INVITED REVIEW ARTICLE

Melioidosis in Malaysia

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SUMMARY

Melioidosis is an important cause of sepsis in the tropics, is caused by an environmental saprophyte - *B.pseudomallei.* It affects mainly adults with underlying predisposing condition such as diabetes. The range of symptoms varies from benign and localized abscesses, to severe community-acquired pneumonia to acute fulminating septicaemia with multiple abscesses often leading to death. *B.pseudomallei* is an intracellular pathogen and some of the virulence mechanisms that govern the complex interaction between the organism and the host have been elucidated. Isolation of *B.pseudomallei* from bodily fluids of patients remains the "gold standard" in diagnosis but a sensitive and specific serological test can lend support to the diagnosis of melioidosis. Ceftazidime is the treatment of choice for severe melioidosis, but the response is slow. Maintenance or eradication therapy for a prolonged period is necessary to prevent relapse and recurrence. Monitoring IgG antibody levels may be useful as a guideline to determine the duration of eradication therapy.

KEY WORDS:
Melioidosis, Burkholderia pseudomallei, Malaysia

HISTORICAL BACKGROUND

Melioidosis caused by *Burkholderia pseudomallei*, a gram-negative soil saprophyte was first described by Whitmore and Krishnaswami in 1912[1] in 38 fatal cases of pneumonia amongst the destitute and morphine addicts in Rangoon, Burma. In 1913 Fletcher recognised the disease in laboratory animals at the Institute for Medical Research in Kuala Lumpur, Malaysia and in 1917 Stanton first described the infection in a human patient from Kuala Lumpur[2] and these authors wrote a short monograph on the disease and its sporadic occurrence in Malaya up to 1932. Since that time, cases have been reported in both man and in a variety of animals such as sheep, buffalo, deer, monkey, gibbon, orang utan, kangaroo, camel, parrot, hamster, zebra and crocodile[3].

The term melioidosis was coined in 1921 by Stanton and Fletcher and is derived from the Greek words "*melis*" meaning "a distemper of asses" and *"eidos"*, resemblance. This was because the disease clinically and pathophysiologically resembled glanders, a chronic and debilitating disease of equines caused by *Pseudomonas* mallei[2].

Melioidosis occurred in Allied and Japanese soldiers during the Second World War in Burma, Malaysia and in Thailand. After the Second World War, sporadic reports of melioidosis appeared in the literature, with 10 cases from Malaysia being reported by Thin *et al* in 1970[4].

The establishment of the Department of Medical Microbiology at the Faculty of Medicine, University of Malaya, saw a resurgence of interest in the disease melioidosis and several publications and reports on less known aspects of the disease in Malaysia, such as demographic details and risk factors, were published[5,6,7].

By the early 1990s there was sufficient interest in melioidosis in the scientific and medical world and it was thought the time was right to bring these researchers together at a forum. Thus the First International Symposium on Melioidosis convened by the Malaysian Society of Infectious Diseases and Chemotherapy, under the Chairmanship of Prof. S D Puthucheary, was held in Kuala Lumpur from April 7-8, 1994. About 100 participants from around the world attended and the papers presented were subsequently edited and published as a book[8].

Burkholderia pseudomallei

This bacterium was known by many names over the past 100 years, generally well known as *Pseudomonas pseudomallei* but in 1992 Yabuuchi and co-workers incorporated it into the new genus *Burkholderia*[9]. This organism is a soil saprophyte and can be readily recovered from water and wet soils in endemic areas.

Burkholderia pseudomallei are motile aerobic, non-spore forming gram negative bacilli. The genome is relatively large, 7.24 Mb, and is divided unequally between two chromosomes (4.07 Mb and 3.17 Mb), with a G+C content of 68%[10].

In the laboratory, *B. pseudomallei* grows aerobically on most agar media, and produces clearly visible colonies within 24-48 hours at 37°C. Ashdown's medium or modifications of it are commonly used and the organism demonstrates differing colonial morphology, with mostly smooth colonies initially and dry or wrinkled colonies on further incubation. Large and small colony variants have also been isolated from blood cultures of patients with previous antibiotic therapy[11]. Gram stain shows gram negative rods described as having bipolar staining. The organism is oxidase positive, uses glucose by an oxidative pathway, and can be identified reliably from its biochemical profile with kit-based systems.

B.pseudomallei exibits resistance to diverse groups of antibiotics, including third generation cephalosporins, penicillins, rifamycins and aminoglycosides. In addition, its

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relative resistance to quinolones and macrolides limits therapeutic options for the treatment of melioidosis. In Malaysia, Puthucheary *et al* in 1987, were the first to publish the antimicrobial susceptibilities on a large collection of 57 isolates of *B.pseudomallei* using 15 chemotherapeutic agents. Significant results were: 86% sensitivity to trimethoprim-sulpahamethoxazole, 84% to chloramphenicol, 58% to tetracycline and 100% to ceftazidime[12]. Almost 20 years later and from the same institution, 80 clinical isolates of *B.pseudomallei*, collected between 1978 and 2003, were tested for *in vitro* susceptibility using the E-test. One hundred percent of the isolates were sensitive to imipenem and meropenem and 97.5% were sensitive to trimethoprim-sulpahamethoxazole[13].

