaths in the year 2020 due to TB (Global Tuberculosis Report, 2021). In Malaysia, the incidence of TB was 92 per 100,000 population, with a mortality rate estimated at 4 cases per 100,000 population per year in 2019 (Avoi & Liaw, 2021). Identified high-risk groups include elderly men, immigrants from Asia and other countries of high prevalence as well as immunocompromised individuals (Chapman & Claydon, 1992).

TB is caused by a group of pathogens which form the *Mycobacterium tuberculosis* complex (MTC) and includes *M. bovis*, *M. tuberculosis*, *M. africanum*, *M. microti*, *M. canetti* and *M. caprae* (Neeraja *et al.*, 2014). They are risk/hazard Group III zoonotic

pathogens that spread to humans through inhalation or ingestion causing tuberculosis. Clinical signs and symptoms of an infected individual vary according to the organ involved; lung involvement may manifest as cough, dyspnea and other signs of low-grade pneumonia with enlarged lymph nodes in advanced cases, gastro intestinal involvement may manifest as abdominal pain and diarrhea while bone involvement may manifest as bone pains.

Although TB is on the rise, a large proportion of cases found at autopsy are unexpected which can be attributed to the low awareness of the possibility of this disease during the lifetime of a patient. Therefore, early diagnosis of TB is necessary for effective treatment. There are various methods available for the diagnosis of TB which include the use of mycobacterial culture, radiological imaging methods, bacteriological as well as histopathological techniques. Mycobacterial culture methods however have a long turn around-time, taking weeks to months. Histopathological techniques using microscopy and Ziehl-Neelsen (ZN) stain is a fast and inexpensive method to identify acid-fast bacilli (AFB).

However, efficacy is operator dependent, resulting in a broad range of sensitivities and specificities. This is when radiological imaging plays an important role in normal or inconclusive cases and the determination of disease activity and complication in addition to being fast.

Newer techniques include the use of rapid nucleic acid methodologies, such as polymerase chain reaction (PCR) and Interferon-Gamma Release Assays (IGRAs). PCR tests offer an alternative robust approach to detect *M. tuberculosis* showing rapid results with good diagnostic accuracy. However, PCR assays may be associated with false-positive and false-negative results. The benefits of IGRAs include the use of antigens that are largely specific for *M. tuberculosis* i.e., no cross-reactivity with BCG with nontuberculous mycobacteria. Limitations include cost and complicated interpretation due to conversions and reversions with inconsistent test reproducibility. (Sawyer *et al.*, 2007; Santos *et al.*, 2010; Achkar *et al.*, 2011; García-Basteiro *et al.*, 2016; Lu *et al.*, 2016; Lewinsohn *et al.*, 2017).

However, despite the numerous laboratory and imaging methods available, diagnosis of TB can sometimes be difficult or missed due to various reasons; possible concurrent conditions masking this disease, difficulty in culturing this slow-growing organism in the laboratory, unidentified reactivation and even the fact that patients might have encountered economic or socio-cultural barriers to accessing health care in the first place (Rastogi *et al.*, 2011; Osman *et al.*, 2021).

The diagnosis of TB during or prior to an autopsy is equally important as during life as undiagnosed tuberculosis can be a health hazard to healthcare workers. Mortuary staff face a risk of being exposed to infectious diseases at autopsy from penetrating sharp injuries, droplet inhalation, ingestion, direct inoculation, through skin breaks or through mucous membranes of the eyes, nose and mouth (Stephenson & Byard, 2019).

It is of utmost importance for the diagnosis or suspicion of TB to be raised prior to an autopsy. This could help forensic pathologists plan the autopsy beforehand, thus reducing the risk of infection to mortuary health care workers. Nevertheless, there is a scarcity of reports looking at the comparison of Post-mortem Computed Tomography (PMCT) and bacteriological culture in the diagnosis of TB. This study aims to calculate the sensitivity and specificity of PMCT imaging done prior to an autopsy in diagnosing pulmonary tuberculosis when compared to bacteriological culture.

MATERIALS AND METHODS

This was a retrospective comparative study conducted within local guidelines and registered with the National Medical Research Registry [NMRR-19-2028-47754]. Subjects included 80 medico-legal autopsy cases at the National Institute of Forensic Medicine, Hospital Kuala Lumpur, Malaysia who had whole body PMCT done in accordance with the Institute's protocol and tissue samples sent for bacteriology tuberculosis culture. Samples that were contaminated or required repeat culture, decomposed subjects, immunocompromised subjects including HIV patients and subjects below 18 years of age were excluded from this study.

