MELIOIDOSIS

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Melioidosis is a tropical infectious disease which is caused by *Burkholderia* (formerly Pseudomonas) *pseudomallei*. The disease is common in Southeast Asia especially in the northeastern part of Thailand and Northern Australia. It is one of the most important causes of fatality from community acquired septicemia.[1] The septicemic form of melioidosis often deteriorates rapidly, and death often occurs within the first few days after hospitalization.[2] Rapid diagnosis and prompt proper antibiotic treatment can reduce the mortality. The first case, a Burmese patient, described by Whitmore A and Krishnaswami CS in 1912,[3] had acute fulminating form, presenting with fever of unknown origin but was proven by autopsy and culture. In Thailand, the first reported cases were two foreign prisoners in 1947 and the first Thai patient was reported in 1955. Presently, the incidence is 137.9 per 100,000 hospital patients in the northeastern part of Thailand.[4]

Bacteriology

Burkholderia pseudomallei (B. pseudomallei) is a small gram negative aerobic bacillus, some has bipolar staining as a safety pin. It is widely distributed as a saprophyte in moist soil and water, particularly in rice fields. The organism survives during the dry season in the clay layer of the soil 25-30 cm from the surface for at least 2 years, and is spreaded by rising water table during the rainy season.[5] It can be found in the area 20^0N-20^0 S of the equator such as Myamar, Loas, Cambodia, Vietnam, Malaysia, Indonesia, Singapore, Philippines, Guam, Australia and Thailand especially in northeastern part.[6,7] In blood agar culture plate, it has hemolysis, white-grey colony color, mud odor and a few days later, the colony shrinks. Identification of this organism is by the characteristic of the colony, odor, gram staining and chemical reaction, which are different from *Pseudomonas cepacia* and *stutzeri*. The special media is TSA-G-CL-B (trypticase

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soy agar-gentamicin 4 mcq/ml-colistin 10 mcq/ml-bile salt 0.1%) which increases growth of *B. pseudomallei* and inhibits growth of the other bacteria. *B. pseudomallei* is an intracellular organism like Salmonella and can survive in the phagocytes and so is facultative intracellular bacteria. It has the glycocalyx that composes of biofilm which protects it from phagocytosis and resistant to antibiotics.

The possible modes of entry are inoculation, inhalation, and oral mucosal entry. The inoculation from soil and water causes skin and subcutaneous infection which is the most likely pathogenesis. The inhalation causes pulmonary infection. The oral mucosal entry causes pharyngitis and cervical lymphadenitis. Up till now, there has not been a report of transmission from animal to human but there was a report of transmission from human to human via sexual act.[8] The clinical symptoms are chronic prostatitis, prostatic abscess and epididymo-orchitis. The natural history of human *B. pseudomallei* infection is unclear. Reactivation is the main mechanism of clinical manifestation, after this organism has entered the body and survives in reticuloendothelial system if cellular immune response is defective or dysfunction, the organism reappears in the blood causing transient bacteremia and acute septicemia or dissemination in compromised host.[9]

In endemic area, seroepidemiological surveys suggests that the majority of people exposed to this organism develops subclinical or asymptomatic infections,[10] commonly in childhood as 80 percent of children had antibodies by the age of four years.[2] There is no evidence of asymptomatic carrier.[11] The occurrence of *B. pseudomallei* infections correspond with the distribution of *B. pseudomallei* in the soil.[4]

