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CLINICAL GUIDELINE FOR DIAGNOSIS AND MANAGEMENT OF MELIOIDOSIS

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SUMMARY

Melioidosis is an emerging infection in Brazil and neighbouring South American countries. The wide range of clinical presentations include severe community-acquired pneumonia, septicaemia, central nervous system infection and less severe soft tissue infection. Diagnosis depends heavily on the clinical microbiology laboratory for culture. *Burkholderia pseudomallei*, the bacterial cause of melioidosis, is easily cultured from blood, sputum and other clinical samples. However, *B. pseudomallei* can be difficult to identify reliably, and can be confused with closely related bacteria, some of which may be dismissed as insignificant culture contaminants. Serological tests can help to support a diagnosis of melioidosis, but by themselves do not provide a definitive diagnosis. The use of a laboratory discovery pathway can help reduce the risk of missing atypical *B. pseudomallei* isolates. Recommended antibiotic treatment for severe infection is either intravenous Ceftazidime or Meropenem for several weeks, followed by up to 20 weeks oral treatment with a combination of trimethoprim-sulphamethoxazole and doxycycline. Consistent use of diagnostic microbiology to confirm the diagnosis, and rigorous treatment of severe infection with the correct antibiotics in two stages; acute and eradication, will contribute to a reduction in mortality from melioidosis.

KEYWORDS: Melioidosis; Clinical guideline; Diagnosis; Antibiotics; *Burkholderia pseudomallei.*

INTRODUCTION

Melioidosis is an emerging infectious disease in Brazil. This potentially fatal bacterial infection is caused by exposure to soil or water contaminated with the bacterial species *Burkholderia pseudomallei*. The disease has been known in the Americas since the middle of the 20th century when there was an outbreak in sheep, goats and pigs in Aruba[24]. Since then sporadic cases of melioidosis have occurred in Ecuador, Panama and possibly in Ceara[1,19]. The recent cluster of melioidosis fatalities in rural Ceara has highlighted the changing epidemiology of this disease. Survey data from Northeastern Brazil indicates that melioidosis is a more important cause of community-acquired sepsis than previously thought[21].

A recurring issue where melioidosis is known but uncommon is how best to confirm the diagnosis. In its most acute, life threatening form the infection has no reliable pathognomonic features[31]. The differential diagnosis is very wide. Baseline diagnostic tests offer little help. The definitive diagnosis depends on culture of the causal bacterial species, *B. pseudomallei*[15] . But even when preliminary culture results are returned from the microbiology laboratory, the result may not be apparent because Gram negative septicaemia has many commoner causes. The appearance of *B. pseudomallei* colonies on an agar plate can be helpful after subculture from blood culture bottles onto solid media, but only after 2-3 days of growth. Younger cultures may not suggest *B. pseudomallei* so that the opportunity to make a definitive early diagnosis may be missed. By this stage, usually 1-2 days after collection of a blood or sputum culture, a severely sick patient may be in respiratory distress or even dead. The methods used to identify Gram negative bacteria in the microbiology laboratory can be very misleading. Some do not reliably identify *B. pseudomallei* at all[9]. Physicians and microbiologists need to know how to look for *B. pseudomallei* to increase their chances of detecting it.

Antibiotic treatment of melioidosis also poses a challenge. Several antibiotics commonly used for Gram negative septicaemia, including Gentamicin, quinolones and third generation cephalosporins have no reliable effect against *B. pseudomallei* infection[13]. The preferred antibiotic treatment of *B. pseudomallei* septicaemia requires costly intravenous agents[3,27]. There is a tendency for early relapse at around 10-14 days after commencement of intravenous antibiotic therapy[2]. Late relapse can occur months or even years after initial septicaemic infection. An additional consequence of this capacity for melioidosis to remain dormant is delayed presentation of acute infection many decades after the initial exposure[18].

In order to raise awareness of the clinical management issues, we present our recommendations for diagnosis, treatment and continuing care of melioidosis based on recent experience and best practice. These guidelines will be subject to revision as diagnostic and therapeutic

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systems improve to meet the challenges posed by this emerging infection.

CASE DESCRIPTION

There are no reliable pathognomonic features of acute or subacute melioidosis. Other infections including tuberculosis and typhoid fever are commonly confused with melioidosis. The commonest clinical presentation of the disease in northern Australia and Southeast Asia where the infection appears to be commonest is septicaemia with or without pneumonia (Table 1)[4]. Other focal organ involvement is common either as a primary source of subsequent septicaemia or as a due to localisation following bloodstream spread. Meningoencephalitis, pneumonia without septicaemia, osteomyelitis, septic arthritis, spinal involvement, localised abscesses in almost any deep organ or superficial soft tissue have all been described. The majority of severe infections occur in patients with a contributory co-morbidity such as uncontrolled diabetes mellitus, chronic renal failure, alcoholic liver disease or chronic lung disease[17] . Overwhelming septicaemia does occasionally occur in previously fit young adults. The subacute end of this disease spectrum usually takes the form of localised superficial soft tissue infection in patients with no associated co-morbidities[15]. Melioidosis can present for the first time after a prolonged pre-patent period or relapse after initially adequate antibiotic therapy followed by a quiescent interval[2,18]. This capacity to develop into a subclinical form, due to sequestration of dormant bacteria within the body was first recognised in veterans of the Viet Nam conflict[22]. A history of soil or surface water exposure and percutaneous inoculation may help identify the patient at risk of melioidosis, but will be absent in a proportion of subsequently diagnosed cases[5].

