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Prognostic Value of Cytokine Concentrations (Tumor Necrosis Factor–α, Interleukin-6, and Interleukin-10) and Clinical Parameters in Severe Melioidosis

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Raised serum concentrations of tumor necrosis factor (TNF)–α, interleukin (IL)–1β, IL-6, or IL-10 are associated with mortality in patients with sepsis, but it is not known whether elevated cytokine levels are independently predictive of mortality. Cytokine assays (TNF-α, IL-6, and IL-10) were performed on admission plasma samples from 172 adult Thai patients with severe melioidosis. Mortality was 31.4%. APACHE II score; septicemia; plasma lactate; TNF-α, IL-6, and IL-10 concentrations; and IL-10/TNF-α and IL-6/IL-10 ratios were each associated with outcome ($P \leq .001$ for all variables). Only the APACHE II score and either IL-6 or IL-10 concentration were independent predictors of mortality, as determined by use of multiple logistic regression (with cytokine concentrations and ratios entered separately). In a multivariate analysis, including both IL-6 and IL-10, the IL-10 concentration was no longer predictive. Therefore, APACHE II scores and either IL-6 or IL-10 concentration may be the most reliable parameters for stratification of patients in future studies of severe gram-negative sepsis.

Bacterial infections prompt the release of cytokines such as tumor necrosis factor (TNF)–α, interleukin (IL)–1, and IL-6, which in turn stimulate peripheral anti-inflammatory responses [1]. Several clinical studies have reported correlations between plasma concentrations of pro- and anti-inflammatory cytokines and outcome in severe systemic infections. Among patients with sepsis, serum or plasma concentrations of TNF-α, IL-1β, IL-6, or IL-10 have each been shown to be significantly increased in patients who died, compared with survivors [2–13], but we do not know whether such elevated cytokine levels can be used as independent predictors of mortality.

Acute melioidosis is a life-threatening systemic infection caused by the gram-negative bacterium *Burkholderia pseudomallei.* This microorganism is found in soil and aquatic environments in Southeast Asia and northern Australia. It is a common cause of community-acquired septicemia in northeast Thailand and has a high associated morbidity and mortality [14]. The high incidence of this single-etiology disease during the rainy season, in a relatively homogeneous population, provides a unique opportunity for studies of severe gram-negative sepsis. We report the results of a large study of the prognostic value of plasma TNF-α, IL-6, and IL-10 concentrations (and their ratios), compared with clinical parameters, in adult Thai patients with melioidosis.

Methods

Patients. The present study was part of a clinical trial comparing the therapeutic efficacy of imipenem and ceftazidime in acute severe melioidosis [15]. The study was conducted from July 1994 through November 1997. Adult Thai patients (aged >14 years) admitted to Sappasitprasong Hospital, Ubon Ratchathani, Thailand, with suspected severe melioidosis were included in this study if they or accompanying relatives gave informed consent. Full clinical details were recorded at study entry. Blood samples were collected for routine hematology and biochemistry (including lactate), as well as suitable specimens for bacteriological diagnosis [16]. Baseline APACHE II scores were calculated. All patients were seen daily thereafter until discharge from the hospital. For cytokine

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Table 1. Median (range) clinical and laboratory indices for 172 patients with melioidosis.

	Survivors	Fatal cases	*P*
Age (years)	50 (21–73)	52 (18–82)	.61[a]
Fever >37.5°C (%)	54.2	63.0	.37[b]
Hypotension[c] (%)	4.6	21.6	.002[b]
Pulse 100 bpm (%)	26.5	70.4	<.001[b]
Respiratory rate >30 (%)	14.0	68.0	<.001[b]
Hematocrit (%)	31 (16–44)	38 (16–51)	.008[a]
WBC count ($\times 10^6$/L)	13,500 (2600–26,900)	11,800 (1800–35,800)	.006[a]
Prothrombin time (seconds)	13 (9–39)	17 (11–88)	<.001[a]
BUN (mg/dL)	21.5 (4.0–144)	50.5 (7.0–246)	<.001[a]
Creatinine (mg/dL)	1.3 (.6–10.6)	2.9 (.7–12.0)	<.001[a]
Alkaline phosphatase (IU/L)	136 (18–1286)	238 (49–912)	.002[a]
Total bilirubin (mg/dL)	0.9 (0.3–15.5)	3.0 (0.6–16.6)	<.001[a]
SGOT (IU/L)	52 (11–6620)	109 (16–3892)	<.001[a]
Albumin (mg/dL)	2.7 (1.4–5.6)	2.2 (1.4–3.9)	<.001[a]
Serum bicarbonate (m*M*)	19 (5–33)	11 (3–27)	<.001[a]
APACHE II score	11 (0–25)	21 (5–38)	<.001[a]
Plasma lactate (m*M*)	1.9 (0.8–5.3)	3.5 (0.9–18.8)	<.001[a]
Bacteremia (%)	44.9	90.7	<.001[b]