The largest collection of 146 clinical isolates of *B.pseudomallei* from Malaysia, when tested for MIC by the E- test gave a sensitivity of 100% for ceftazidime, imipenem and meropenem; 99.3% for amoxicillin-clavulanate; 97.9% for chloramphenicol; 88.4% for trimethoprim-sulpahamethoxazole and 82% for ciprofloxacin (unpublished data, See KH Masters thesis). Although it is reassuring that the drugs of choice had no problems with resistance, nevertheless we have reported that a single infectious clonal population of *B.pseudomallei* from a patient, did contain subpopulations with differing ceftazidime and amoxicillin-clavulanate susceptibilities and that these were associated with a single nucleotide substitution in the *penA* gene[14].

Ecology and Distribution

Burkholderia pseudomallei is an environmental saprophyte and melioidosis is endemic in southeast-Asia and tropical Australia but the global distribution boundaries of melioidosis continue to expand well beyond these traditionally recognised endemic regions. In Malaysia the organism has been isolated from soil and water from all states in West Malaysia by Strauss and co-workers in 1969[15]. Samples were taken from primary and secondary forests, wet rice fields and recently cleared areas. The isolation rates were lower from the forested areas compared to the cleared areas of wet rice fields and newly planted oil palm plantations. Soil moisture was an important criterion in the isolation of the organism and the incidence of melioidosis peaks during the months of heavy rainfall in Thailand and Australia: this is because the water table rises to the surface carrying with it the bacteria that normally reside below the surface. In Malaysia, although the percentage of water and soil specimens positive for *B.pseudomallei* was higher during increased rainfall[15], there appeared to be only a slight increase in the number of cases during the wet monsoon period. Our findings of correlation of rainfall and the occurrence of 125 patients with culture proven melioidosis, over a 30 year period, support the hypothesised general association between melioidosis and rainfall. But the correlation appeared less strong than those reported in other centres, which may be due to other factors, including the different local patterns of rainfall intensity[16], as well as increased exposure to the organism during ploughing and planting of the rice paddies in endemic areas[17].

Soil and surface waters are highly complex ecosystems in which a vast range of physical, chemical and biological factors interact. But the organism is found to persist in some tropical regions better than others, such as hyper endemic foci or "hot spots" such as north-eastern Thailand and East Malaysia where more cases are reported than from the rest of the country[17] but the exact reasons for this are far from clear. The tenacity of this soil saprophyte to survive in a hostile environment should not be underestimated. There is a homeostatic balance in nature between man, organism and the environment and any disturbance of this by logging, clearing of large tracts of vegetation will upset this equilibrium with drastic consequences for man, animals and the environment[17].

Epidemiology

In a comprehensive review of 98 septicaemic and 43 non-septicaemic cases studied over a period of 35 years in our institution, we found a bimodal distribution of age in both groups of patients (Fig.1). Age of patients ranged from 17 days to 79 years. The increase in the 10-30 year group possibly reflects the greater environmental exposure during play or outdoor recreational activities. The peak age-specific incidence occurred from 41 – 59 years for both males and females in Malaysia but in Thailand the peak age-specific incidence was 50-59 years for women and 60-69 years for men[18], whilst in Singapore the risk of melioidosis increased steadily with age, being maximal in those over 65 years[19]. In every published case series on melioidosis, males have outnumbered females but the proportions varied considerably (ratio of male: female from 5:1 to 1.4:1). This likely reflects involvement in activities which lead to exposure to contaminated soil and water. In Malaysia the M: F ratio was found to be 3.2:1[11].

Ethnic differences in susceptibility to melioidosis were suggested by studies in Singapore where morbidity rates were highest in Indians, lowest in Chinese and intermediate in Malays in both the general population and the military. In Malaysia, the morbidity rate was highest amongst the Indians, lowest in Malays and intermediate in the Chinese[11]. However, it is possible that the groups differed in their frequency of activities resulting in occupational and environmental exposure to soil and water and so no firm conclusions can be drawn.

Modes of acquisition

Three modes of acquisition, i.e., inhalation, ingestion and inoculation are recognised for *B.pseudomallei*, but the relative contribution of each are yet to be determined. As with other infectious diseases, it is likely that these factors as well as the size of the inoculums are responsible for the pattern and severity of disease. Inhalation was initially thought to be the primary mode of acquisition, based on studies of U.S. soldiers in Vietnam, where it was noted that helicopter crews seemed to have a high incidence of the disease. This and the long incubation period resulted in melioidosis acquiring the sobriquet "the Vietnamese time bomb"[20], but it seems logical to assume that melioidosis is acquired mainly by contact with contaminated soil and water through penetrating wounds or existing skin abrasions, ulcers, burns or by inhalation of dust particles, by aspiration of contaminated water during near-drowning episodes[21] iatrogenic inoculation and by laboratory accidents[22].

