Short Report: Prevalence of Melioidosis in Patients with Suspected Pulmonary Tuberculosis and Sputum Smear Negative for Acid-Fast Bacilli in Northeast Thailand

Pornpan Suntornsut, Kriangsak Kasemsupat, Santi Silairatana, Gumphol Wongsuvan, Yaowaruk Jutrakul, Vanaporn Wuthiekanun, Nicholas P. J. Day, Sharon J. Peacock, and Direk Limmathurotsakul*

Mahidol–Oxford Tropical Medicine Research Unit, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand; Department of Medicine, Udon Thani Hospital, Udon Thani, Thailand; Department of Medical Technology, Udon Thani Hospital, Udon Thani, Thailand; Center for Clinical Vaccinology and Tropical Medicine, Nuffield Department of Clinical Medicine, University of Oxford, Churchill Hospital, Oxford, United Kingdom; Department of Medicine, Cambridge University, Addenbrooke's Hospital, Cambridge, United Kingdom; Department of Microbiology and Immunology, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand; Department of Tropical Hygiene, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand.

Abstract. The clinical and radiological features of pulmonary melioidosis can mimic tuberculosis. We prospectively evaluated 118 patients with suspected pulmonary tuberculosis who were acid-fast bacilli (AFB) smear negative at Udon Thani Hospital, northeast Thailand. Culture of residual sputum from AFB testing was positive for Burkholderia pseudomallei in three patients (2.5%; 95% confidence interval [CI] 0.5–7.3%). We propose that in melioidosis-endemic areas, residual sputum from AFB testing should be routinely cultured for B. pseudomallei.

Melioidosis is a serious infectious disease caused by the Tier 1 Select Agent and Gram-negative bacillus, Burkholderia pseudomallei. Naturally acquired melioidosis is highly endemic in northeast Thailand where it is the third most common cause of death caused by infectious diseases after human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS) and tuberculosis, and in northern Australia where it is the commonest cause of fatal community-acquired bacteremic pneumonia. Melioidosis is also increasingly reported from many countries across Asia, regions of South America, various Pacific and Indian Ocean islands, and some countries in Africa including Nigeria, Gambia, Kenya, and Uganda. Death from melioidosis reaches 80% in those who are not treated with effective antimicrobial drugs.

Melioidosis can manifest with a variety of clinical presentations including sepsis, pneumonia, arthritis, and internal organ abscesses, and has been termed “the great mimiccer” because it can be confused with a range of diseases. The most notable example is tuberculosis, with an estimated 10% of melioidosis patients presenting with chronic respiratory symptoms and chest radiography mimicking pulmonary tuberculosis. In reported cases, failure of clinical improvement after the administration of anti-tuberculosis drugs led to bacteriological culture of sputum, broncho-alveolar lavage, or blood, and the detection of B. pseudomallei. Although it is clear that melioidosis can mimic clinical features of tuberculosis, patients presenting with suspected tuberculosis in Thailand where melioidosis is highly endemic are not systematically screened for melioidosis. Here, we evaluated the use of culturing sputum samples taken from individuals in Thailand with suspected tuberculosis that were smear negative for acid-fast bacilli (AFB) to detect B. pseudomallei.

THE STUDY

A cross-sectional study was conducted at Udon Thani Hospital, Udon Thani, northeast Thailand, between August 2012 and February 2013. Inclusion criteria were adult patients (≥ 18 years of age) with suspected pulmonary tuberculosis who had three consecutive sputum samples taken that were AFB smear negative. Cases were initially identified during daily visits to the routine diagnostic microbiology laboratory. We excluded patients with confirmed pulmonary tuberculosis based on a positive AFB smear or sputum culture positive for Mycobacterium tuberculosis. Culture for M. tuberculosis was performed by the National Health Security Office (NHSO), Thailand, using both solid and liquid culture medium, according to standard procedures. Sputum culture for M. tuberculosis is recommended by NHSO for the following: 1) patients with suspected multidrug-resistant tuberculosis; including suspected cases who are household contacts of multidrug-resistant tuberculosis cases, individuals with HIV/AIDS, and prisoners, 2) patients with pulmonary tuberculosis who have been treated for 3 months but remain sputum AFB smear positive, and 3) those who relapse with pulmonary tuberculosis, or whose treatment is interrupted for 2 or more months. All participants were enrolled after they gave written informed consent.

In this study, residual sputum from AFB testing was cultured to detect the presence of B. pseudomallei as described previously. In brief, all available residual sputum specimens were collected and inoculated into 7 mL of modified Ashdown broth (containing 10 g/L of typtone soya broth, 40 mL/L of glycerol, 5 mL/L of 0.1% crystal violet, and 50 mg/L of colistin). These were incubated at 37°C in air for 48 hours, after which 10 μL of the upper layer was streaked onto Ashdown agar. Plates were then incubated at 37°C in air and examined daily for 4 days, and colonies of presumptive B. pseudomallei identified as described previously. Patients with a sputum culture positive for B. pseudomallei were contacted and appropriate treatment of melioidosis initiated as soon as possible. This study was approved by the ethical committees of Udon Thani Hospital, and the Faculty of Tropical Medicine, Mahidol University, Thailand.