NOTE. BUN, blood urea nitrogen; SGOT, serum glutamic oxaloacetic transaminase; WBC, white blood cell.

[a] Mann-Whitney *U* test.

[b] χ^2 test with Yates correction for continuity.

[c] Systolic blood pressure <90 mm Hg.

assays, EDTA-trasylol blood samples were collected before the start of specific antimicrobial therapy. Samples were centrifuged immediately, and plasma was stored at −70°C until assays could be performed. Results for those patients not shown subsequently to have culture-proven melioidosis were excluded from this analysis.

Assays. Cytokines were measured by ELISA. TNF-α was measured by use of a commercially available assay for human TNF-α, according to the manufacturer's instructions (Pelikine, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam). IL-6 was measured by use of anti–human IL-6 monoclonal antibody (MAb) (clones MQ2-13A5 and MQ2-39C3; Pharmingen, San Diego). IL-10 was measured by use of anti–human IL-10 MAb (clones JES3-9D7 and JES3-12G8; Pharmingen). According to the manufacturer's specifications, these ELISAs are specific for the relevant cytokine. The detection limits for these assays were 2–3 pg/mL (TNF-α), 5–10 pg/mL (IL-6), and 2–4 pg/mL (IL-10).

Statistical analysis. For comparison of groups, the Mann-Whitney *U* test was applied, and the α level was set at 0.05. The Spearman rank correlation test was used to determine correlation coefficients. Proportions were compared by use of the χ^2 test with Yates correction for continuity. Univariate and multivariate logistic regression analyses were performed by entering outcome as the dependent variable and clinical and cytokine parameters as independent variables. In the multivariate models, variables with a $P < .10$ in the univariate analysis were entered. Cytokine and lactate concentrations did not conform to a normal distribution and were log-transformed for the univariate/multivariate analyses. All statistical analyses were performed by use of the statistical computing package SPSS for Windows, version 8.0 (SPSS, Chicago) and SAS version 6.12 (SAS Institute, Cary, NC).

Results

Patient characteristics. Two hundred and ninety-six patients with suspected melioidosis were enrolled, of whom 214 had culture-proven melioidosis. Of these 214 patients, 178 had cytokine samples collected at baseline, but 6 were deemed nonassessable (final outcome unknown). The analysis was restricted therefore to the remaining 172 patients (80.4%). Samples were not collected from the other 36 melioidosis patients for a variety of reasons, but no patient categories were excluded under the study protocol. The median age of the assessable patients was 51 years (range, 18–82 years; interquartile [IQ] range, 41–59 years), and 97 (56.4%) were male. Underlying diseases were common: 83 patients (48%) had diabetes mellitus, and 20 (11%) had chronic renal failure. One patient had a human immunodeficiency virus infection. Blood cultures were positive for *B. pseudomallei* in 102 (59.3%) patients. The overall mortality was 31.4% (54 patients), but mortality was 48.0% for patients with positive blood-culture results.

Eighty-one patients received ceftazidime as the first-line therapy. There were no significant differences between the 2 treatment arms for age ($P = .61$), sex ($P = .96$), positive blood cultures ($P = .28$), fever clearance time ($P = .8$), or mortality ($P = .99$). Therefore, the results from the 2 treatment groups were pooled.