Predisposing or risk factors

It has been recognised that *B. pseudomallei* behaves as an opportunistic pathogen. Exposure to the organism is widespread and yet disease is not that common, occurring predominantly in those with underlying predisposing conditions suggesting that the susceptibility of the host is an important factor. The majority of patients with clinically apparent melioidosis are recognised as having underlying diseases: 76% in Malaysia[7], 88% in Australia[23] and 53% in Thailand[18]. The difference in the percentages between the studies is probably accounted for by the definition of underlying diseases and the extent to which they were sought. In Malaysia, northeast Thailand and Singapore, diabetes mellitus was the most frequently reported predisposing condition with up to 60% of patients having pre-existing or newly diagnosed type 2 diabetes[18]. Studies in Thailand clearly showed that the prevalence of diabetes mellitus in patients with culture proven melioidosis was significantly higher than in patients with septicaemia due to other bacteria. Renal failure, renal calculi, retroviral infections, malignancy, steroid therapy, alcoholism, occupational exposure, trauma and parenteral drug abuse were also confirmed as important predisposing factors both in Malaysia and Thailand [7, 24].

The precise nature of the predisposing immune dysfunction is poorly understood. The underlying conditions described above lead to a wide range of immune deficits including phagocytic defects, diminished humoral and cellular responses and diminished cytokine production. It has also been postulated that insulin deficiency may contribute directly to the association of melioidosis with diabetes mellitus [25]. Underlying disease was seldom reported in cases from the Singapore Armed Forces, and in children and young adults from East Malaysia, suggesting that a substantial exposure to *B. pseudomallei* will cause infection even in healthy individuals.

Clinical Manifestations

The infection is a collection of disease states and the clinical entity of melioidosis is virtually impossible to define as the spectrum of signs and symptoms can range from benign skin and soft tissue infections to a rapidly fulminant and fatal septicaemia. Due to this wide array of clinical signs and symptoms, the causative bacterium *Burkholderia pseudomallei* has been called "the great mimicker."

The incubation period of melioidosis has not been accurately defined in melioidosis. Estimates are possible of the time taken for wound sepsis to appear following trauma or motor vehicle accidents. After a laboratory accident an incubation period of 2 days was recorded. Currie *et al* [26] give an incubation period of one to 21 days (mean 9 days) in 25 cases where a clear incubation period could be determined between the inoculating injury and the onset of symptoms.

In all series, the lung was the most commonly affected organ, either presenting with cough and fever resulting from a primary lung abscess or pneumonia, or secondary to septicaemic spread (blood-borne pneumonia). Sputum is often purulent but seldom blood-stained. Large or peripheral lung abscesses may rupture into the pleural space to cause empyema [27]. Thus, melioidosis is usually perceived as an acute pulmonary illness characterized by prostration and marked toxicity that is often out of proportion to objective physical findings or chest radiographic findings. However, melioidosis has been recognized to give rise to inapparent infections, transient bacteraemia, asymptomatic pulmonary infiltration, acute localised suppurative lesion, acute pulmonary infection, disseminated septicaemic infection, non-disseminated septicaemic infection or chronic suppurative infection. Since melioidosis is a multi-system disease and the signs and symptoms are non-specific, the clinical classification of melioidosis has been controversial. The subdivision into acute, subacute and chronic melioidosis is unsatisfactory as there exists a clinical continuum as the less acute or localised forms may rapidly progress to the septicaemic form especially if there is concomitant debilitating illness. The same is true of a classification by organ involvement as more than one organ may be involved [7]. Melioidosis has a high mortality rate of 30-70%. Both this and the severity of the acute manifestations of the disease appear to depend on whether the patient is septicaemic or not. Therefore a more functional and useful clinical classification for melioidosis would be into septicaemic and non-septicaemic melioidosis [11]. The overall mortality of non-septicaemic melioidosis is 5-20%, which is very much lower than that of septicaemic melioidosis.

Septicaemic melioidosis may present in many forms, from a simple bacteraemia with no obvious focus of infection to the most severe form of disseminated bacteraemia with fulminant shock and multi-organ involvement. It is almost always community-acquired and patients often present simply with a history of fever (median duration 6 days with a range of several days to several months) and often with no evidence of a focus of infection. In one of the largest series of 1000 culture proven cases seen at the Sappasithiprasong Hospital in northeast Thailand, 15% of the patients did not have an obvious primary site of infection [27]. This may be true at the time of admission to hospital, nevertheless a thorough septic screen must be implemented including a chest X-ray and an ultrasound of the abdomen.

