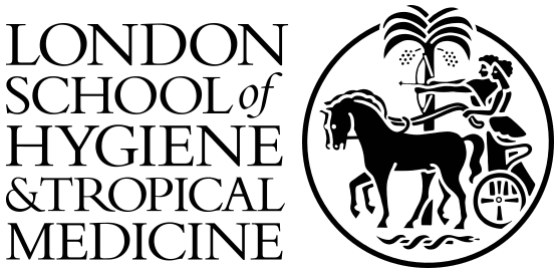

Cheng, Allen C; Currie, Bart J; Dance, David AB; Funnell, Simon GP; Limmathurotsakul, Direk; Simpson, Andrew JH; Peacock, Sharon J; (2013) Clinical Definitions of Melioidosis. AMERICAN JOURNAL OF TROPICAL MEDICINE AND HYGIENE, 88 (3). pp. 411-413. ISSN 0002-9637 DOI: https://doi.org/10.4269/ajtmh.12-0555

Am. J. Trop. Med. Hyg., 88(3), 2013, pp. 411–413
doi:10.4269/ajtmh.12-0555

Perspective Piece

Clinical Definitions of Melioidosis

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Abstract. Clinical definitions of melioidosis and inhalation-acquired melioidosis (*Burkholderia pseudomallei* infection) are described together with the evidence used to develop these definitions. Such definitions support accurate public health reporting, preparedness planning for deliberate *B. pseudomallei* release, design of experimental models, and categorization of naturally acquired melioidosis.

INTRODUCTION

The purpose of this article is to provide case definitions of melioidosis (*Burkholderia pseudomallei* infection) and describe the evidence on which these are based. There are two definitions that reflect the purpose for which they may be used. The first is a simple case definition for use by clinicians, public health reporting, and epidemiological and clinical studies. The second is a clinical definition of inhalational melioidosis, which has value for preparedness planning for deliberate *B. pseudomallei* release, design of experimental models, and categorization of routes of infection for naturally acquired melioidosis.

CASE DEFINITIONS

Definition of melioidosis. Reaching a definite diagnosis of melioidosis is very straightforward when one or more clinical specimens are culture-positive for *B. pseudomallei*. This organism is not thought to be a member of the normal microbiota,[1] and detecting even a single colony of *B. pseudomallei* in any specimen taken from a patient with clinical features consistent with an infective process should be interpreted as a clinically significant result. Culture is imperfect, however, with one study reporting an estimated diagnostic sensitivity of around 60%.[2] Only culture-proven cases are collected by national reporting systems, but having some guidance in place to support the diagnostic process for patients with suspected melioidosis who are culture-negative is of considerable clinical importance. Criteria are proposed for this patient group in Table 1, which is an adaptation of criteria reported elsewhere.[2] Evidence of exposure to *B. pseudomallei* (residence or past travel to endemic area, known outbreak, or laboratory accident) and the presence of major risk factors for melioidosis (diabetes mellitus, chronic renal disease, hazardous alcohol use, chronic pulmonary disease, thalassemia, steroid therapy, and malignancy) should be considered alongside these criteria to increase or decrease the weight of clinical suspicion. Serological evidence of exposure is not included in this definition, because background seropositivity is high in endemic regions and the available serological tests are poorly standardized.[3,4] However, serological testing may be more specific in returned travelers and after laboratory exposures outside the endemic area.[5] Polymerase chain reaction (PCR) is being increasingly used for confirmation of identity of bacterial cultures, but assays using detection of nucleic acid directly from clinical specimens are not sufficiently validated for routine use. Problems that need to be overcome include better DNA extraction procedures from blood, serum, or tissue matrices to increase PCR sensitivity of blood cultures[6–8] and careful selection of probe targets so that they are specific enough to detect all strains of *B. pseudomallei* but not so well-conserved that they detect *B. mallei* or other *Burkholderia* species.[9] In the event of a deliberate release of *B. pseudomallei*, confirmation based on a culture-confirmed diagnosis would provide definitive evidence of case burden, but the same problems of imperfect sensitivity of culture and PCR assays are likely to prevail.