A total of 118 patients were enrolled, from whom 278 sputum samples were cultured for B. pseudomallei. Three, two, and one sputum specimens were available for culture from 68, 24, and 26 patients. Overall, 77 (65.3%) were male, and the median age was 56 years (interquartile range [IQR] 46–66 years,
None of the sputum cultures were positive for \textit{Burkholderia pseudomallei} presenting in northeast Thailand (43%). We propose that in melioidosis-endemic areas, residual sputum from AFB testing can provide the correct diagnosis and institution of appropriate antimicrobial therapy for patients with pulmonary melioidosis. Early diagnosis and treatment can reduce the risk of death caused by melioidosis, and avoid unnecessary treatment with anti-tuberculosis drugs that can lead to adverse drug reactions. The case fatality rate in these three patients (33%) is comparable with the overall case fatality rate in melioidosis patients presenting in northeast Thailand (43%). \textsuperscript{2} We propose that in melioidosis-endemic areas, residual sputum from AFB testing should be routinely cultured for \textit{B. pseudomallei}.

Of the 118 patients, 3 (2.5%; 95% confidence interval [CI] 0.5–7.3\%) were sputum culture positive for \textit{B. pseudomallei}. None of the sputum cultures were positive for \textit{M. tuberculosis}. A clinical description of each melioidosis case is provided in Table 1. Cases 1 and 2 had a rapid clinical deterioration after enrollment, and were admitted to the hospital where they were treated with parenteral ceftazidime. Clinical review of case 3 led to the decision that parenteral treatment of acute melioidosis was unnecessary, and oral treatment of melioidosis was prescribed. Patient 1 died, whereas the other two cases completed melioidosis treatment and recovered (Table 1).

The study has a number of limitations. Not all sputum specimens for AFB smear were available for our study because some samples were completely used for mycobacterial culture, and some were accidentally discarded. Furthermore, we did not evaluate the prevalence of melioidosis in tuberculosis patients who were smear positive. Nonetheless, this is the first study to prospectively evaluate the prevalence of pulmonary melioidosis in patients with suspected pulmonary tuberculosis who are sputum AFB smear negative in Thailand. Our estimated prevalence is much higher than that observed in Port Moresby, Papua New Guinea, in which only 1 of 529 patients who were smear positive. Nonetheless, this is the first study to prospectively evaluate the prevalence of pulmonary melioidosis in patients with suspected pulmonary tuberculosis who are sputum AFB smear negative in Thailand. Our estimated prevalence is much higher than that observed in Port Moresby, Papua New Guinea, in which only 1 of 529 patients with suspected pulmonary tuberculosis had sputum culture positive for \textit{B. pseudomallei}\textsuperscript{12}; the prevalence reported here probably represents a minimum estimate in Thailand because the sensitivity of \textit{B. pseudomallei} culture is less than perfect. \textsuperscript{13}

Overall, we show that culture for \textit{B. pseudomallei} using residual sputum from AFB testing can provide the correct diagnosis and institution of appropriate antimicrobial therapy for patients with pulmonary melioidosis mimicking tuberculosis. Early diagnosis and treatment can reduce the risk of death caused by melioidosis, and avoid unnecessary treatment with anti-tuberculosis drugs that can lead to adverse drug reactions. The case fatality rate in these three patients (33\%) is comparable with the overall case fatality rate in melioidosis patients presenting in northeast Thailand (43\%). \textsuperscript{2} We propose that in melioidosis-endemic areas, residual sputum from AFB testing should be routinely cultured for \textit{B. pseudomallei}.

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**Table 1** Details of three patients with sputum culture positive for \textit{Burkholderia pseudomallei}

<table>
<thead>
<tr>
<th>Gender and age</th>
<th>Underlying disease</th>
<th>Presenting symptoms</th>
<th>Chest x-ray*</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>M 70yr</td>
<td>Diabetes and hypertension</td>
<td>Cough and fever for 30 days</td>
<td>Infiltration in RUL and cavity in LUL</td>
<td>Two days after enrollment, developed high-grade fever and death. Admitted to the hospital and treated with parenteral ceftazidime. Died 5 days after admission. Probable cause of death was multiple organ failure caused by melioidosis.</td>
</tr>
<tr>
<td>F 55yr</td>
<td>Diabetes</td>
<td>Cough for 14 days and fever with dyspnea for 1 day</td>
<td>Infiltration in RUL</td>
<td>One day after enrollment, developed high fever and severe dyspnea. Admitted to hospital and treated with parenteral ceftazidime. Died 5 days after admission. Probable cause of death was multiple organ failure caused by melioidosis.</td>
</tr>
<tr>
<td>M 34yr</td>
<td>None</td>
<td>Cough for 30 days and fever for 7 days</td>
<td>Moderately thickened wall cavity with subjacent infiltration in RUL</td>
<td>No signs of clinical deterioration in review. Received 20 weeks of oral eradicative treatment and made a complete recovery.</td>
</tr>
</tbody>
</table>

*RUL = right upper lobe; LUL = left upper lobe.

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Authors’ addresses: Pornpan Suntornsut, Gumphol Wongsuvan, Vanaporn Wuthiekanun, and Direk Limmathurotsakul, Faculty of Tropical Medicine, Mahidol University - Mahidol–Oxford Tropical Medicine Research Unit, Bangkok, Thailand, E-mails: pornpan@tropmedres.ac, gumphol@tropmedres.ac, lek@tropmedres.ac, and direk@tropmedres.ac. Kriangsak Kasemsupat, Santi Silairatana, and Yaowaruk Jutrakul, Udon Thani Hospital - Department of Medicine, Udon Thani, Thailand, E-mails: kriangsak_kp@hotmail.com, snaultuiz@gmail.com, and yaowa_jul@yahoo.com. Nicholas P. J. Day, University of Oxford - Clinical Medicine, Bangkok, Thailand, E-mail: nickd@tropmedres.ac. Sharon J. Peacock, University of Cambridge, Department of Medicine, Addenbrookes Hospital, Cambridge, UK, E-mails: sharon@tropmedres.ac or sp10@sanger.ac.uk.

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