Septicaemia of abrupt onset may rapidly progress with dissemination of the primary focus of infection, frequently evidenced clinically by the subsequent development of multiple subcutaneous abscesses, multiple nodular lesions visible on chest X-ray, joint swelling and myositis. Patients with the septicaemic form usually have a rapidly progressive course particularly if there is concomitant debilitating illness, such as uncontrolled diabetes mellitus, haematological malignancies and disorders, solid tumours, collagen vascular disease, renal disease, retroviral infections and alcoholism. Up to 25% of these patients may be hypotensive with signs of organ dysfunction on admission[28] and multi-organ involvement was seen in 25-30% of cases. In the series mentioned earlier, the lungs were the commonest site (50%) when only a single organ was involved, other sites being CNS (brain abscess), joints, prostate or testes, liver and/or spleen[27]. Renal involvement such as pyelonephritis, perinephric abscess, skin and soft tissue sepsis, cellulitis accompanied by regional lymphangitis and multiple superficial abscesses were also recorded in septicaemic patients. Unlike other pyogenic infections, haematologic dissemination to the viscera involved the spleen more frequently than kidney or liver in

the form of multiple abscesses[29]. Other uncommon conditions seen were pericardial effusion, meningitis, empyema of the gall bladder, abscess of the parotid gland, abscess of tail of pancreas, and mycotic aneurysms[30,31]. Paranasal sinus infections such as acute ethmoiditis and frontal sinus empyema have also been described [32]. Melioidosis of the central nervous system is uncommon, but macroscopic brain abscess[33] and a case of epidural abscess of the spine in Malaysia[34] have been reported from our institution.

Localised melioidosis may occur in the form of acute suppurative lesions, superficial and deep abscess such as in the psoas muscle and the parotid glands, cellulitis, chronic otitis media and sepsis following burns, trauma or motor vehicle accidents [11]. Parotid abscesses and cervical lymphadenopathy are seen commonly in children [35]. Localised osteomyelitis has been described in 10 patients from Thailand whose ages ranged from 28 to 62 years with 7 of them having underlying predisposing conditions. The vertebrae were involved in 4, the proximal humerus in 4 and the proximal femur and tibia in two patients respectively. Osteomyelitis of the skull although very rare, has been observed (SD Puthucheary, unpublished observations). It must be emphasised that *B. pseudomallei* has the potential to cause pyogenic or granulomatous inflammation at virtually at any site in the body. Two manifestations of melioidosis that are occasionally very dramatic are the rapid progression of respiratory failure, and profound weight loss. Our report on the clinical and pathological study of six cases of respiratory failure in melioidosis suggested that a combination of acute necrotising pneumonia and the acute respiratory distress syndrome appeared to be responsible [36].

Nearly all clinical studies have come from Thailand, Malaysia, Singapore and northern Australia and the overall mortality in adults ranged from 40-65% [7,18]. In northern Australia the mortality in severe melioidosis has been falling in recent years with earlier recognition and intensive care treatment [10] and is expected to also decrease in other endemic areas.

Radiographic findings

We found aproximately 50% of all septicaemic cases to have lung involvement. In a series of 50 septicaemic cases from Malaysia, 46% had pneumonia[7]. In the 1,000-case series seen in northeast Thailand, 66% of the cases had radiological abnormalities. Unilateral pulmonary shadowing (56%) was more common with predominant involvement of the right lung. More than one lobe was affected in one out of five patients. The right upper and lower lobes were the common sites of involvement [27]. Bilateral pulmonary shadowing was seen in 44% of the cases in northern Australia. A cavitating lesion was present in 14% of the cases. These cavities in melioidosis, unless very large, usually do not have an air-fluid level [37].

Fibro-nodular infiltrates with or without cavitation in the upper-lobe (i.e. single lobe) can be quite similar to that of pulmonary tuberculosis and in chronic pulmonary melioidosis the appearance may closely simulate that of tuberculosis, but the tendency for the apices to be uninvolved makes the possibility of melioidosis more likely, especially in cases with minimal or no signs of fibrosis. The nodular lesions in melioidosis are neither discrete nor as uniformly distributed as in disseminated tuberculosis or fungal infections [38, 39].

In severe septicaemic cases of melioidosis there is a rapid appearance of diffuse, fluffy alveolar infiltrates in both lungs within one to two days, leading to the acute respiratory distress syndrome and inevitable death usually due to primary respiratory failure[36]. Visceral abscesses are common in melioidosis[40] and an ultrasound of the abdomen should be performed in all culture proven cases. Hepatic abscesses are usually multifocal, unlike the large solitary abscess found in other pyogenic infections. The presence of miliary or micro abscesses in the liver and spleen seen at autopsy [41] may not be easily evident on ultrasound examination and a CT scan is indicated, as the presence of micro abscesses will have important implications for both management and prognosis.

Relapse and recurrence

Relapse and recurrent infections are not uncommon in melioidosis, especially in hosts who are immunocompromised, and occur in spite of appropriate and prolonged antimicrobial therapy. Relapse is the reappearance of signs and symptoms after initial clinical response while still on antimicrobial therapy. A recurrent infection or recrudescence is a new episode of melioidosis caused by the same organism after convalescence and full clinical recovery. Relapse and recurrence are potential problems in patients who survive acute melioidosis. Such infections are assumed to be due to failure by the host to eliminate the organism during the initial infection. In northeast Thailand, the overall relapse rate was from 15-30% per year of survivors of severe melioidosis despite treatment for at least two months (and longer in patients with persisting abscesses or osteomyelitis). The same organ was involved in 44% of the relapsed cases [42].