Clinical and laboratory parameters. Clinical and laboratory baseline data are shown in table 1. There were significant differences between survivors and nonsurvivors for many of these parameters. APACHE II scores were used in the subsequent analyses because they combine many of these variables.

Cytokines at study entry. Baseline cytokine concentrations

(median, range, and IQ range) are shown in figure 1. The median baseline TNF-α level was 3.6 pg/mL (range, <2–752.7 pg/mL; IQ range, <2–14.1 pg/mL). In 81 (47.1%) patients, the TNF-α concentration was below the detection limit. The median baseline IL-6 level was 227.2 pg/mL (range, <5–745,000 pg/mL; IQ range, 82.6–708.2 pg/mL), and the median IL-10 level was 33.3 pg/mL (range, <2–5730 pg/mL; IQ range, 13.5–125.2 pg/mL). IL-6 and IL-10 levels were undetectable in only 3 (1.7%) and 5 (2.9%) patients, respectively. Median baseline cytokine levels were significantly higher in nonsurvivors than in survivors ($P < .001$ for each cytokine, as shown in figure 1). TNF-α was detectable in 39.8% of surviving patients at baseline, compared with 81.5% of those who died ($P < .001$).

There were strong correlations between the cytokine levels at study entry (table 2), as well as among baseline plasma lactate concentrations, APACHE II scores, and each cytokine level (table 2). Median baseline cytokine levels were significantly higher in patients who were bacteremic on admission to hospital, compared with those whose blood-culture results were negative: TNF-α, 7.2 pg/mL (IQ range, <2–24.8 pg/mL) versus <2 pg/mL (IQ range, <2–5.2 pg/mL), respectively ($P < .001$); IL-6, 645.9 pg/mL (IQ range, 212.2–5008 pg/mL) versus 77.4 pg/mL (IQ range, 21.1–245.4 pg/mL), respectively ($P < .001$); and IL-10, 75.9 pg/mL (IQ range, 22.5–241.0 pg/mL) versus 15.5 pg/mL (IQ range, 8.7–35.2 pg/mL), respectively ($P < .001$). TNF-α was detectable in 66 (64.7%) bacteremic patients at baseline, compared with 25 (35.7%) who had negative blood-culture results ($P < .001$). Baseline concentrations of all 3 cytokines were similar between the 2 antibiotic treatment arms (TNF-α, $P = .43$; IL-6, $P = .73$; and IL-10, $P = .49$).

Determinants of outcome: univariate and multivariate analyses. We tested the influence on survival of the following variables: age; sex; APACHE II score; presence of bacteremia; plasma lactate concentration (>4.0 mmol/L); undetectable TNF-α levels; log-transformed concentrations of TNF-α, IL-6, and IL-10; and the ratios between the log-transformed concentrations of the cytokines.

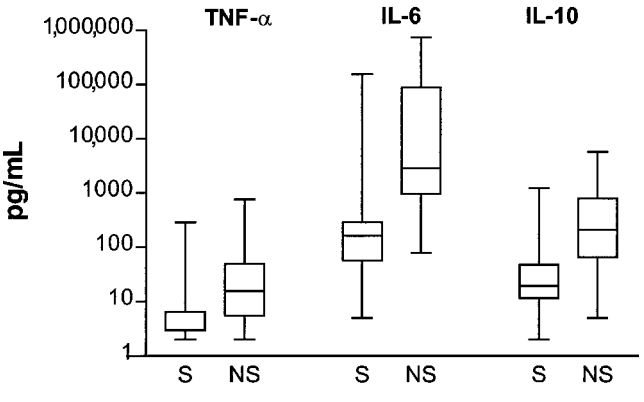

Figure 1. Plasma tumor necrosis factor (TNF)–α, interleukin (IL)–6, and IL-10 concentrations in survivors (S) and nonsurvivors (NS). Horizontal lines inside boxes indicate median values; boxes represent 25th–75th percentiles; and bars represent ranges. Difference between survivors and nonsurvivors in all cases: $P < .001$.

Table 2. Correlations between cytokine and lactate concentrations and APACHE II scores (Spearman rank correlation test coefficients).

