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Role and Significance of Quantitative Urine Cultures in Diagnosis of Melioidosis

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Melioidosis is associated with significant mortality in countries in which it is endemic. Previous studies have demonstrated that quantitative *Burkholderia pseudomallei* counts in blood are predictive of mortality. Here we examine the relationship between outcomes and quantitative *B. pseudomallei* counts in urine. A total of 755 patients presenting to Sappasithiprasong Hospital, Ubon Ratchathani, northeast Thailand (in the northeast part of the country), with melioidosis between July 1993 and October 2003 had quantitative urine cultures performed within 72 h of admission. Urine culture results were divided into the following groups: (i) no growth of *B. pseudomallei* from a neat sample or pellet, (ii) positive result from a centrifuged pellet only ($<10^3$ CFU/ml), (iii) detection of between 10^3 CFU/ml and 10^5 CFU/ml from a neat sample, or (iv) detection of $\geq 10^5$ CFU/ml from a neat sample. The overall in-hospital mortality rate was 45%. Patients with negative urine cultures had the lowest death rate (39%). Mortality rates rose with increasing *B. pseudomallei* counts in urine, from 58% for those with positive spun pellets only to 61% for those with between 10^3 CFU/ml and 10^5 CFU/ml and 71% for those with $\geq 10^5$ CFU/ml. This was independent of age, presence of bacteremia, known risk factors for melioidosis such as diabetes, and the prior administration of antibiotics. The presence of *B. pseudomallei* in urine during systemic infection is associated with a poor prognosis.

Melioidosis, the disease caused by the gram-negative bacillus *Burkholderia pseudomallei*, is associated with significant mortality in countries in which it is endemic. The case fatality rate is approximately 50% for Thai adults and 19% for patients in northern Australia (2, 9). Delineating predictors of a poor outcome following admission provide clinical tools with which to identify high-risk patients. Previous studies have demonstrated that the clinical severity (1), bacterial count in the blood (8), and magnitude of the inflammatory response (6) are predictive of mortality for patients with melioidosis. Defining colony counts in blood is straightforward but is only applicable to patients with positive blood cultures upon presentation (50% of patients, in our experience) and is relatively expensive for resource-poor regions of the world.

Quantitative urine culture is a standard, inexpensive diagnostic test that is widely undertaken to provide laboratory evidence of urinary tract infection. The presence of $\geq 10^5$ CFU/ml in urine culture is used to distinguish between a contaminated sample and one from a patient with urinary tract sepsis and is generally accepted as the gold standard for laboratory diagnosis (5). This interpretation is not applicable to patients with suspected melioidosis. *B. pseudomallei* is not a member of the human commensal flora, and the isolation of even a single colony in urine is both significant and sufficient to confirm the diagnosis of melioidosis. Furthermore, the significance of *B. pseudomallei* counts in urine is poorly understood. Although the urinary tract may be the primary source (especially in nephrolithiasis) of the organism or may become involved in the infective process, it is unclear whether the standard cutoff of $\geq 10^5$ CFU/ml indicates an infection of the bladder, kidneys, or prostate. For this study, we explored the relationship between urinary symptoms and positive urine culture results for *B. pseudomallei*, determined whether the *B. pseudomallei* count in urine represents a marker for mortality risk, and defined the role of this simple, inexpensive technique in the diagnosis of melioidosis.

MATERIALS AND METHODS

Patients were recruited prospectively between July 1993 and October 2003 by a study team at Sappasithiprasong Hospital, Ubon Ratchathani, Thailand (located in the northeastern part of the country). Patients with suspected melioidosis were actively sought during twice-daily rounds of the medical and intensive care wards, together with passive surveillance of the surgical and pediatric wards as a part of eligibility screening for clinical trials. Detailed clinical information, including the treatment regimen, was recorded. Microbiological specimens for blood culture and throat swabs were taken from all patients, and urine, pus, and sputum were collected when available. All culture-proven melioidosis patients who had urine cultures within 3 days of admission were enrolled in the study.

Urine was collected into a sterile container and transported immediately to our on-site microbiology research laboratory. Using a sterile calibrated loop, we streaked 1 μl of fresh, unprocessed urine onto one half each of a MacConkey agar plate and an Ashdown's agar plate and then incubated the plates at 37°C for 2 days or 4 days, respectively. The remaining urine sample was centrifuged at 3,000 rpm for 5 min, excess supernatant was removed, and the pellet was cultured

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on one half of an Ashdown's agar plate. *B. pseudomallei* was identified by standard methodology as previously described (3).

