Multivariate Analysis of 9 Disease-Associated Variables for Outcome Prediction in Patients With Sepsis

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Objective: To assess the ability of 9 clinical or biological variables to predict outcome (survival or nonsurvival) using multiple regression and classification analyses.

Design: Prospective, descriptive cohort study with no interventions.

Setting: Surgical intensive care unit of a tertiary care hospital and a medical school research laboratory.

Patients: Eighteen patients with a documented source of infection who met currently accepted criteria for sepsis syndrome or septic shock.

Main Outcome Measures: Prediction of survival or nonsurvival based on analysis of clinical (Multiple Organ Dysfunction score, Acute Physiology and Chronic Health Evaluation III scores) and biological (plasma levels of cortisol, interleukin 6, interleukin 10, phospholipase A2, soluble tumor necrosis factor receptor p75, and monocyte membrane tumor necrosis factor receptor levels) variables, with comparison of predicted and actual outcomes.

Results: Plasma interleukin 6, interleukin 10, and phospholipase A2 concentrations were not significantly different between survivors and nonsurvivors. By standard, forward stepwise, and backward stepwise multiple regression analyses, only monocyte membrane tumor necrosis factor receptor levels measured at the onset of sepsis significantly predicted outcome in all 3 analyses. However, by both standard and backward stepwise analyses, Multiple Organ Dysfunction scores based on evaluation at the onset of sepsis and 24 hours later were also significant predictors of outcome. Classification analysis showed that assignment to outcome group was statistically significant when based on monocyte membrane tumor necrosis factor receptor levels determined at the onset of sepsis or on Multiple Organ Dysfunction scores assessed 24 hours after sepsis was diagnosed.

Conclusion: Although these findings were based on a relatively small cohort, both multiple regression and classification analyses indicated that only monocyte membrane tumor necrosis factor receptor levels are able to discriminate survivors from nonsurvivors at the onset of sepsis.

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There is consensus that systemic inflammation is the sine qua non for both sepsis and systemic inflammatory response syndrome (SIRS) and that this inflammation is the result of a cascade of more proximal cell-derived mediators.1-2 The identification of such mediators presumably would permit the development of rational strategies for early diagnosis and intervention. Unfortunately, and despite considerable efforts and great expense, no cell- or mediator-based diagnostic test or therapeutic compound has proved to be of more than marginal value in sepsis or SIRS.1-13

Appropriate classification of patients with sepsis or SIRS to outcome categories according to indexes based on overall health (including age), acute physiological characteristics, severity of injury, and the extent of organ system dysfunction has been reported.14-21 However, there is controversy regarding the robustness of such classification schemes, especially at times shortly after the onset of sepsis or SIRS. Unfortunately, it is at just such early time points that outcome classification may be most critical for assigning those with an unfavorable prognosis to aggressive, novel, and resource-intensive therapies.22

Previous reports have shown that peripheral-blood monocyte tumor necrosis factor receptor (MTNFR) levels were significantly lower at diagnosis of sepsis in patients who subsequently did not survive.23,24 However, in those studies, no efforts were made to quantitate the MTNFR level as a predictor variable for outcome. In the

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PATIENTS AND METHODS

PATIENTS AND EXPERIMENTAL DESIGN

All studies were approved by the appropriate institutional review board. Informed consent was obtained from each patient or a designated relative.

Eighteen patients in the surgical intensive care unit were studied. Entry criteria were those of sepsis syndrome plus documentation of a source of infection as described by Ziegler et al.7 A 10-mL arterial blood sample was obtained from each patient on entry to the study (baseline) and at 24 and 72 hours. An aliquot of blood was removed and immediately evaluated for MTNFR level. The remaining blood was centrifuged at 900g and the plasma was removed, aliquoted, and frozen (−70°C) for subsequent assays.

BIOLOGICAL ASSESSMENTS

Plasma interleukin 6, interleukin 10, phospholipase A2, and soluble tumor necrosis factor receptor p75 were quantified by sandwich enzyme-linked immunosorbent assay.11,23 Plasma cortisol concentrations were determined by direct radioimmunoassay.26 As reported previously,23,24,27,28 MTNFR levels were assessed by first lysing the erythrocytes in an aliquot of whole blood. Leukocytes were obtained with biotinylated tumor necrosis factor α followed by counterstaining with phycoerythrin-conjugated streptavidin. Using flow cytometry, monocytes were identified by their simultaneous side and forward light scatter intensities on a 2-parameter dot plot. An elliptical gate was placed over the monocyte cluster, and the gated cells were then analyzed for fluorescence intensity as measured by mean channel fluorescence.

CLINICAL ASSESSMENTS

On entry to the study (baseline) and at 24 and 72 hours, patients were examined and assigned a Multiple Organ Dysfunction (MOD) score according to the method of Marshall et al14 and an Acute Physiology and Chronic Health Evaluation III score as described by Knaus et al.15

DATA ANALYSIS

Data were analyzed using STATISTICA (StatSoft, Tulsa, Okla). Two-way (outcome × time), repeated-measures analyses of variance were used for assessing overall effects of each dependent variable. Variables that showed statistically significant differences on outcome by analysis of variance were then subjected to standard, forward stepwise, and backward stepwise multiple regression (R) analyses for identification of predictor variables. The α was set at .05 for all analyses. Analyses by χ2 were used for determining significance in 2 × 2 classification tables.