Post-mortem computed tomography (PMCT) scan

An unenhanced PMCT scan was performed at the Institute prior to any manipulation of the body using a 64-slice (Toshiba Aquilion 64 TSX-101A, Japan) multi-detector computed tomography (MDCT) scanner, a machine wholly dedicated for autopsy use. Examinations were performed in a cranio-caudal direction from head to toe using 1.0 mm slice thickness for the head region and 2.0 mm slice thickness for the thorax, abdomen and pelvis down to the toes. Scans were performed using pre-defined scanning protocols: 120 kVp, 250 mA, FOV 500 (LL), 1.0 x 32 raw detector collimation and 0.844/standard pitch.

Image analysis of DICOM PMCT images was performed using INFINTT Healthcare Monitor (PACS version 3091, Korea) via multiplanar reconstructions (MPR) by two radiologists who were blinded to the pathological findings. One of the radiologists had forensic imaging experience of more than 10 years, while the other was a specialist in respiratory imaging.

Imaging findings suggestive of active TB included acinar or air-space nodules, centrilobular nodules, or clustered nodules, thick-walled cavity with surrounding consolidation, air fluid levels in tuberculous cavities, enlarged ipsilateral or mediastinal lymph nodes with central necrosis, conglomeration of lymph nodes as well as effusion or emphysema (Bhalla *et al.*, 2015) (Figures 1 and 2).

Mycobacterial culture

Lung tissue samples collected during autopsy were decontaminated and concentrated prior to inoculation onto the egg-based Ogawa culture medium and then subjected to incubation at 37°C. The cultures were examined and results recorded weekly, for 8 intervals. *Mycobacterium tuberculosis* usually grows as a buff-colored, dry colony, which would be very distinctive under normal room lighting observation (Global Laboratory Initiative Working Group, 2014).



Figure 1. Axial CT scan image of the thorax in mediastinal window showing enlarged mediastinal lymph nodes in the right paratracheal and pre-tracheal regions (red arrow).

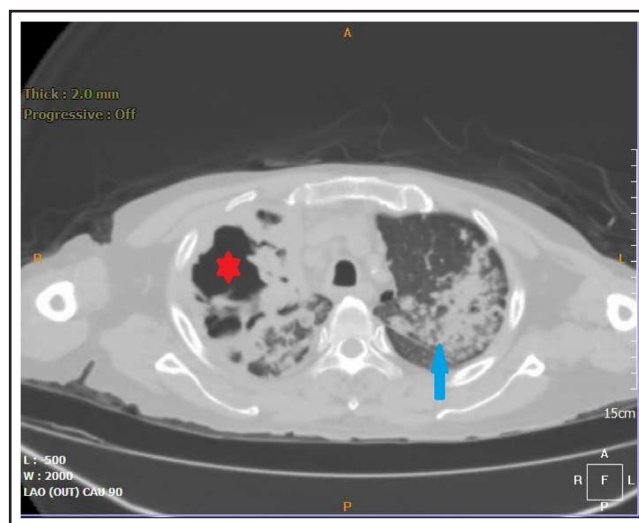


Figure 2. Axial CT scan thorax in lung window showing a thick-walled cavity (red star) and multiple air space nodules (blue arrow).

Table 1. Comparison between the reporting by Radiologist 1 and 2 with the TB culture

		Culture		Total
		Yes	No	
Radiologist 1	Yes	22	17	39
	No	11	30	41
	Total	33	47	80
Radiologist 2	Yes	25	26	51
	No	8	21	29
	Total	33	47	80

RESULTS

Postmortem CT imaging diagnostic findings were compared with the reference “gold standard” of mycobacterial culture and identification. Statistical analysis was performed using Excel 2013 (Microsoft, Redmond, Washington, USA) and Statistical Package for Social Sciences 24.0 (SPSS, Chicago, Illinois, USA) software. The sensitivity, specificity, positive predictive values (PPV) and negative predictive values (NPV) with 95% exact confidence intervals were calculated. Statistical analysis was also used for comparison of proportions using chi-square (χ^2) test and a test of agreement (kappa) with a confidence level of 95% and a p value < 0.05 considered significant.

