Am. J. Trop. Med. Hyg., 91(6), 2014, pp. 1176–1178
doi:10.4269/ajtmh.14-0354

Short Report: Predictors of Severe Disease in Melioidosis Patients in Kuala Lumpur, Malaysia

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Abstract. The predictors of severe disease or death were determined for 85 melioidosis patients in Kuala Lumpur, Malaysia. Most of the patients were male, > 40 years old, and diabetic. Severe disease or death occurred in 28 (32.9%) cases. Lower lymphocyte counts and positive blood cultures were significant independent predictors of severe disease, but age, presentations with pneumonia, inappropriate empirical antibiotics, or flagellin types of the infecting isolates were not. Knowledge of local predictors of severe disease is useful for clinical management.

Melioidosis is endemic in southeast Asia and northern Australia. Clinical manifestations are diverse, with the most severe forms often leading to death. Mortality rates vary widely between settings (for example, between Australian [19%] and Thai [50%] patients).[1] This may be because of differences in patient populations, strain virulence, environmental factors, and healthcare facilities. It is not certain whether variations within virulence coding genes influence severity of melioidosis. The flagellin protein in *Burkholderia pseudomallei* is involved in mobility, invasion, and virulence.[2] The flagellin (*fliC*) gene has been used to type *B. pseudomallei* by polymerase chain reaction (PCR) -restriction fragment-length polymorphism (RFLP).[3] However, flagellin types have yet to be correlated with clinical presentation. It is important to predict which patients are most at risk of severe disease to institute earlier interventions, such as appropriate antibiotics and intensive care unit (ICU) admission. Therefore, the objectives of this study were to determine clinical and laboratory predictors of severe melioidosis, including flagellin types.

This retrospective study reviewed clinical and laboratory data of patients with a first presentation of culture-positive melioidosis from any site from August of 1988 to June of 2010 at a teaching hospital in Kuala Lumpur, Malaysia. A standard data collection form was used. Severe disease was defined as death or requirement of ICU admission, ventilation, or inotropic support. Appropriate empirical antibiotics were defined as treatment started before culture results were available using any of the antibiotics recommended for melioidosis treatment, which are ceftazidime, imipenem, meropenem, coamoxiclav, doxycycline, chloramphenicol, and cotrimoxazole.[4] Approval was obtained from the hospital's Medical Ethics Committee (reference no. 733.8).

Flagellin gene typing was carried out on available stocked isolates of *B. pseudomallei* as described in an earlier study,[3] which included isolates of 24 patients included in this study. Bacterial isolates were recovered from stocks on Mueller–Hinton or blood agar at 37°C for 24–48 hours. Colonies were suspended in 500 μL sterile distilled water, heated at 100°C for 30 minutes, cooled down in ice, briefly vortexed, and centrifuged at 12,000 rpm for 1 minute. The supernatant was used as the DNA template for amplification of the *fliC* gene using the primer pairs BC6E (5′-ACCAACAGCCTGCAGCGTATC-3′) and BCR14 (5′-TTATTGCAGGAGCTTCAGCAC-3′).[5] A 50-μL reaction mixture was prepared containing 5 μL 10× *Taq* buffer with KCl, 2 mM $MgCl_2$, 0.2 mM each dATP, dCTP, dGTP, and dTTP, 0.5 μM primers, 2.5 U *Taq* DNA polymerase (Fisher Scientific, Pittsburgh, PA), and 2.5 μL template DNA. PCR was performed in a MyCycler thermal cycler (Bio-Rad, Hercules, CA) at 95°C for 5 minutes followed by 35 cycles of 95°C for 1 minute, 60°C for 1 minute, and 72°C for 1.5 minutes with a final extension at 72°C for 10 minutes.

After confirmation of the product size by agarose gel electrophoresis, the amplified products were subjected to digestion using two restriction enzymes: FastDigest *Msp*1 and *Sau*96I (*Cfr*13I; Fisher Scientific); 2 μL 1× FastDigest Green Buffer was mixed with 1 μL each of the enzymes, 10 μL amplified DNA, and 2 μL sterile water, and the mixture was then incubated for 15 minutes at 37°C. The final products of restriction digestion were separated by electrophoresis on a 2.5% agarose gel pre-stained with ethidium bromide and visualized using a UV illuminator (Syngene, Cambridge, UK).