Pathogenesis

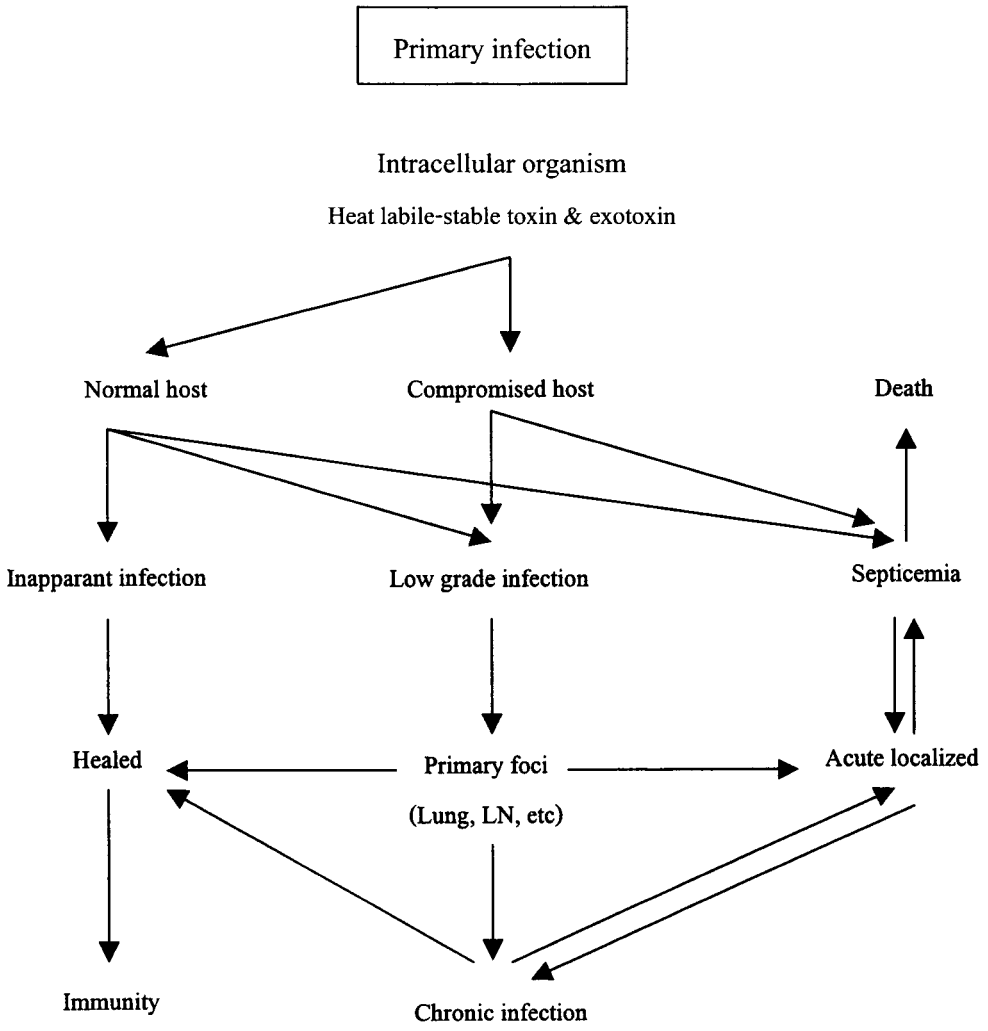
The pathogenesis of melioidosis is as in figure 1.

Cliniical classification and clinical features

In 1989, Thai national workshop on melioidosis classified melioidosis into 6 groups according to the clinical features,[12] as in table 1.

1. Disseminated Septicemic Melioidosis (DSM)
2. Non-disseminated Septicemic Melioidosis (NSM)
3. Localized Melioidosis (LM)
4. Transient Bacteremic Melioidosis (TM)
5. Probable Melioidosis (PM)
6. Subclinical Melioidosis (SM)

Clinical picture of DSM consists of multiple organs involvement, positive hemoculture, rapid progression, 89 percent with septic shock and mortality rate 85-95 percent. With inappropriate management, most patients died within 24-72 hours after admission. Clinical picture of NSM consists of few organs involvement, positive hemoculture, 5 percent with septic shock and mortality rate of 20-30 percent. The clinical manifestation of septicemic form can not be differentiated from septicemia caused by other organisms.[13] Clinical picture of LM consists of few organs involvement, negative hemoculture, slow progression, and mortality rate of 10-20 percent. Inappropriate treatment may progress to septicemia. Clinical picture of TM consists of only transient positive hemoculture without organ involvement.



Clinical picture of PM consists of abnormal foci in internal organ that may be melioidosis, with melioid titer being positive more than or equals to 1:160. Clinical picture of SM is only positive melioid titer more than or equals to 1:40 without organ involvement and negative hemoculture.