For the purposes of analysis, urine culture results were divided into the following four groups based on the presence and number of *B. pseudomallei* cells: (i) no growth of *B. pseudomallei* from a neat sample or pellet, (ii) culture of *B. pseudomallei* from the centrifuged pellet only ($<10^3$ CFU/ml), (iii) detection of between 10^3 CFU/ml and 10^5 CFU/ml, or (iv) detection of $\geq 10^5$ CFU/ml in a neat sample. Because renal tubular acidosis is endemic to this region, we specifically examined the profiles of a subgroup of patients with nondiabetic renal disease. Statistical analysis was performed with the statistical program Intercooled STATA, version 8.0 (College Station, Tex.). The trend across ordered groups was tested with a χ^2 test for trend. Logistic regression analysis was used to examine the independence of factors associated with quantitative urine cultures and the prognostic value of quantitative urine cultures for the survival outcome. Ethical approval for all clinical trials was obtained from the Ministry of Public Health, Royal Government of Thailand.

RESULTS

During the 10-year study period, 1,950 patients were admitted to Sappasithiprasong Hospital with culture-confirmed melioidosis. Quantitative urine cultures were performed within 72 h of admission in 822 of these cases 230 (28%) of which were positive for *B. pseudomallei.* Fifty-eight positive cases were excluded because no record had been made of the quantitative urine culture results, and nine cases were subsequently excluded because of missing outcome data. For the remaining 755 patients included in the study, the median age was 48 years (range, 1 to 89 years), and 57% of the patients were male. Diabetes ($n = 363$; 48%) and renal disease, including chronic renal failure and renal calculi ($n = 165$; 22%), were common comorbidities. Blood cultures were performed in 727 of the 755 cases, 487 (67%) of which were positive. The overall in-hospital mortality rate was 45%.

Urine cultures that were positive for *B. pseudomallei* were analyzed from 171 patients (23%). Of these, 52 (30%) were positive from the spun pellet only ($<10^3$ CFU/ml), 51 (30%) contained between 10^3 CFU/ml and 10^5 CFU/ml, and 68 (40%) contained $\geq 10^5$ CFU/ml. The urine culture was the only positive direct specimen in 70 cases. Nineteen of these (27%) were culture positive from the spun pellet only, and eight of these patients had negative blood cultures.

Positive urine cultures were significantly associated with increasing age, the presence of urinary symptoms, preexisting renal disease, and positive blood cultures upon both univariate and multiple-variable analyses. For both of these models, patients with diabetes and those who had received antibiotics which are active against *B. pseudomallei* prior to urine culture had lower rates of urine culture positivity (Table 1). The same factors predicted bacterial concentrations in the urine (data not shown). Only 41 patients (24%) with positive urine cultures had urinary symptoms.

The mortality rate rose with increasing bacterial quantities in urine. For melioidosis patients with negative urine cultures, the fatality rate was 39% (228 of 584 patients). In contrast, patients with positive urine cultures from the spun pellet ($<10^3$ CFU/ml) had a 58% mortality rate (30 of 52 patients), patients with positive urine cultures with between 10^3 and 10^5 CFU/ml had a 61% mortality rate (31 of 51 patients), and patients with positive urine cultures with $>10^5$ CFU/ml had a 71% mortality rate (48 of 68 patients).

In a multiple-variable model, a positive blood culture, a clinical diagnosis of pneumonia, and age were significantly associated with mortality, and diabetes and the prior administration of an effective antibiotic regimen were associated with survival. A positive urine culture was predictive of mortality independent of the prior receipt of active antibiotics, age, diabetes, blood culture positivity, and the presence of pneumonia. Furthermore, the magnitude of the urinary bacterial concentration was correlated with an increased risk of mortality compared to that of patients with negative urine cultures (χ^2 test for trend; $P < 0.001$) (Table 2).

There were 116 patients with nondiabetic renal disease. These patients had a higher rate of positive urine cultures (39% versus 20%; $P < 0.001$) but a similar rate of blood culture positivity (69% versus 64%; $P = 0.29$) as patients without renal disease. Nondiabetic renal disease was associated with a higher mortality rate (65% versus 41%; $P < 0.001$).