Data were analyzed with the Statistical Package for the Social Sciences, version 18 (IBM, Armonk, NY) to determine predictors for severe disease. Univariate logistic regression was first carried out. Crude odds ratios (ORs) with 95% confidence intervals (95% CIs) were calculated, and variables with $P < 0.2$ were then subjected to multivariate logistic regression using stepwise selection. Adjusted ORs (aORs) with 95% CIs were calculated, and variables with $P \leq 0.05$ were considered significant. The final model was assessed with the Hosmer and Lemeshow goodness-of-fit test and the area under the curve of the receiver operating characteristic curve.

In total, 132 patients had positive cultures for melioidosis. Medical records were not available for 47 (35.6%) cases, leaving 85 patients included in the study. Of these patients, 28 (32.9%) patients had severe disease (Table 1). Most patients were male (75.3%) and > 40 years old (69.4%). The mean age was 46.4 years (range = 9–80 years). The distributions of the major ethnic groups were significantly different among the melioidosis cases compared with the general population (Indian: 40% versus 9.3%; Malay: 31.8% versus 40.6%; Chinese: 22.3% versus 39.1%).[6] Diabetes mellitus was the most common associated risk factor for melioidosis ($N = 57$; 67.1%), like in other studies.[7–10] In Malaysia, Indians have the highest prevalence of diabetes[11] and the poorest glycemic control,[12] which may explain the overrepresentation of Indians among the cases. However, ethnicity did not predict severe disease. Other risk factors for melioidosis were renal dysfunction ($N = 14$; 16.5%), increased alcohol intake ($N = 13$; 15.3%), chronic lung disease/asthma ($N = 11$; 12.9%), immunosuppression ($N = 11$; 12.9%), and recent jungle trekking ($N = 3$; 3.5%).

For 70 patients for whom occupation history was available, the most commonly reported jobs were commercial vehicle

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TABLE 1
Selected results of univariate and multivariate logistic regression analyses

Predictor	*n* (%)	No. with severe disease, *n* (%)	Crude OR (95% CI)	*P* value	Adjusted OR (95% CI)	*P* value
Age, years	85	28 (32.9)	1.03 (1.00–1.07)	0.028	–	NS
Gender						
Male	64 (75.3)	21 (32.8)	1		–	–
Female	21 (24.7)	7 (33.3)	1.02 (0.36–2.92)	0.97		
Ethnicity						
Indian	34 (40.0)	11 (32.4)	0.72 (0.10–4.93)	0.74	–	–
Malay	27 (31.8)	9 (33.3)	0.75 (0.11–5.32)	0.77		
Chinese	19 (22.3)	6 (31.6)	0.69 (0.09–5.29)	0.72		
Other	5 (5.9)	2 (40.0)	1			
Any risk factors for melioidosis						
No	7 (8.2)	2 (28.6)	0.8 (0.15–4.41)	0.80	–	–
Yes	78 (91.8)	26 (33.3)	1			
Pneumonia						
No	47 (56.0)	10 (21.3)	1		–	NS
Yes	37 (44.0)	18 (48.7)	3.51 (1.36–9.07)	0.010		
Bacteremia						
No	42 (49.4)	7 (16.7)	1		1	
Yes	43 (50.6)	21 (48.8)	4.77 (1.74–13.08)	0.002	4.05 (1.31–12.53)	0.015
Empirical antibiotics						
Appropriate	20 (24.1)	9 (45.0)	1	0.23	–	–
Inappropriate	63 (75.9)	19 (30.2)	0.53 (0.19–1.48)			
Lymphocyte count	73	26 (35.6)	0.91 (0.84–0.98)	0.008	0.91 (0.84–0.99)	0.023
Platelets	78	24 (30.8)	0.995 (0.991–0.999)	0.018	–	NS
Serum bilirubin	72	25 (34.7)	1.02 (1.00–1.04)	0.041	–	NS
Serum alanine transaminase	74	21 (28.4)	1.01 (1.00–1.02)	0.019	–	NS
Serum urea	83	27 (32.5)	1.06 (1.00–1.12)	0.052	–	NS
Flagellin type of isolate						
I	30 (75.0)	19 (63.3)	1		–	–
II	6 (15.0)	2 (33.3)	0.29 (0.05–1.85)	0.19		
III	4 (10.0)	3 (75.0)	1.74 (0.16–18.80)	0.65		

NS = not significant.

drivers ($N = 13$; 18.6%), construction workers ($N = 8$; 11.4%), and work involving plants and soil ($N = 8$; 11.4%). These occupations involve exposure to soil, dust, and wind, increasing exposure to the environmental *B. pseudomallei*. In Brazil, there is a significant association between construction workers and *B. pseudomallei* seropositivity,[13] which may be caused by the liberation of organisms into the air during soil excavation.[14]

The majority of the patients presented with pneumonia (44.0%) and fever (41.7%) followed by skin or soft tissue infections (27.4%). In total, 43 (50.6%) patients had bacteremia. The median duration of illness before hospital visit was 14 days (range = 0–360 days), and the median length of hospital stay was 20 days (range = 0–210 days).