Clinical features of melioidosis vary, ranging from localized, benign disease to fulminant septicemia. In Thailand, septicemia is present in 57.4 percent, pulmonary infection in 44.9 percent, skin-soft tissue infection in 13.6 percent and hepato-biliary infection in 11.2 percent. In the northeastern part of Thailand, B. pseudomallei is a common cause of community acquired septicemia. The clinical presentation of septicemic melioidosis varies between a simple febrile illness to fulminant septicemia. Three main factors that influence clinical features and outcome depend on the balance between the virulence and density of

Table 1 Clinical classification and mortality in melioidosis

Type of melioidosis	Incidence (%)	Clinical features	Mortality (%)
1. Disseminated Septicemic Melioidosis (DSM)	45	Positive hemoculture, disease involves multiorgans	85 - 95
2. Non-disseminated Septicemic Melioidosis (NSM)	12	Positive hemoculture, diseasse involves one or few organs	20 - 30
3. Localized Melioidosis (LM)	42	Negative hemoculture, diseaseusually involves single organ	10 -20
4. Transient Bacteremic Melioidosis (TM)	1	Transient bacteremia, self-limited, benign course	-
5. Probable Melioidosis (PM)	?	IHA* positive, clinical features compatible with melioidosis	-
6. Subclinical Melioidosis (SM)	very common	IHA* positive, no clinical symptoms of infections	-

* IHA : indirect hemagglutination

organism, immune status of host, and type of treatment.[14] The density of organisms in the infection site and the amount of endotoxin and exotoxin may play major roles in clinical manifestations of disease. More than half of the patients had underlying conditions such as diabetes, chronic renal failure, urolithiasis and liver cirrhosis.[15,16] The disease tends to be severe in patients with defective immune response. Further, the quiescent infection may be reactivated and develops into acute and severe forms of infection when immune response is impaired. In septicemic melioidosis, half of the patients had no obvious source of infection and most patients die within the first 5 days of conventional treatment with chloramphenicol, tetracycline, kanamycin, and co-trimoxazole.

Organ involvements in melioidosis are pulmonary, gastrointestinal, urogenital, skin and soft tissue. Pulmonary involvements are multiple metastatic pneumonia, acute lobar pneumonia, subacute or chronic lung infection, pleural effusion or empyema.[17] Pulmonary involvement are different from tuberculosis

such as sparing of apical part of the lung, no calcification or hilar adenopathy, no fibrosis, rapid progression to cavity, miliary spreading but bigger size than tuberculosis.[17] Urogenital involvements are urinary tract infection or renal abscess. Skin and soft tissue involvements are superficial erythematous pustules of skin, cutaneous abscess, chronic ulcer and lymphadenitis.

Gastrointestinal involvement includes abscesses of solid internal organs. In endemic area, the abscesses were found in the spleen in 72.8 percent of cases, liver 45.7 percent, kidney 12.3 percent, prostate gland 2.5 percent and multiple organs 76 percent.[18] Liver abscess must be differentiated from amoebic liver abscess in adult, staphylococcal liver abscess in children and other organism. Pyogenic liver abscess caused by *B. pseudomallei* is among the most common pathogen in the northeastern part of Thailand.[19] Most of the melioidosis liver abscesses had clinical course as subacute to chronic. The positive physical signs that help to differentiate melioidosis from the others were splenomegaly and pneumonia.[19] Typical ultrasonographic findings of melioidosis liver abscess are multiple and involvement of both lobes of the liver, associated splenic and or kidney abscesses.[19,20] Typical cartwheel appearance in ultrasonography found in only 40 percent. The identification method of the organism is percutaneous liver aspiration under ultrasound or computed tomography guidance with fresh smear, gram's staining and culture. This method may cause post-aspirated septicemia and death thus the proper antibiotics should be used before percutaneous liver aspiration. Splenic abscess is uncommon but it should make one think of melioidosis in endemic area.

Investigations

Recent developments in various aspects of laboratory diagnosis of melioidosis have exhibited potentials for significant improvement that leads to the rapid diagnosis.