In Malaysia, the rate of relapse or recurrence was found to be approximately 13% over a period of 5 years, an underestimate due to cases being lost to follow-up. Numerous episodes of infections after initial recovery occurred in several patients followed-up over many years, the longest period of follow-up being 5 years (SD Puthucheary, unpublished observations). Chaowagul *et al* [42] during their study period were not aware of any cases of relapse in children, which is similar to our experience in Malaysia. This suggests that relapse is less common in children and is consistent with the observation that the acute prognosis is better in the younger age group.

This infection has the potential for prolonged latency and recurrence into a fulminating form which can present acutely. Factors thought to contribute to this include the survival of *B. pseudomallei* in protected sites such as within phagocytic cells[43] and in sealed abscesses where the organisms can evade the host immune response. Other reasons for recurrence are the ability of *B. pseudomallei* to form glycocalyx, biofilms and microcolonies in infected tissues where antimicrobials are unable to penetrate [44]. Factors that influence the likelihood of relapse and recurrence include clinical severity at original presentation and the type and length of parentral and oral antimicrobial treatment. Patients

with severe infections have an overall higher risk of relapse than those with localised infections.

But the most important factor for relapse is non-compliance of maintenance (eradication) oral antimicrobial therapy. Therefore any patient with a previous history of melioidosis presenting with fever or symptoms of sepsis, should be suspected of having a relapse or recurrence and empirical antibiotic therapy effective against *B. pseudomallei* should be instituted without delay. With improvements in the management and treatment of severe melioidosis, a greater proportion of patients now survive and return to the community. Therefore relapse of melioidosis will become more significant. The adage, "once a melioidosis, always a melioidosis" seems to hold true for many infected patients [29].

In the majority of cases, relapse or recurrence is due to reactivation of the original infecting strain, and we [33] demonstrated this with isolates recovered from four patients over a period of 3 months to 5 years, using pulsed-field gel electrophoresis (PFGE). Isolates from each patient were identical but PFGE patterns from different patients were distinctly different [45]. These findings also imply *in vivo* genomic stability of *B. pseudomallei* in the presence of selective pressures of antimicrobial therapy and host defence mechanisms Ribotyping and PFGE provided further differentiation between isolates from 5 patients with clearly distinguishable episodes of melioidosis suggesting that repeated episodes of infections in melioidosis are due to the original infecting strain, although reinfection with strains of similar types from the environment cannot be ruled out. We concluded that some patients with melioidosis may be infected by more than one strain of *B. pseudomallei* and that infection with one strain does not prevent concurrent infection with another strain. Only one of the 13 patients in this report harboured two strains of different ribotypes [46].

Diagnosis of Melioidosis

In endemic areas, a high index of suspicion and a good travel history in patients from non-endemic regions are important factors involved in establishing a diagnosis. Melioidosis should be considered in the differential diagnosis of any febrile illness if the presenting features are those of fulminant respiratory failure, if multiple pustular or necrotic or subcutaneous lesions develop, or if there is a radiological pattern of tuberculosis from which tubercle bacilli cannot be demonstrated, especially in a patient who is immunocompromised, has diabetes mellitus, and either resides or has travelled to endemic areas [11]. The diagnosis of melioidosis can be missed or dismissed when such histories are not adequately acquired or the time between possible exposure and presentation is considered too great [48]. History of occupational and recreational exposure should be sought and abrasions and trauma, however minor, must be elucidated as these may be considered too trivial for medical attention[17]. Asymptomatic carrier state is not known and so recovery of the organism from specimens such as throat swabs and urines indicates active disease [48].

Isolation of *B.pseudomallei* from bodily fluids of patients remains the "gold standard" in diagnosis and requires the use of selective media for non-sterile specimens. The organism is not fastidious and will grow on almost all routine media, but with non-sterile specimens *B.pseudomallei* can be overgrown by contaminating flora due to paucity of the organisms especially from deep-seated abscesses and infected tissues. Therefore incubation of the culture aerobically at 37^{0}C for 3-5 days may be necessary. Recognition and identification of *B. pseudomallei* depends very much on awareness and familiarity with the cultural characteristics of the organism. The colonial morphology can vary with the medium used and source of the strain[11]. Many tests based on molecular detection of *B.pseudomallei* have been described, but few have been field tested.

Serology

In situations where patients are critically ill with fulminating sepsis or when infections are deep seated and no specimens are available, serology may be sufficiently rapid to facilitate aggressive and appropriate treatment and management of patients. But serological diagnosis of melioidosis has been hampered by factors such as raised antibody levels in the population of endemic regions, the presence of subclinical and asymptomatic infections, poorly standardised antigens and probable cross reactions with other organisms. We developed an indirect immunofluorescent test using whole cells of *B.pseudomallei* as the antigen[49], and evaluated its diagnostic and prognostic value in the detection of total antibodies (IgG and IgM) to *B.pseudomallei*. This test was found to be rapid and useful as it required only a day to complete. A cut-off value of 1:80 was used to differentiate between true infections from background titres due to basal antibody levels in endemic areas. This study also demonstrated the need to include melioidosis as one of the differential diagnosis in patients with PUO in endemic regions, and in military personnel, eco-tourists and others returning home from endemic areas [50].