The following variables of interest did not predict severe disease: age, flagellin type, ethnicity, any risk factor for melioidosis, and inappropriate empirical antibiotics (Table 1). The final multivariate regression model showed two independent predictors of severity, lower lymphocyte counts (aOR = 0.91; 95% CI = 0.84–0.99) and presence of positive blood cultures (aOR = 4.05; 95% CI = 1.31–12.53), which were also found in other studies.[10,15] This model had satisfactory fit and discrimination (goodness-of-fit $P = 0.80$; area under the curve = 0.77). T-cell lymphocytes and natural killer cells are depleted in acute melioidosis.[16] Because both are important sources of interferon-γ, which plays an important role in resistance to *B. pseudomallei*,[17] lower lymphocyte counts may result in a poorer response to acute infection. Other reported independent predictors of severity include Acute Physiology and Chronic Health Evaluation II (APACHE II) scores, interleukin-6 (IL-6), pneumonia, low platelet counts, fever, urea levels, hypoxia, and altered sensorium.[7,10,18]

In total, 63 (75.9%) patients were started on inappropriate empiric antibiotics before diagnosis, although 56 (65.9%) patients eventually received appropriate intensive-phase therapy after diagnosis. Of these patients, 47 (83.9%) patients were prescribed ceftazidime, and 9 (16.1%) patients were given a carbapenem, although the proportion of patients on a carbapenem who had severe disease was higher (77.8%) compared with those on ceftazidime (23.4%). Of 65 (76.5%) patients who survived, 54 (85.8%) patients were discharged with melioidosis eradication therapy, and most of these (63.6%) patients were prescribed a two- or three-drug combination therapy. Surprisingly, there was a non-significant higher rate of severe disease in patients with appropriate empiric therapy (45.0%) compared with those with inappropriate therapy (30.2%). This finding was also reported in another Malaysian center,[19] and it is contrary to the experiences of other centers, where incorrect empiric therapy was associated with worse outcomes.[20–22] It may be that the Malaysian patients presented late with advanced disease, and appropriate treatment had less impact on outcome. Another possibility is that those presenting with severe disease were more likely to be suspected of having melioidosis and started on specific antimelioid therapy.

Flagellin typing results of 24 isolates were reported in an earlier study.[3] Another 16 isolates were typed in this study, making a total of 40 isolates. Of these isolates, 30 (75%) patients had flagellin allelic type I, 6 (15%) patients had flagellin allelic type II, and 4 (10%) patients had flagellin allelic type III, but there was no association with severity of disease. Other studies have also failed to find a link between molecular types with disease presentation,[23,24] suggesting that host factors are more important in determining the course of the disease.

Cheng and others[15] developed a scoring system for predicting mortality in melioidosis in Darwin, Australia based on seven criteria: presence of pneumonia, age, lymphocyte count, serum urea, bilirubin, creatinine, and bicarbonate. A cutoff of score of three stratified the patients into two groups, low risk (mortality < 10%) and high risk (mortality > 40%), with a positive predictive value (PPV) of 44.6% and a negative predictive value (NPV) of 91.4%. The scoring system was evaluated using 67 cases in our study for which at least six data values were available, giving a similar PPV of 57.9% and a similar NPV of 86.2%. This scoring system may, therefore, be useful in Malaysian settings.

In conclusion, patients with melioidosis presenting to our center commonly were older, were male, had diabetes mellitus or renal disease, and presented with fever and pneumonia. Severe morbidity and mortality rates were high (32.9%). Lower lymphocyte counts and positive blood cultures during admission were significantly associated with severe melioidosis. Study of the local factors influencing severity of melioidosis is important, because they likely vary between locations and will benefit clinical interventions.

Received June 9, 2014. Accepted for publication August 19, 2014.

Published online September 22, 2014.

Acknowledgments: We acknowledge the staff of the Microbiology Diagnostic Laboratory of University Malaya Medical Centre, Kuala Lumpur, who were involved in the processing of the clinical samples.

Financial support: The study was funded by PPP Grant P0042/2009B and High-Impact Research Grant E000013-20001 from University Malaya.

Disclaimer: The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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