1. Detection of specific antibody

The first serodiagnosis for detecting melioid antibody in serum is indirect hemagglutination antibody test (IHA). The IHA test on admission is a sensitive but not specific, as a significant proportion of the healthy population are also seropositive. A negative IHA result is of value in excluding a diagnosis of melioidosis, but high titer does not mean *B. pseudomallei* infection. This method can detect melioid antibody in approximately 70 percent within the first 2 weeks, peak at 4-5 months and persistence for 6-9 months. The melioid antibody level is not correlated with symptom, severity of disease, and prognosis but this level is increased after treatment may relapse. The interpretation of IHA melioid antibody titer is as table 2.[21,22]

2. Detection of bacterial antigens

Many methods of bacterial antigen detection in blood and urine have been developed and studied such as polyclonal or specific monoclonal antibody to B. pseudomallei exotoxin by ELISA.[23-27]

3. Bacterial isolation

In laboratory, the colony in culture media looks like contaminants, which is easily misdiagnosed. Bacterial isolation by culture from clinical specimens such as blood, sputum, urine or pus is reliable and

Table 2: IHA melioid antibody interpretation

Melioid antibody titer	Interpretation	Recommendation
< 1 : 80	Negative	
1 : 80 - 1 :320	Suggestive	Treatment if clinical not well
> 1 : 320	Very likely	Empirical treatment and looking for source of infection
> 1 : 1,280	Acute infection	

sensitive but it needs to take around 3-5 days to obtain the results. Two ways for improvement are the culture media and the use of automated culture systems. *B. pseudomallei* grows easily in common bacterial culture media but tends to be overgrown by other bacteria. The special media is TSA-G-CL-B (trypticase soy agar-gentamicin 4 mcq/ml-colistin 10 mcq/ml-bile salt 0.1%) which increases growth of *B. pseudomallei* and inhibits growth of the other bacteria. The growth of *B. pseudomallei* in blood culture system using an automated BacT/Alert automated blood culture system can be detected within less than 48 hours, of which more than half are detectable within the first 24 hours.[13] The shorter time of detection of the bacterial growth in blood cultures may reflect a higher bacterial level in the patient at the time blood was taken, and may be responsible for the poor clinical outcome.[28]

4. Detection of bacterial genetic materials

Specific DNA probe or polymerase chain reaction (PCR) for *B. pseudomallei* was developed and used to test in clinical specimens. Some data were able to detect the bacteria in blood from the major of septicemic melioidosis patients, as sensitive as conventional blood culture method.[29,30]

5. Radiologic findings

Ultrasonography or computed tomography of abdomen can be used to identify disease localization especially abscess formation. Ultrasonography is easier and requires less time than computed tomography. Typical ultrasonographic appearance is Swiss cheese or cartwheel especially in the liver and spleen. Abscess formation tends to be multiple small cavities in the liver parenchyma and can be found in the liver, spleen and kidney. Splenic abscess is an unusual occurrence in most infections, but more than one-half of the patients with melioidosis liver abscess also had an abscess in the spleen. Thus, the finding of a splenic abscess is a strong diagnostic indicator of melioidosis.[31,32]

Diagnosis

Awareness is a good way for diagnosing this disease because its clinical manifestation is similar with other organism include tuberculosis.[33] In endemic area, the setting of underlying risk with multiple organ

involvement and septicemia should be managed as melioidosis.[34]

Clinical characteristics that should make one aware of the diagnosis of melioidosis.

1. Rapidly progressive septicemia and or septic shock with no identifiable source.

2. Patients with severe sepsis within 2-3 days of post burn or injuries that had contact with natural water, soil or mud.

3. Rapidly progressive community acquired pneumonia especially in the near drowning.

4. Acute bilateral metastatic pneumonia in non-drug addict person.

5. Multiple abscesses of internal organs especially multiple organs such as liver, spleen, renal, lung, and brain.

6. Chronic or subacute pulmonary infiltration or lung abscesses with previous diagnosis of tuberculosis and non-response to treatment within 2-3 weeks.

7. Chronic or subacute infections of skin, soft tissue, muscle, lymph node, and parotid gland with small bipolar staining gram negative bacilli in discharge by Gram's stain.