Pathogenesis and Virulence

B.pseudomallei like many soil bacteria is a difficult organism to kill. It can survive in triple distilled water for years [51] and yet it has the ability to transcend the environmental saprophytic state to become a pathogen of humans and animals. Melioidosis is a fascinating infection in terms of pathogenesis. The outcome of the host pathogen interaction ranges from asymptomatic seroconversion to rapidly fatal and fulminant sepsis. Between these extremes the infection may run a chronic or relapsing course, or remain latent for many years before reactivation into an active infection. This outcome will depend on the interplay of several factors such as the size of the inoculum, the virulence of the infecting strain and the susceptibility of the host as well as possible as yet unknown genetic factors [11].

We set about studying aspects that contribute to the suspected virulence of the organism:

i) The mucoid colonial morphology suggests the presence of slime or extracellular polysaccharide layer. Ruthenium-red stained preparations of bacterial cultures viewed by electron microscopy revealed three morphologically distinct variants; one with a very marked and another with a less electron-dense layer surrounding the cell wall, and a third layer devoid of such a structure [52]. Subsequently it has been shown that *B.pseudomallei*

produces a highly hydrated glycocalyx polysaccharide capsule, an important virulence determinant that helps to form slime. This capsule facilitates formation of microcolonies in which the organism is both protected from antibiotic penetration and phenotypically altered [26].

ii) Intracellular penetration and survival of *B.pseudomallei.* We demonstrated by transmission electron microscopy, the internalisation of *B.pseudomallei* by human macrophages via conventional phagocytosis enclosed within membrane-bound phagosomes [53].

iii) We further showed by a quantitative approach, that phagolysosome fusion occurred slowly and inefficiently in monocytes of patients with melioidosis, leading to an increased number of intracellular organisms compared with monocytes obtained from healthy donors[54]. Our observations in this study suggest that a small number of *B.pseudomallei* are able to overcome the microbicidal armamentarium of the human host cell, to persist and multiply or remain latent in a dormant state, giving rise to relapse and recurrence at a later date.

iv) Nitric oxide, a major microbicidal mechanism in phagocytic cells, together with other reactive nitrogen intermediates produced during the respiratory burst is cytotoxic and inhibits replication of many intracellular pathogens. The activation state of macrophages can be determined by measuring the generation of 8-iso-PGF2α, a bioactive product of free radical induced lipid peroxidation. We demonstrated that macrophages obtained from melioidosis patients generated significantly lower levels of nitric oxide and 8-iso-PGF2α compared to macrophages obtained from normal subjects [56].

Burkholderia pseudomallei isolates produce a number of factors that may contribute to the progression of disease such as secretory virulence determinants: protease, catalases, peroxidases, superoxide dismutase, lipase, phospholipase C (lecithinase) and hemolysins as well as at least one siderophore. Cell-associated virulence determinants, quorum sensing, type III secretion systems and flagella have also been implicated. Resistance to complement-mediated bacteriolysis is also a key virulence determinant as described by Ismail *et al* [56].

The immune response

Burkholderia pseudomallei produce a humoral antibody response during all stages of the disease including asymptomatic seroconversion. We used 2 types of antigen preparation, a culture filtrate antigen and a whole cell antigen, and two different tests, an ELISA and an immunofluorescent antibody test (IFAT) to detect antibody levels in the sera of various groups of subjects and patients [50,57,58,59].

Collectively, these studies demonstrated:

i) Serology was useful and rapid in the presumptive diagnosis of both septicaemic and non-septicaemic melioidosis;

ii) The need to include melioidosis as one of the differential diagnosis in patients with fevers in endemic regions;

iii) Strong IgG, IgA and IgM responses were produced by melioidosis patients to the culture filtrate antigen throughout the infection;

iv) Analysis of IgG isotypes demonstrated that IgG1 followed by IgG2 were the predominant subclasses involved in the humoral response;

v) *In vivo* killing / clearing of the pathogen was not evident despite elevated Th1 response as demonstrated by the presence of IgG1 antibody response;

vi) Septicaemic patients, mainly adults, maintained high levels of antibody for many years, suggesting the continuous sequestration of the organism or bacterial products;

vii) The non-septicaemic patients were mainly children where initial high levels of antibodies were found to taper down and eradication antimicrobials were stopped when titres dropped to below diagnostic levels. They remained symptom free on follow up for up to 2 years;

viii) These studies provide evidence that monitoring either IgG or IgG+IgM antibody levels in patients under maintenance /eradication therapy may be useful as a guideline to determine the duration of this therapy.

It has been recognised that serodiagnosis is problematic in areas of endemicity due to background seropositivity. We demonstrated that if a suitable cut-off titre is used to exclude background antibody levels, then a sensitive and specific serological test can lend support to the diagnosis of melioidosis[50, 59].