RESULTS

There were no significant differences by analysis of variance between survivors and nonsurvivors for plasma interleukin 6, interleukin 10, or phospholipase A2 levels, so these variables were excluded from further analyses. The remaining variables were analyzed for fit with the expected normal distributions and found to be acceptable, with no values for any variable being greater or less than 3 SDs from their respective means.

Standard, forward stepwise, and backward stepwise multiple R analyses were performed, and the adjusted R² values were greater than 0.8 in all 3 analyses, indicating the presence of significant predictor variables in the data set. Only MTNFR level at baseline was a significant predictor of outcome in all 3 multiple R analyses. However, MOD scores at baseline and at 24 hours also manifested significant predictive abilities in the standard and backward multiple R analyses.

Correlations between MTNFR level at baseline, MOD scores at baseline and at 24 hours, and the standardized predicted values were compared using the backward stepwise model (Figure). (The Figure shows scatterplots of single predictor variables vs standardized predicted values. The latter are the standardized predicted outcomes based on the multiple regression equations, which are weighted linear combinations of the predictor variables. Because in these analyses outcome is dichotomous [either survivor or nonsurvivor], any standardized predicted value that is >0 represents a predicted survivor and any value <0 represents a predicted nonsurvivor.) The superiority of MTNFR level at baseline as a predictor of outcome is apparent for this model (r = 0.79) as it was for the other 2 models (data not shown).

The Table shows a classification analysis for outcome using backward stepwise multiple R as the model and MTNFR level at baseline, MOD score at baseline, and MOD score at 24 hours as predictor variables. Backward stepwise multiple R was chosen as the model because the correlations between predictor variables and the standardized predicted values were highest in this model (the Figure). The MTNFR level at baseline was again the best single predictor of outcome.

COMMENT

Previous reports23,24 suggested that the MTNFR level might be a reasonable predictor of outcome in patients with sepsis. However, this suggestion was based on simple inspection of the MTNFR levels, which seemed to be uniformly low for nonsurviving patients and relatively normal for patients who ultimately survived. This was contrasted with, for example, plasma soluble tumor necrosis factor receptor concentrations, which were low in normal controls, middling in surviving patients, and high in nonsurviving patients. Thus, it

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was suggested that the relatively dichotomous nature of MTNFR levels in surviving vs nonsurviving patients might make this variable a good predictor of outcome in patients with sepsis.

The present results support the notion of the MTNFR level as a predictor of outcome. Only MTNFR level at baseline was a significant predictor of outcome in standard, forward, and backward multiple R analyses. However, MOD scores at baseline and at 24 hours were also significant predictors in the standard and backward models. Although MTNFR level at baseline was the strongest predictor, the overall predictability improved greatly when this variable was combined with others in the multiple R analyses. This is shown in the Figure, where the nonsurvivors (closed circles) without exception had MTNFR levels less than 0 and survivors without exception had MTNFR levels greater than 0. Thus, when multiple weighted variables including MTNFR level at baseline and MOD scores were considered in the multiple R analyses, prediction of outcome was perfect in this small cohort.

It is not surprising that the MTNFR level was a significant early predictor of outcome. Previous reports have shown that a significant decline in MTNFR levels occurs as little as 1 hour after the administration of endotoxin to normal controls. Furthermore, modulation of MTNFR levels may be one of the most sensitive indicators of the presence of proinflammatory mediators. In a large number of studies performed with the normal human endotoxemia model, changes in MTNFR levels can still be detected, albeit attenuated, in the presence of proinflammatory cytokine antagonists and cytokine and endotoxin neutralizing agents.

The cohort evaluated in this study was small. Because extreme outliers can have profound effects in multiple R analyses, especially those with small numbers of subjects, all variables were checked for homogeneity of variance. A general rule of thumb is that a data point be considered an outlier when it is greater or less than 3 SDs from its respective mean. In the present study, no data for any variable met this criterion, so there is little concern that outliers influenced the results of the multiple R analyses.

The standard predicted value of 0 in the backward multiple R model was used to estimate the MTNFR level at baseline that best discriminates between survivors and nonsurvivors (Figure). With the use of this value, a classification analysis was then performed (Table). By this technique, the sensitivity and specificity were 75% and 100%, respectively. This compares favorably with the Multiple Organ Failure Scoring System of Hebert et al, where these values were 51% and 87%, respectively. With the use of the same method, MOD score at baseline did not classify patients as well as did MOD score at 24 hours. This analysis has been reported by Marshall et al and Barie and Hydo. The latter investigators found that daily
MOD scores in patients whose stay in the surgical intensive care unit was more than 21 days did not distinguish survivors from nonsurvivors until day 2 of the stay. However, from day 2 on, MOD scores were predictive of outcome.

In conclusion, both multiple R and classification analyses indicate that the MTNFR level is perhaps the earliest significant predictor of 28-day mortality in patients with sepsis. It is proposed that cellular biologically based indicators such as MTNFR levels will likely enhance the early prediction of SIRS- or sepsis-related complications and mortality.

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