This study was conducted to evaluate the accuracy of radiological PMCT findings as compared to mycobacterial culture results from known lung tissue samples sent after an autopsy. Inter-observer agreement between the two assigned senior radiologists was analysed in this study so as to avoid any bias incurred. True positive agreement was high at 93.6% (95% CI: 81.4–98.3), while the true negative agreement was at 65.1% (95% CI: 49.0–78.5) with a kappa value of 0.594 ± 0.08 (95% CI: 0.433–0.756).

PMCT findings were positively associated with acid-fast organisms in 23.5 out of 33 cases (71.2%). The average sensitivity and specificity were calculated taking into account the findings from both radiologists. PMCT diagnostic method demonstrated a relatively high average sensitivity at 71.3% (95% CI: 52.7–84.9) but a relatively low average specificity at 54.3% (95% CI: 39.5–68.4) based on the protocol set in this study (Table 1). This study also showed relatively low positive predictive values (PPV) of 52.7% (95% CI: 37.4–67.5) and relatively high negative predictive values (NPV) of 72.8% (95% CI: 54.7–85.9).

DISCUSSION

Tuberculosis (TB) remains a global emergency and is the second commonest infectious disease worldwide causing death, thus early diagnosis of TB is crucial to avoid transmission of *Mycobacterium tuberculosis* (Global Tuberculosis Report, 2021). Both in the living and after death, TB is diagnosed using radiological imaging, as well as bacteriological and histopathological techniques. Rapid nucleic acid methodologies, such as polymerase chain reaction (PCR), may also be used when appropriately validated (Sawyer *et al.*, 2007; García-Basteiro *et al.*, 2016). However, traditional mycobacterial culture remains the gold standard method for routine confirmation of infection.

There is a study looking at the performance of the various diagnostic methods available whereby six different diagnostic methods for TB were evaluated in parallel using 167 samples. Compared to bacteriological culture, estimates of sensitivity for histopathology was 77.8%, gross pathology 72.2%, PCR for MPB70 gene 66.7%, detection of acid-fast bacilli (AFB) in tissue contacts

smears 55.6% and in histopathology slides 16.7% (estimated specificity was 96.7%, 100%, 100%, 94.4% and 100%, respectively). Combining gross pathology with stained smears in parallel increased estimated sensitivity to 94.4% (94.4% specificity). Four probable bacteriological culture false-negative samples were identified by Discriminant Function Analysis. Recalculating the parameters considering these samples as infected generated estimated values for sensitivity of bacteriology and histopathology at 81.8%, gross pathology 72.7%, PCR for MPB70 gene 63.6%, detection of AFB in tissue contacts smears 54.5% and in histopathology slides 13.6% (estimated specificity was 100% for gross pathology, PCR, bacteriology and detection of AFB in histopathology slides, 96.7% for histopathology and 94.4% for stained smears) (Santos *et al.*, 2010). However, culture methods have a long turnaround-time, taking up to two months and molecular methods have high cost and technology requirements. Since culture results need several weeks, this frequently causes delays in isolating infectious patients. Thus, imaging plays an important role in the early detection and evaluation of TB (Bhalla *et al.*, 2015).

A study was also initiated during a TB outbreak, whereby both chest digital radiography (CDR) and CT were taken of close contacts (n = 35 cases) for screening purposes. The chi-square test and ROC curve were used to evaluate and calculate the missed pulmonary lesions from CDR and the nature of these lesions. Xiwei Lu's standards were used to classify pulmonary tuberculosis signs, which were divided into the type of cavity with spread, consolidation, nodules, and interstitial lesions. Abnormal shadow detection rates of chest CDR and CT were 22.9% (8/35) and 40% (14/35) respectively, the difference was statistically significant ($\chi^2 = 16.154$, $P < 0.01$) (Lu *et al.*, 2016).

Another study proved that both high-resolution CT and Magnetic Resonance Imaging (MRI) correctly diagnosed 50 patients with culture-proven pulmonary tuberculosis and identified pulmonary abnormalities in all patients. There were no significant differences between the two techniques in terms of identifying the location and distribution of the lung lesions, though the higher resolution of MRI did allow for better identification of parenchymal inhomogeneity, caseous, and pleural or nodal involvement (Busi Rizzi *et al.*, 2011). In comparative to CT findings, the accuracies of low-radiation dose digital tomosynthesis (DTS) and radiography in depicting mycobacterial disease were 97% and 89%, respectively, for observer 1 ($P = 0.039$) and 99% and 93%, respectively, for observer 2 ($P = 0.031$) (Kim *et al.*, 2010). In a separate study, overall sensitivity of thoracic CT scan and chest X-ray were 78.9% and 63.2%, respectively which show no significant difference. CT scan was observed to detect lymphadenopathy, nodule/nodular infiltration, collapse and pleural effusion or thickening significantly better than chest X-ray (Bolursaz *et al.*, 2015).