TABLE 1. Variables associated with positive urine cultures ($n = 171$) for 755 patients with melioidosis[a]

Variable	No. of patients	Univariate odds ratio (95% CI)	Multiple-variable odds ratio (95% CI)
Age		1.01 (1.002, 1.02)	1.01 (1.001, 1.02)
Diabetes	363	0.49 (0.34, 0.69)	0.54 (0.38, 0.80)
Urinary symptoms[b]	123	1.9 (1.3, 2.9)	1.7 (1.1, 2.7)
Prior administration of active antibiotics[c]	161	0.42 (0.25, 0.69)	0.51 (0.31, 0.87)
Preexisting renal disease	165	2.1 (1.5, 3.1)	1.6 (1.1, 2.4)
Positive blood culture	487	2.1 (1.4, 3.1)	2.1 (1.4, 3.2)

[a] Gender and the presence of pneumonia were not significantly associated with positive urine cultures in a univariate or multiple-variable models.
[b] Presence of urinary frequency, dysuria, hematuria, or flank or back pain.
[c] Meropenem, imipenem, ceftazidime, or coamoxiclav.

DISCUSSION

This study has demonstrated that the presence and number of *B. pseudomallei* organisms in the urine of patients presenting with melioidosis have predictive value in terms of identi-

TABLE 2. Variables associated with in-hospital mortality ($n = 337$) for 755 patients with melioidosis[a]

Variable	No. of patients	Univariate odds ratio (95% CI)	Multiple-variable odds ratio (95% CI)
Age		1.02 (1.006, 1.03)	1.02 (1.01, 1.03)
Diabetes	363	0.41 (0.30, 0.55)	0.36 (0.25, 0.50)
Pneumonia	362	1.7 (1.3, 2.3)	2.2 (1.6, 3.1)
Positive blood culture	487	5.0 (3.6, 7.1)	5.8 (4.0, 8.5)
Prior administration of active antibiotics[b]	161	0.45 (0.31, 0.65)	0.58 (0.38, 0.89)
Negative urine culture	584	1.0	1.0
Positive urine culture	171	2.7 (1.9, 3.9)	2.0 (1.3, 2.9)
Positive spun pellet only ($<10^3$ CFU/ml)	52	2.1 (1.2, 3.8)	1.3 (0.70, 2.5)
Positive (10^3 to 10^5 CFU/ml)	51	2.4 (1.3, 4.3)	1.5 (0.77, 2.9)
Positive ($>10^5$ CFU/ml)	68	3.7 (2.1, 6.5)	3.3 (1.8, 6.1)

[b] Meropenem, imipenem, ceftazidime, or coamoxiclav.
[a] Gender and the presence of urinary symptoms were not significantly associated with mortality in a univariate or multiple-variable models.

fying patients with poor outcomes. This correlation is independent of age, bacteremia, and the timeliness of antibiotic administration. An increasing urinary bacterial concentration was associated with an increasing mortality rate, suggesting that the concentration in urine may be a surrogate marker for the overall bacterial load. The lack of association between diabetes and mortality has been noted previously (1) and may be related to the fact that distinct factors are involved in the acquisition of infection versus those influencing the outcome of disease. In addition, diabetic patients may manifest mild disease at lower levels of exposure to *B. pseudomallei* or may receive appropriate antibiotics earlier for presumed melioidosis.

Genitourinary tract infections were previously thought to be infrequent in Thai patients compared with other population groups, such as those in northern Australia, where prostatic involvement has been described for 19% of male patients (2). During the 10-year period of this study, only 41 of 755 patients (5%) for whom urine culture was performed had both a positive urine culture and the clinical features of urinary tract sepsis. Since many patients did not have urine cultures performed and since patients with urinary symptoms were more likely to have cultures performed, it is likely that the true rate of urinary involvement is lower than that described in this study. The finding of asymptomatic bacteriuria has been considered to represent renal filtration of bacteria present in the blood, previously termed "spillover." We are currently investigating this assumption in a prospective study of simultaneous quantitative urine and blood cultures.

Chronic renal failure and/or renal calculi are well-recognized risk factors for melioidosis. In northeastern Thailand, renal tubular acidosis is endemic and is strongly associated with nephrolithiasis (4, 7). We found that patients with nondiabetic renal disease were more likely to have involvement of the urinary tract, probably reflecting calculi acting as a nidus of infection.

The observation that urinary symptoms are often absent from melioidosis patients with positive urine cultures indicates the utility of urine culture for the routine screening of patients with suspected disease. For one-third of patients with bacteriuria, *B. pseudomallei* would have been missed by standard urine culture techniques using undiluted urine since the cultures were positive for the spun urinary pellet alone. This indicates that the diagnostic sensitivity can be increased by using concentrating techniques prior to urine culture.

In conclusion, quantitative urine culture is a simple and inexpensive technique that has clinical utility for predicting the outcome of melioidosis independent of blood culture positivity and other potential confounders.

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