Definition of inhalational melioidosis. The purpose of this diagnostic subcategory is to provide a more focused definition for biothreat-related research and assist those organizations who develop guidelines on emergency response after a deliberate release. This definition has five criteria, all of which must be met.

(1) Development of respiratory symptoms (e.g., cough, breathlessness, pleuritic chest pain) in the preceding 4 weeks.
(2) Presence of sepsis, defined as two or more of: body temperature below 36°C or above 38°C, heart rate greater than 90 beats/minute, respiratory rate greater than 20 breaths/minute, and white cell count of less than 4×10^9 or greater than 12×10^9 cells/L or more than 10% band forms (immature white blood cells).
(3) Evidence of alveolar infiltrate on chest radiograph within 48 hours of admission. In the event that there are previous radiographic records available for the individual, the infiltrate should be new (not present on previous radiographs).
(4) No evidence of percutaneous inoculation injury in an appropriate setting (contaminated soil, mud, pooled surface water in endemic area, or needle stick injury with pure culture) and evidence of opportunity for inhalational

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TABLE 1
Criteria for the diagnosis of naturally acquired melioidosis

	Definition
Definite melioidosis	One or more clinical samples culture-positive for *B. pseudomallei*
Probable melioidosis	Evidence of one or more abscesses that would be consistent with a diagnosis of melioidosis* but culture not performed or negative for *B. pseudomallei*, or culture negative for *B. pseudomallei* on first presentation but represented to hospital within 1 month with culture-proven melioidosis
Possible melioidosis	Clinically suspected melioidosis improved after treatment with an effective antimicrobial regimen for melioidosis (ceftazidime/carbapenem drug/amoxicillin-clavulanate) or clinically suspected melioidosis but the patient died before improvement was observed
Not melioidosis	Definite alternative diagnosis for manifestations leading to suspected melioidosis or resolution of clinical features of suspected melioidosis without treatment with antimicrobial drugs with activity against *B. pseudomallei*

* Evidence of splenic and/or hepatic abscesses with appearance on ultrasound characteristic for melioidosis (Swiss cheese appearance or small dispersed abscesses) or parotid or prostatic abscess in a melioidosis-endemic region where *B. pseudomallei* is the most probable cause.

exposure (e.g., recent severe weather event, known aspiration of surface water, or known exposure to aerosolized *B. pseudomallei*).

(5) Isolation of *B. pseudomallei* from any sterile or nonsterile body site.

Evidence for each of the criteria. *Criterion 1.* Melioidosis pneumonia, as with other bacterial pneumonia, usually presents with acute respiratory symptoms. Although subacute presentations similar to tuberculosis are well-described, chronic melioidosis is found in < 15% of cases.[10]

Criterion 2. Sepsis syndrome is an adverse systemic response to an infection that includes fever, rapid heart rate and respiratory rate, low blood pressure, and abnormal white blood cell count. Severe sepsis, often used as an inclusion criterion in clinical trials,[11] is the presence of sepsis with sepsis-related organ dysfunction, and septic shock is defined as the presence of sepsis with sepsis-related organ dysfunction and persistent hypotension unresponsive to fluid resuscitation. Sepsis syndrome is a common but not universal manifestation of melioidosis in humans; chronic melioidosis, in particular, may not be associated with significant systemic inflammation. In an unpublished study based in Thailand, sepsis criteria were present in 90% of patients with melioidosis and 93% of patients that died with melioidosis (Cheng AC, unpublished data). In a prospective melioidosis study in Darwin, Australia, 116 of 540 (21%) patients with melioidosis had septic shock on presentation, and 88 (76%) of these patients presented with pneumonia and septic shock.[12] In a marmoset model of inhalational melioidosis, fever, leucocytosis, and abnormal liver function were present within 24 hours of exposure to aerosolized *B. pseudomallei*, with asthenia and dyspnea most pronounced by 48 hours.[13] With lower inoculating doses (< 10 cfu), the time to clinical symptoms was slightly longer. The clinical definition for inhalational melioidosis proposed herein uses the widely accepted Systemic Inflammatory Response Syndrome (SIRS) consensus criteria to identify the presence of sepsis.[14]