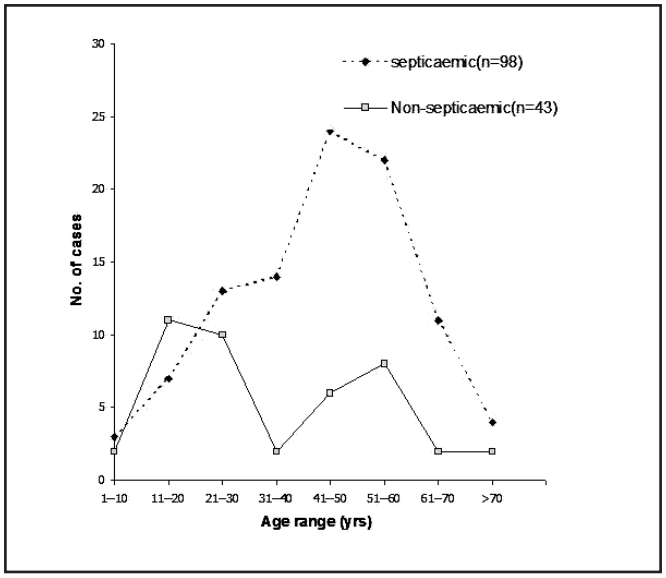
Histopathology

Histopathology was used to study human tissues infected with *B.pseudomallei.* The lesions which varied from acute to chronic granulomatous inflammation were not tissue specific. In 5 autopsy cases, the inflammations were usually focal or diffuse acute necrotising inflammation with varying numbers of neutrophils, macrophages lymphocytes and "giant cells". Numerous gram negative, non-acid fast, intra- and extracellular bacilli were also present. Intracellular bacteria within macrophages and "giant cells" were so numerous as to resemble "globi." In 14 surgical cases, biopsies showed acute inflammatory lesions, no different from acute inflammation due to other causes. However, the inflammation was either an acute-on-chronic inflammation with a focal granulomatous component, or was purely granulomatous in character. Bacilli were difficult to demonstrate in surgical biopsies even with the gram stain[60].

Apart from pathological changes of the inflammatory lesions caused as a result of *B.pseudomallei* infection, there may also be other associated pathological changes especially in the lungs of patients with acute respiratory failure. The lungs, in few of these cases where autopsy was performed, exhibited changes typical of acute respiratory distress syndrome (ARDS) with inflammation and fibrinoid hyaline membranes in the alveolar spaces [36].

Treatment and Management

Severe septicaemic melioidosis or patients with a provisional diagnosis of septicaemic melioidosis should be treated with parentral antimicrobial therapy. The conventional drug regimen was a combination of high dose chloramphenicol, doxycycline and trimethoprim-sulphamethoxazole respectively. Present day treatment consists of high dose intravenous ceftazidime (100-120 mg/kg/day) in three divided doses alone[61] or in combination with co-trimoxazole



(8-12 and 40-60mg/kg/day). This combination has been shown to be far more effective in lowering the mortality rates than the conventional regimen or ceftazidime monotherapy for septicaemic melioidosis[62]. Monotherapy with ceftazidime may be indicated in cases of simple bacteraemia with no obvious focus of infection but the clinical condition of the patient should dictate this decision.

Carbapenems kill *B.pseudomallei* more rapidly than do cephalosporins, and imipenem proved equivalent to ceftazidime in a large randomised trial; imipenem (50-60 mg/kg/day) is a safe and effective treatment for acute severe melioidosis and maybe considered an alternative to ceftazidime[63]. Even with appropriate antimicrobial therapy, the mean time to resolution of fever was approximately 9 days, but patients with large abscesses or empyema often have fluctuating fevers for more than one month and therefore parenteral antimicrobial therapy should be given for at least 10-14 days and continued until there is clear evidence of symptomatic improvement[64]. This may take several weeks, particularly when visceral abscesses are present; fever persisting for more than one week is common and does not necessarily imply treatment failure. We have successfully treated an adult patient with acute and severe melioidosis with 12 weeks of imipenem and trimethoprim–sulphamethoxazole (SD Puthucheary, unpublished observations). Physicians who are not experienced in the management of melioidosis often switch antibiotic treatment prematurely, fearing the emergence of drug resistance, but resistance to ceftazidime is rare. Enlargement of an abscess or appearance of new abscesses, especially in skeletal muscle, or seeding to a joint, is not uncommon in the first week of treatment, and is not necessarily a sign of failure[10].

Localised infections do not always need parenteral antimicrobials. Oral therapy with the conventional antimicrobial agents, ampicillin-sulbactam or amoxycillin-clavulanic acid can be used from the beginning. But an initial short course of parenteral antibiotics followed by oral drugs may sometimes be indicated especially in the older aged patients with underlying predisposing or immunocompromised states. Children with simple and uncomplicated wounds following trauma usually do well with oral therapy for 6-8 weeks[11].

Adjunctive or supportive therapy

The objective of this therapy is to reduce the in-hospital mortality of patients with severe and septicaemic melioidosis, which is usually seen in the older-aged patients. Supportive and symptomatic treatment of shock to maintain good tissue perfusion and oxygenation are of paramount importance. If possible surgical drainage of abscess should be carried out. Complications such as septic shock, acute renal failure and acute respiratory distress syndrome (ARDS) are commonly seen in severe melioidosis[36]. Therefore, these patients should be nursed in a high dependency location or in intensive care units. Patients with uncontrolled diabetes mellitus should be given continuous infusion of insulin. Partial splenectomy should be considered rather than total splenectomy, when indicated, for large solitary or a perforated abscess. Surgical curettage and debridement of affected muscle and bone is essential in musculoskeletal melioidosis in addition to antimicrobials.

