Prognostic value of cytokine concentrations (tumor necrosis factor-alfa, 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 ($P < .001$ 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
assays, EDTA-trasylol blood samples were collected before the start of specific antimicrobial therapy. Samples were centrifuged immediately, and plasma was stored at \(-70^\circ \text{C}\) 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-\(\alpha\) was measured by use of a commercially available assay for human TNF-\(\alpha\), 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 ELISAAs are specific for the relevant cytokine. The detection limits for these assays were 2–3 pg/mL (TNF-\(\alpha\)), 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 \(\alpha\) level was set at 0.05. The Spearman rank correlation test was used to determine correlation coefficients. Proportions were compared by use of the \(\chi^2\) 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 non-assessable (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
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.

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 mM 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; \( P = .001 \)) 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; \( P = .001 \)). 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 >4.0 mM: OR, 4.12, \( P = .032 \); septicemia: OR, 4.00, \( P = .025 \); APACHE II score, OR, 1.20, \( P = .001 \)) 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, 1.75–6.93; \( P < .001 \)), 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; \( P = .21 \)).
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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References


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