Criterion 3. Pneumonia is the most common presenting feature of human melioidosis. Given the route of infection, the majority of cases with inhalational melioidosis might reasonably be expected to present with pneumonia and clinical and radiological signs of pulmonary involvement. Nonetheless, in a minority of cases, inhalation of *B. pseudomallei* may present with sepsis without radiological evidence of pulmonary consolidation. Thus, including the criterion for pulmonary consolidation in the clinical definition of inhalational melioidosis is likely to result in a higher specificity but lower sensitivity of the definition. The time to appearance of signs of pulmonary involvement has also been considered in the clinical definition. In a primate model of inhalational melioidosis, the highest bacterial densities were seen in the lungs at 22 hours after exposure (10^6 organisms/gram tissue), with multifocal necrotizing pneumonia evident at this time.[13] It is also well-recognized that in patients with pneumonia, abnormal findings may not be seen on the initial chest X-ray. Therefore, extending the time for radiological abnormalities to 48 hours after admission will improve the sensitivity of the clinical definition of inhalational melioidosis.

Criterion 4. The proportion of naturally acquired melioidosis cases in which the route of infection is inhalation (or aspiration) compared with percutaneous inoculation or ingestion remains entirely unclear.[15] Indeed, it has not definitively been shown that humans may acquire melioidosis naturally by the inhalation of aerosols, although cases have been described after near drowning, during which contaminated water was aspirated directly into the lungs and may also have been swallowed. The observation that helicopter crews seemed to be at increased risk of melioidosis during the Vietnam war has prompted a hypothesis that inhalation of contaminated dust may be a route of acquisition of melioidosis.[16] Pneumonia is more common in the monsoonal season, with severity correlating to rainfall in the previous 14 days.[17] A recent case-control study in Thailand found that exposure to rainfall was associated with melioidosis in residents of endemic areas (Limmathurotsakul D, personal communication). Small animal studies have found higher virulence when *B. pseudomallei* was administered by inhalation compared with the percutaneous route.[18,19] More recent work in a marmoset model found that inhalation of < 10 cfu *B. pseudomallei* was associated with lethal infection.[13]

Criterion 5. Inclusion of culture positivity in the definition of definite melioidosis is consistent with best clinical practice, but the available evidence does not provide guidance on whether microbiological culture relating specifically to the definition of inhalational melioidosis should be restricted to respiratory secretions or broadened to other sample types. *B. pseudomallei* may rapidly become disseminated, and the site of culture positivity may not reflect the clinical manifestations of disease. Furthermore, patients with melioidosis who have respiratory manifestations may not have culture-positive respiratory secretions. A study conducted in northeast Thailand of over 700 patients with culture-proven melioidosis who had at least one sputum culture performed reported that two-thirds of patients with radiological abnormalities had a positive sputum culture but that one-third had

a sputum culture that was negative.[20] Possible explanations include poor-quality sputum leading to a false-negative sample, a radiological abnormality associated with miliary spread (akin to miliary tuberculosis), and radiological changes caused by acute respiratory distress syndrome. In the same study, one-half of patients with a negative sputum culture but radiological changes had a throat swab taken, which was positive in 34% of cases.[20] This result provides evidence that sputum culture may be falsely negative. Limiting culture positivity to respiratory secretions in the clinical definition of inhalational melioidosis would increase specificity and may be suitable for the design of experimental models and studies that define the relationship between infection route and clinical manifestations of melioidosis. In the event of a deliberate release associated with inhalation of *B. pseudomallei*, it is likely that culture of the respiratory tract would be positive, but the knowledge that false-negative sputum culture could occur has influenced the decision to extend the definition to culture positivity of any site in this situation. Early inhalational melioidosis may not be associated with sputum production, and throat swabs should be taken in all suspected cases together with blood and urine cultures.

Received September 6, 2012. Accepted for publication December 8, 2012.

Acknowledgments: D.A.B.D., D.L., and S.J.P. are supported by the Wellcome Trust. S.J.P. is also supported by the National Institute for Health Research Cambridge Biomedical Research Centre.

Financial support: This work was supported by the Health Protection Agency, Microbiology Services Department, United Kingdom, through funds provided by the Biomedical Advanced Research and Development Authority, Department of Health and Human Services (Contract number HHSO1002011000008I and Task Order HHSO10033001T).

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