	TNF-α	IL-6	IL-10	Lactate
IL-6	.47			
IL-10	.59	.72		
Lactate	.39	.54	.55	
APACHE II	.37	.59	.49	.39

NOTE. $P < .001$ for all correlations. IL, interleukin; TNF, tumor necrosis factor.

The APACHE II score, a high plasma lactate, being septicemic, having an undetectable TNF-α level, and increased TNF-α, IL-6, and IL-10 concentrations were all significantly associated with outcome (table 3). The ratios IL-6/IL-10 and IL-10/TNF-α were also significantly associated with outcome.

Multiple logistic regression analyses were performed, comparing APACHE II score, septicemia, and a lactate concentration >4.0 m*M* with each of the log-transformed cytokine concentrations or their ratios. Since the cytokine concentrations were all highly intercorrelated (table 2), the cytokines (or their ratios) initially were each entered into separate multiple logistic regression models, together with the above variables.

In these models, both IL-6 (odds ratio [OR], 3.60; 95% confidence interval [CI], 1.76–7.38; $P \leq .001$) and IL-10 (OR, 4.31; 95% CI, 1.75–10.61; $P = .002$) concentrations were independent predictors of mortality, together with the APACHE II score (OR, 1.17; 95% CI, 1.07–1.28; $P \leq .001$). The presence of septicemia or a high lactate concentration was not independently predictive. The plasma TNF-α concentrations did not predict mortality independently ($P = .23$), and a high IL-10/TNF-α ratio was not associated with an increased risk of death ($P = .12$). The log-transformed IL-6/IL-10 ratio was associated significantly with mortality (OR, 2.27; 95% CI, 1.08–4.78; $P = .031$), but in this model the other variables tested (lactate >4 m*M*: OR, 4.12, $P = .032$; septicemia: OR, 4.00, $P = .025$; APACHE II score, OR, 1.20, $P \leq .001$) were each associated significantly with outcome. Dichotomized by the median value (8.32), higher IL-6/IL-10 ratios were associated in the univariate analysis with a 3.46-fold increased risk of mortality (95% CI, 1.73–6.93; $P < .001$), but this was not confirmed as an independent risk in the multivariate analysis ($P = .06$). Thus, for every point increase in APACHE II score, the risk of mortality increased by 17%, and every 10-fold increase in IL-6 or IL-10 concentration (i.e., 1 log increase) resulted in a 3.6- or 4.3-fold higher risk of mortality, respectively. In an additional multivariate analysis including both IL-6 and IL-10 (log-transformed), the APACHE II score (OR, 1.16; 95% CI, 1.06–1.27; $P = .001$) and IL-6 (OR, 2.70; 95% CI, 1.19–6.16; $P = .018$) concentration remained independent predictors of outcome, but the IL-10 concentration was no longer predictive (OR, 2.06; 95% CI, 0.67–6.34; $P = .21$).

Table 3. Prognostic indices derived from univariate analysis.

	P	OR (95% CI)
Age	.44	1.01 (.98–1.03)
Sex (female vs. male)	.88	0.95 (0.50–1.82)
APACHE II score	<.001	1.28 (1.18–1.38)[a]
Septicemic melioidosis	<.001	12.0 (4.5–32.3)
Lactate >4 m*M*	<.001	19.3 (6.8–54.9)
Undetectable TNF-α	<.001	0.15 (0.07–0.33)
Log TNF-α	.0011	4.76 (1.87–12.15)[b]
Log IL-6	<.001	7.24 (3.77–13.88)[b]
Log IL-10	<.001	12.20 (5.58–26.70)[b]
Ratio log IL-10/TNF-α[c]	.001	4.44 (1.79–11.01)
Ratio log IL-6/IL-10	<.001	4.20 (2.37–7.45)

NOTE. CI, confidence interval; IL, interleukin; OR, odds ratio; TNF, tumor necrosis factor.

[a] For every point increase in APACHE II score.

[b] Per log increase in concentration.

[c] Only calculated for patients in whom TNF-α was detectable.