8. The compromised host with fever of unknown origin, pleural effusion, pericardial effusion, septic arthritis, osteomyelitis, peritonitis, meningitis, mastoiditis, or pharyngocervical lymphadenitis which had high melioid antibody level.

Treatment

DSM, NSM and LM are to be treated, PM should be treated, and TM is not to be treated but should be followed up closely.

Medical treatment should consist of a combination antibiotics especially in septicemic form[35] such as ceftazidime, cefoperazone/sulbactam[36], co-amoxiclav[37,38], piperacillin, imipenam[39,40], co-trimoxazole, tetracycline, doxycycline, chloramphenicol, kanamycin. The recommendation of antibiotics regimen in endemic area include intravenous ceftazidime alone or plus intravenous co-trimoxazole, or intravenous co-amoxiclav or high dose of intravenous imipenam or intravenous cefoperazone/sulbactam plus intravenous co-trimoxazole for septicemic form,[41-44] and oral doxycycline plus oral co-trimoxazole for non-septicemic form.

Dosage of antibiotics are, ceftazidime 2 g IV q 8 hours or 120 mg/kg/day in monotherapy or 100 mg/kg/day in combination with other drugs. Co-trimoxazole 2 ampules IV q 8 hours or 8 mg/kg/day of trimethoprim. Doxycycline 100 mg IV q 12 hours or 4-6 mg/kg/day. Co-amoxiclav 2.4 gm IV loading then 1.2 gm IV q 4 hours. Imipenam 1 gm IV q 8 hours or 60 mg/kg/day.

The response to treatment is slow. The median fever clearance time is 5.5 days (1-50 days) from starting appropriate therapy.

Duration of optimal treatment is not known. Data from many studies, melioidosis had overall relapse rate of 5-20 percent per year of follow up.[45] The factors influencing the likelihood of relapse include clinical severity at initial presentation, type of parenteral and oral antibiotics used, and the survival of *B. pseudomallei* in protected sites, such as within phagocytic cells in sealed abscess. Usual recommendation is at least 3 months duration and 6 months if osteomyelitis is present.[1,45] In septicemic form, give

antibiotic intravenously initially and switching to oral maintenance if clinical picture improves and no fever for 48-72 hours. In non-septicemic form, the oral antibiotics can be used from the beginning. The effective oral maintenance antibiotics treatment such as doxycycline plus co-trimoxazole or high dose amoxicillin plus clavulanic acid.[46,47] Dosage of oral antibiotics are doxycycline 4 mg/kg/day or 100 mg oral bid; Co-trimoxazole 8 mg/kg/day of trimethoprim; Co-amoxiclav 375 mg 2 tablets oral qid plus amoxycillin 250 mg oral qid.

Splenectomy is recommended in patient who has splenic abscess because these patients will commonly have recurrence after stopping antibiotic.

For follow up the clinical picture is used along with serum C-reactive protein (CRP). CRP is correlated with serum specific IgM antibodies. Serial CRP can help to identify the proper duration of treatment and occult or unsolved infection.[48]

The prevention of exposure to *B. pseudomallei* exposure in the community is very difficult as the organism is widely distributed in the environment. An effective vaccine may be a possible preventive measure in the future. The lipopolysaccharide II of *B. pseudomallei* would be a potentially useful component of a vaccine developed against fatal melioidosis.[49,50] The diabetic rice farmer would be the most appropriate target population for such a trial of prevention.

Conclusion

Melioidosis is an important tropical infectious disease especially in Southeast Asia and Northern Australia. With increasing world wide travel of both human and other animal, the potential exists for melioidosis to spread to new and fertile pastures. Specific diagnosis of melioidosis requires awareness on the part of clinicians, and the existence of a laboratory capable of rapidly isolating and identifying *Burkholderia pseudomallei.* In endemic area, the septicemic patient who has underlying diabetes or chronic renal failure that have multiple organs involvement, should be managed as melioidosis. Rapid diagnosis and prompt proper antibiotic treatment are the way to reduce the mortality especially in septicemic form.

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