The use of imaging especially PMCT in clinical and academic practice has increased throughout the world, and it is now globally accepted as a means of providing valuable information to ascertain the cause of death and as an adjunct to autopsy. This is because in addition to its ability to provide the cause of death, PMCT also alerts pathologists to possible dangerous communicable infections such as tuberculosis, allowing them to take adequate protective measures during autopsy (Thali *et al.*, 2003; Roberts *et al.*, 2012; McLaughlin *et al.*, 2016).

In our study of 80 cases, there was a total of 33 cases with TB culture positive results while 47 cases had TB culture negative results. PMCT findings were positively associated with acid-fast organisms in 23.5 out of 33 cases (71.2%). Our study also showed that PMCT had a sensitivity of 71.3% and specificity of 54.3% (95% CI: 39.5–68.4) in diagnosing TB based on the protocol set in this study. About 22 cases (66.7%) of the TB culture positive cases were specifically certified as tuberculosis related death as the final cause of death. The other eight cases were certified as general lung

infection or sepsis and the remaining three cases due to ruptured ectopic pregnancy, Hodgkin Lymphoma and coronary artery embolism while four out of the 47 cases with TB culture negative results cases were certified as TB related death. This showed that TB culture has a high agreement with the final cause of death which concurs with literature that TB culture is the gold standard method for diagnosis of pulmonary tuberculosis.

This study investigated the sensitivity and specificity of PMCT in diagnosing TB taking culture as the gold standard. This study did not look at the sensitivity and specificity of bacteriology culture. The gold standard for TB diagnosis is either isolation of *M. tuberculosis* by culture or detection of *M. tuberculosis* specific nucleic acids by molecular methods (Achkar *et al.*, 2011).

Nevertheless, there are some limitations for the TB culture method as it is totally dependent on the sampling methods by the medical officers who perform the postmortem, specimen storage protocol as well as the transporting medium used for the tissue samples prior to the reception at the laboratory. All these factors may affect the TB culture results which may be misinterpreted as negative for pulmonary tuberculosis.

Similarly, there are certain features that may not be specific for TB on radiological imaging and differential diagnosis of non-tubercular infections, non-infectious diseases e.g., sarcoidosis and serositis or even malignancies like lymphoma and carcinoma can present with cavitating lung lesions and hilar lymphadenopathy.

This study however showed that there was relatively good agreement between radiological CT findings and bacterial culture, suggesting that radiological examination is a relatively reliable tool for preliminary screening and possible diagnosis of TB prior to a postmortem examination as histopathological examination takes a couple of days and mycobacterial culture a couple of weeks before results are available.

Based on its sensitivity and specificity, PMCT would be beneficial in reducing the risk of transmission of TB to health workers during autopsy. This is because when compared to laboratory diagnostic tests that may take a longer time, PMCT findings could trigger the possibility of such a contagious disease much earlier than other mentioned methods. Thus the health inspector can be notified earlier based on the suspicion of TB raised from PMCT and autopsy findings while awaiting the mycobacterial culture results, This will allow the next of kin and close contact persons to be screened for pulmonary TB much earlier and help reduce the spread of tuberculosis in the community.

Diagnosis of incidental TB in community deaths reflects the burden of disease within the community and while public health efforts worldwide are being directed towards reducing the spread of TB, forensic autopsy is a vital tool in detecting unknown TB cases in the community. Forensic autopsy data are thus indispensable for disease intelligence and prevention as these data can help public health map disease surveillance systems, and implement public awareness programs for prevention (Mucheleng'anga *et al.*, 2022).

CONCLUSION

PMCT is beneficial and should be used as a standard protective measure for the early detection of potentially infective cases to prevent inadvertent exposure as shown by the relatively high average sensitivity and specificity when compared to the gold standard of mycobacterial culture. Although radiologists tend to over diagnose pulmonary tuberculosis, this may be beneficial in triggering more precautions to be taken by the forensic pathology team. The use of PMCT can also be replicated for other highly infectious diseases like the COVID-19 infection as done at our Institute.

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Conflict of Interests

This research did not receive any funding. The authors of this manuscript declare that there is no conflict of interest that could potentially be construed to affect the material contained in the manuscript that is being submitted to this Journal.

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