Discussion

Melioidosis, or infection with *B. pseudomallei,* is a common cause of community-acquired sepsis in northeast Thailand. In-hospital mortality remains ~40%–45%, despite specific antimicrobial therapy with ceftazidime, and ~60% of patients admitted to hospital with melioidosis are bacteremic [17, 18]. The disease provides an opportunity to study gram-negative sepsis of a single etiology, in a large number of patients from a relatively homogeneous population [14]. Previous clinical studies of gram-negative sepsis have frequently been complicated by the multiple etiologies of the infections, small numbers of patients, or the inclusion of patients at varying stages of disease [19]. Melioidosis is therefore a suitable infection in which to study the determinants of mortality in severe gram-negative sepsis in humans.

Elevated levels of the cytokines TNF-α [5, 6, 10] and IL-6 [2, 5, 8, 13] have been shown previously to be associated with mortality in severe sepsis, and elevations in TNF-α, IL-6, IL-8, and interferon-γ concentrations have been associated with death among patients with melioidosis [20–22]. Simple clinical and laboratory variables may be better predictors of mortality than TNF-α concentrations, for patients with septic shock [6, 13], but the independent predictive value of IL-6 or IL-10 concentration has never been established in models that included clinical parameters as well as other pro- and anti-inflammatory cytokines. It has also been reported recently that high admission ratios of the pro- and anti-inflammatory cytokines IL-10 and TNF-α were associated with death among febrile patients with community-acquired infections [4]. The IL-10/TNF-α ratio proved to be a better predictor of mortality than either IL-10 or TNF-α concentration alone, but the authors did not report how many patients had undetectable plasma TNF-α levels. Furthermore, the patient population studied was very heterogeneous, and no evidence was provided to indicate that the anti-inflammatory cytokine profile could predict disease outcome independently of clinical parameters. Another recent report proposed that a genetically determined intrinsic anti-inflammatory profile (i.e., a high IL-10/TNF-α ratio during in vitro whole blood stimulation) might influence mortality in meningococcal disease [23].

The patients enrolled in this study were treated with either ceftazidime or imipenem. It is possible that this may have influenced patient outcomes, but mortality was similar between the 2 treatment arms, as were baseline levels of the assayed cytokines; therefore, we think that antibiotic therapy is unlikely to be an important confounder. As in earlier studies of septic patients, high levels of TNF-α, IL-6, and IL-10 (and elevated IL-10/TNF-α and IL-6/IL-10 ratios) were associated with mortality in melioidosis in univariate analyses, in addition to the APACHE II score [2–8, 10–13, 24]. The fact that the APACHE II score predicts mortality is not surprising, since this scoring system is designed specifically to stratify acutely ill patients by risk of death [24]. However, in multivariate analyses, we have shown that both IL-6 and IL-10 levels were risk factors for mortality among patients with melioidosis, independent of the APACHE II score (although the IL-10 levels were not independently predictive of mortality, if included in the same analysis as IL-6). A high IL-6/IL-10 ratio was also associated with mortality, although less strongly than each cytokine alone; a similar finding for severe malaria has been reported recently [25]. Neither TNF-α levels alone nor the IL-10/TNF-α ratio (i.e., an anti-inflammatory profile) were independent risk factors for mortality. However, in this study, the value of these TNF-α–containing parameters is limited, as nearly half the patients in the present study had undetectable TNF-α levels. TNF-α is generally considered to be one of the most important proinflammatory mediators of sepsis. However, the half-life in blood of TNF-α is short, and even significant tissue production may not be reflected accurately by systemic concentrations [26]. Systemic concentrations of TNF-α have been predictive of mortality in some studies [5, 6, 10] but not in others [2, 4, 27]. Those studies that have compared TNF-α concentrations with clinical and laboratory parameters did not demonstrate that TNF-α was an independent predictor of mortality [6, 13].

The present data do not allow us to determine whether the increased mortality in melioidosis patients with higher IL-6 or IL-10 levels is a direct result of these cytokines or whether the elevated levels are merely a reflection of greater disease severity. However, the clinical significance of these findings is that APACHE II scores and either IL-6 or IL-10 concentration are independent predictors of mortality in melioidosis and, by extrapolation to other forms of severe gram-negative sepsis, are therefore probably the most reliable parameters to stratify patients in future sepsis trials. There is a great need for such parameters, given the heterogeneity of the study populations and infections usually seen in sepsis trials [28].

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