Table 1
Clinical presentations of melioidosis

Commonest acute presentations
Pneumonia
Pneumonia with septicaemia
Septicaemia
Other presentations
Soft tissue infection: cellulitis, fasciitis, skin abscess/ulcer
Bone and joint infection: osteomyelitis, septic arthritis
Genitourinary: prostatic abscess
CNS infection: cerebral abscess, meningoencephalitis, encephalomyelitis
Facial: suppurative parotitis
Ocular infection: conjunctival ulcer, hypopyon, orbital cellulitis
Incidental finding
asymptomatic seroconversion

DIAGNOSTIC INVESTIGATIONS

Confirmation of a diagnosis of melioidosis depends heavily on the clinical microbiology laboratory, and specifically on recovering a *B. pseudomallei* isolate by culture from blood, sputum, cerebrospinal fluid or other bacteriology specimen[15]. The bacterial count in venous blood (CFU/mL) can reach high levels during septicaemia and *B. pseudomallei* grows easily so that conventional blood culture methods are an effective means of establishing a bacteriological diagnosis[28]. Samples from non-sterile sites are less helpful because the numbers of *B. pseudomallei* may be much lower, and can be overwhelmed by larger numbers of commensal species. This problem can be overcome by use of selective agar to suppress commensal bacteria. The media used for this are Ashdown's Selective agar (ASA) or *B. pseudomallei* Selective Agar (BPSA)[7,29]. ASA was developed for clinical bacteriology use, but may inhibit the growth of some strains of *B. pseudomallei*, particularly those that are excessively mucoid. BPSA was developed to improve recovery of mucoid strains without reducing recovery of the classic wrinkled type. A laboratory discovery pathway has been developed for *B. pseudomallei*[10]. This comprises three stages (Table 2): an initial screen of easily performed tests, confirmatory phenotypic methods and finally, definitive genotypic confirmation. Substrate utilisation panels are used in many laboratories to confirm the identity of suspected *B. pseudomallei*[6,16,25]. These appear to perform well when there is an expectation that the isolate is indeed *B. pseudomallei*, or when evaluating a collection of isolates already identified by substrate utilisation. However, neither the manual nor the automated substrate utilisation panels used to identify unknown Gram negative bacilli from

Table 2
Laboratory criteria for confirmation of *B. pseudomallei* infection

Definitive:
culture of a Gram negative bacillus from blood, other sterile fluid or sputum sample with the following features
ALL of ..
Oxidase positive
Gentamicin resistant
Polymyxin (Colistin) resistant
PLUS one or more of ..
B. pseudomallei agglutinating antibody positive
B. pseudomallei-specific PCR positive
B. pseudomallei 16s DNA sequence positive
Supportive, but not definitive:
Gram negative bacillus from blood, other sterile fluid or sputum sample without any positive confirmatory tests in a clinical setting consistent with melioidosis
Gram negative bacillus from blood, other sterile fluid or sputum sample in a clinical setting consistent with melioidosis, with positive first line tests (oxidase +, GM/POLY R) a contradictory bacterial identification panel (e.g. API, Vitek etc) but awaiting definitive test
A fourfold or greater rise in *B. pseudomallei* antibodies by IHA or ELISA in a clinical setting consistent with melioidosis
A single very high *B. pseudomallei* antibody titre in a clinical setting consistent with melioidosis

blood cultures are fully reliable[9,14]. Where molecular tests are not available, substrate utilisation panels should be used with caution after taking into account the results of simple preliminary tests such as Gram stain, oxidase and antibiotic susceptibility pattern[6,10]. Alternatively, they can be used to supplement other identification tests, or they may be used in reference laboratories as part of classical bacteriological identification procedures that rely on polyphasic determinative bacteriological methods[10]. All suspected *B. pseudomallei* isolates should be stored on agar slopes or in glycerol broth at -70 ºC for future reference laboratory work, even when preliminary results suggest that the isolate in question is another bacterial species. We still encounter occasional referred clinical isolates that have been identified as another bacterial species and that subsequently prove to be *B. pseudomallei*. Moreover, our current discovery pathway will probably require amendment when the more recent PCR protocols have been evaluated. Isolates referred for definitive identification and molecular typing should be transferred to reference laboratories in accordance with current UN/IATA guidelines. International transfers must also comply with the respective national quarantine regulations.

Serological evidence of infection can be obtained by indirect haemagglutination assay (IHA)[30], but seroconversion is unlikely to occur early enough to affect treatment choices during the admission phase of a severe, acute infection. False negatives do occur and though ELISA-based or indirect immunofluorescent (IFAT) tests have improved the sensitivity and specificity in some centres, they have not completely resolved this problem[26]. Seroconversion or a single high IHA titre (e.g. > 160) in the absence of a positive culture is therefore regarded as supportive rather than definitive evidence of melioidosis[15]. The level of titre regarded as diagnostic will vary in different locations according to local melioidosis epidemiology.