Maintenance or eradication therapy

Patients who have recovered from melioidosis require long term oral maintenance therapy and follow-up because of the high risk of relapse, latency and recurrence which may lead to an acute, often fulminating, fatal infection. Long courses of oral maintenance therapy have been recommended in order to eradicate melioidosis in an approach similar to antituberculous therapy. The combination of oral chloramphenicol, doxycycline and co-trimoxazole is the most widely used maintenance therapy for melioidosis[42] but carries a significant risk of serious chloramphenicol or sulphonamide toxicity and cannot be used in children or pregnant women. Amoxycillin-clavulanic acid is a good alternative as it has good activity against *B. pseudomallei.* In a comparative trial co-amoxyclav was found to be safer and better tolerated but may be less effective than the oral "conventional" regimen[65].

There are no well-established guidelines for the duration of maintenance antibiotic therapy although 3 to 6 months has been mentioned in many reports[66]. Treatment courses of less than 8 weeks for maintenance therapy in survivors of severe disease are clearly insufficient as the relapse rate was 23%[42]. With an increase to 20 weeks of oral therapy the relapse rate was reduced significantly to 10%[65].

In our experience, relapse or recurrence of signs and symptoms have occurred while patients were on maintenance therapy and also after completing 6 months of antibiotics, with signs and symptoms appearing between 2 and 6 years later. In this group the antibody levels, measured (using an IFAT) at intervals of one to two months from initial presentation, remained high[50]. These were mainly adult patients with diabetes mellitus and other immunocompromised states. Adults with localised non-septicaemic infections showed a reduction in antibody titres over a period of 20-28 months. This decrease in titre on follow-up suggests that the infection was either being

resolved or arrested. In contrast, among patients who had developed septicaemia, the titres remained at a high level over a period of one to three years, suggesting the continuous sequestration of antigen or organisms in an intracellular or cryptic site in the host. In children with melioidosis, the initial high antibody levels were found to taper down during maintenance therapy and antibiotics were stopped when the titres decreased to below diagnostic levels. The children were followed up for an average of two years and remained symptom free. Thus we believe that serological follow-up may be useful as a guideline to determine the optimal duration of maintenance antibiotic therapy for this persistent and potentially lethal infection. Life-long follow-up and counselling, particularly regarding compliance, should be a requirement in the prevention of relapse and recurrence [59].

Empirical Therapy

As *B.pseudomallei* is intrinsically resistant to most beta-lactams and aminoglycosides, it is often untreated by empirical broad-spectrum antibiotic therapy (such as ampicillin and gentamicin) for patients admitted with suspected septicaemia. In our series of 50 septicemic melioidosis cases, 76% of the patients did not receive appropriate empirical antibiotic therapy effective against *B. pseudomallei.* Another situation is when unconfirmed cases of pulmonary melioidosis may be empirically treated with anti-tuberculous drugs [7]. In most Southeast Asian countries both diseases are usually endemic and therefore awareness is essential. Amoxycillin-clavulanic acid is appropriate as empirical broad-spectrum antibiotic in areas where *B. pseudomallei* is a recognised pathogen, but once the diagnosis has been confirmed, ceftazidime remains the treatment of choice in acute presentations [64].

Prevention and Prophylaxis

There is no effective vaccine available that protects against *B.pseudomallei* infection and the prospect of one in the immediate horizon seems remote! In the endemic regions of southeast Asia, the sites most likely to yield *B.pseudomallei* are cleared, cultivated and irrigated agricultural lands, but exposure to this organism is very difficult to prevent in rural rice growing areas. It would be ideal for persons engaged in occupational or recreational activities to take simple precautionary measures such as covering all open wounds with waterproof dressings and wearing boots and gloves during outdoor activities. In endemic areas where *B.pseudomallei* is an ubiquitous environmental saprophyte, it is expected that clinical diagnostic laboratories would process samples at a BSL-2 level facility, and safe laboratory practice will serve to minimise the risk of exposure. Accidental exposure in the laboratory to *B.pseudomallei* must be reported to the laboratory safety officer and post exposure management should be offered [67].

CONCLUSION

In Malaysia the true incidence and epidemiology is still unknown and thus there is a compelling need for it to be made a notifiable disease [68]. This disease remains greatly under-diagnosed in the tropics and hence there is a need for greater awareness and improved diagnostic microbiology services, which will enable early and rapid diagnosis and treatment to overcome the high mortality rates. This environmental saprophyte of tropical and subtropical countries is not going to disappear. Therefore more work need to be carried out on the distribution and incidence of melioidosis in Malaysia. There has been increased interest in melioidosis since *B.pseudomallei* has been designated as a potential agent for biological warfare and terrorism by the CDC (www.cdc.gov/od/sap) and hopefully there will be growing research interest in all aspects of melioidosis.

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