IMMEDIATE AND CONTINUATION THERAPY

Clinical trials have shown a significant reduction in mortality by early intervention with a suitable intravenous antibiotic, and comparable results with other, newer agents. Ceftazidime was the first antibiotic to produce clear improvements in mortality (Table 3)[27]. There are theoretic reasons why a carbapenem should be used instead for severe infections[11], and both Imipenem and Meropenem have been shown to be at least as good as Ceftazidime[3,23]. Supplementary antibiotic agents may have a place in treating persistent bacteraemic infection, or reducing the risk of early relapse but it is not yet clear which agent is most suitable or when it should be introduced. Other measures that may also be important for specific patients with severe, acute infection include correction of metabolic acidosis, ketosis, diabetic control and oxygenation[12]. All these factors are likely to assist phagocytic function and possibly reduce the risk of late relapse. There is a lack of consensus on exactly how long to continue intravenous antibiotics and what to continue for oral eradication therapy. These issues will only be resolved by carefully planned clinical trials.

CONCLUSION

Melioidosis presents a diagnostic challenge to both the physician and the diagnostic laboratory scientist. The poor sensitivity and speed of current methods of laboratory diagnosis of melioidosis will continue to frustrate the primary diagnostician for some time to come. The 2003 melioidosis case cluster in Ceara, in which three of the four presumed cases succumbed to infection, illustrated these problems[20,21]. No bacteriological diagnosis was possible in the first case. The Gram negative bacillus from blood culture in the second case didn't survive long enough for definitive identification by the state public health laboratory, and the one surviving case had no positive culture but a delayed seroconversion by IHA. A definitive, culture-based diagnosis was only made in the third case after several days of processing an unfamiliar Gram negative isolate whose initial appearance resembled *Yersinia pestis* on the Gram stain. This result came too late to prevent a fatal outcome, but did alter the choice of antibiotic therapy for the one survivor. We believe that increased awareness of the varied clinical presentation of melioidosis and correct choice of diagnostic methods will result in faster diagnosis and a better prognosis for those with acute, septicaemic disease. This must be matched by enhanced clinical laboratory capability, including the development of a melioidosis reference laboratory. The capacity to deliver melioidosis reference tests is under development in Ceara, where the majority of recent cases have presented. Finally, effective antibiotic therapy is available for this potentially fatal infection. Use of the recommended regimes will reduce the mortality from this disease and help prevent its subsequent relapse.

Table 3
Antibiotic therapy for culture confirmed melioidosis

Severe, acute melioidosis including septicaemia, with or without pneumonia, central nervous system infection and other invasive forms of the disease:
EITHER Ceftazidime (adult) 2-3 g or 40 mg/kg/dose every eight hours intravenously for 2-4 weeks PLUS Co-trimoxazole (Trimethoprim-Sulphamethoxazole) 10/50 mg/kg (up to 320/1600 g) every 12 hours
OR Meropenem 1 g or 25 mg/kg every eight hours intravenously for ≥ 2 weeks
Eradication phase 1 Trimethoprim-Sulphamethoxazole 8/40 mg/kg every 12 hours for ≥ 12-20 weeks 2 Doxycycline 4 mg/kg/day plus Trimethoprim-Sulphamethoxazole 8/40 mg/kg every 12 hours for ≥ 12-20 weeks 3 Chloramphenicol 40 mg/kg/day plus Doxycycline 4 mg/kg/day, Trimethoprim-Sulphamethoxazole 8/40 mg/kg every 12 hours for ≥ 12-20 weeks

RESUMO

Recomendações clínicas para o diagnóstico e tratamento da melioidose

Melioidose é uma infecção emergente no Brasil e em países vizinhos da América do Sul. O amplo espectro de apresentação clínica

inclui pneumonia adquirida na comunidade, septicemia, infecção do sistema nervoso central e infecção de partes moles de menor severidade. O diagnóstico depende essencialmente da identificação microbiológica. *Burkholderia pseudomallei*, a causa bacteriana da melioidose, é facilmente cultivada em sangue, escarro e em outras amostras clínicas. Entretanto, *B. pseudomallei* pode ser difícil de identificar com segurança e também ser confundido com outras bactérias Gram negativas. Os exames sorológicos podem dar suporte a um diagnóstico de melioidose, mas não fornece um diagnóstico definitivo por si só. A realização de investigação laboratorial seqüenciada pode ajudar a reduzir o risco de não reconhecer isolados incomuns de *B. pseudomallei*. O tratamento antibiótico recomendado para infecção severa é Ceftazidima ou Meropenem endovenosos por várias semanas, seguido por um tratamento oral com uma combinação de Sulfametoxazol-Trimetopim e Doxiciclina por até 20 semanas. O uso consistente do diagnóstico microbiológico e o tratamento rigoroso da infecção severa com antibióticos adequados nas duas etapas, aguda e de erradicação, contribuirão para a redução da mortalidade por melioidose.

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