The Potential Utility of Blood-Derived Biochemical Markers as Indicators of Early Clinical Trends Following Severe Traumatic Brain Injury

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Key words
- Biomarkers
- D-dimer
- MMP-9
- S-100B
- Trauma
- Traumatic brain injury

Abbreviations and Acronyms
AUC: Area under the curve
CNS: Central nervous system
CT: Computed tomography
GCS: Glasgow Coma Scale
MMP: Matrix metalloproteinase
NPV: Negative predictive value
PPV: Positive predictive value
ROC: Receiver operating characteristic
TBI: Traumatic brain injury

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OBJECTIVE: **Severe traumatic brain injury (TBI) is a dynamic neuropathologic process in which a substantial proportion of patients die within the first 48-hours. The assessment of injury severity and prognosis are of primary concern in the initial management of severe TBI. Supplemental testing that aids in the stratification of patients at high risk for deterioration may significantly improve posttraumatic management in the acute setting.**

METHODS: **This retrospective study assessed the utility of both single-marker and multimarker models as predictive indicators of acute clinical status after severe TBI. Forty-four patients who sustained severe TBI (admission Glasgow Coma Scale [GCS] score ≤8) were divided into two cohorts according to a dichotomized clinical outcome at 72 hours after admission: Poor status (death or GCS score ≤8) and improved status (GCS score improved to >8). Threshold values for clinical status prediction were calculated for serum S-100B, matrix metalloproteinase-9, and plasma D-dimer, upon admission and at 24 hours after TBI by the use of receiver operating characteristic analysis. Performance characteristics of these single-marker predictors were compared with those derived from a multimarker logistic regression analysis.**

RESULTS: **Biomarkers with the greatest predictive value for poor status at 72 hours included serum S-100B on admission, as well as plasma D-dimer and serum S-100B at 24 hours, for which, associations were strongly significant. Multimarker analysis indicated no substantial improvement in prediction accuracy over the best single predictors during this time frame.**

CONCLUSION: **In conjunction with other clinical, physical, and radiologic evidence, blood-derived biochemical markers may serve to enhance prediction of early clinical trends after severe TBI.**

INTRODUCTION

Severe traumatic brain injury (TBI) is a dynamic neuropathologic process in which the primary mechanical injury triggers a heterogeneous complex of vascular, metabolic, cellular, and molecular consequences that promote neurologic deterioration and secondary brain injury (33). The mortality rate associated with severe TBI has been reported between 30% and 50%, with approximately 90% of deaths occurring within 48 hours of insult (28). Therefore, the early assessment of injury severity is of significant importance in the management of patients who have sustained severe TBI (18). Despite the substantial burden of this disease process, however, optimally reliable outcome predictors after head trauma are lacking. Because the early assessment of neurological injury often presents a significant challenge in the intensive care unit setting, the use of biochemical markers may be of value in the identification of patients at greater risk for deterioration and in the guidance of immediate posttraumatic therapeutic strategies (5).

The analysis of disease-specific biomarkers in modern medicine has revolutionized the diagnostic, prognostic, and therapeutic approach of various human pathologies. For example, in myocardial infarction, the evaluation of troponin, creatine kinase MB, D-dimer, and brain natriuretic peptide plays an important diagnostic role (9). In the case of TBI, however, the brain itself introduces multiple unique challenges to the identification of a single reliable marker of injury. Among these include the distribution of heterogeneous cell populations within the central nervous system (CNS) and their respective tolerance or resistance to injury, the overall complexity of ischemic and neuroinflammatory cascades, and the presence of the blood-brain barrier (22). Furthermore, the majority of markers are nonspecific for cerebral injury; rather, they represent various components of the ischemic and neuroinflammatory cascades (22). Thus, despite statistical associations between cerebral injury and individual markers of inflammation, glial activation, and neuronal injury, no single marker has possessed the characteristics required to

demonstrate stand-alone diagnostic or prognostic value (22).

The primary aim of this investigation was to evaluate tendencies in select biomarker profiles that could serve as posttraumatic indicators of early clinical trends after severe TBI. Cut-off values for serum S-100B, matrix metalloproteinase-9 (MMP-9), and plasma D-dimer were established, on admission and at 24 hours, to predict clinical status at the 72-hour time point. Performance characteristics of these single-marker predictors were compared with those derived from a multimarker model to assess the predictive value of multimarker monitoring in the early posttraumatic period.

MATERIALS AND METHODS

Patient Population

Ethical and institutional approval for the study was granted by the University of Miami Institutional Review Board prior to study commencement, and informed consent was obtained from either the patient's health care surrogate or closest family member. A retrospective analysis of biochemical data was conducted on patients who were admitted to Ryder Trauma Center at Jackson Memorial Hospital, between the dates of June 2003 and February 2005, with severe TBI (Glasgow Coma Scale [GCS] score $\leq$8) after initial resuscitation. All patients included in this study were victims of severe TBI—any mechanism of injury—between the ages of 16 and 64 years, and were admitted within 3 hours of injury. Patients outside of the stated age range or those who presented after 3 hours after injury, as well as patients with a history of acute meningitis, cerebral vasculitis, or any other recently documented CNS infection, were excluded from the analysis. All patients were sedated, intubated, mechanically ventilated, and managed according to a protocol adapted from the guidelines for the management of severe TBI proposed by the Brain Trauma Foundation (4). Corticosteroids were not used in the treatment of these patients.

Blood Sampling and Biochemical Measurements

Venous blood samples were obtained from each patient upon admission to Ryder Trauma Center (study entry), as well as at the 24-hour, 48-hour, and 72-hour post-admission time points. Two 5-mL samples were collected, during routine blood drawing from an existing indwelling vascular catheter that had been placed during the patient's admission. All venous serum samples were centrifuged at 1800 g for 7 minutes at room temperature. Plasma supernatant fractions from peripheral venous blood samples were then separated and stored at −80°C until samples were sent to Alere (formerly Biosite Incorporated, San Diego, California, USA) for batch analysis.

Assessment of Clinical Outcomes

Clinical outcomes after severe TBI in this study were measured at 72 hours after admission and were defined by survival, as well as by assessment using the GCS to evaluate the degree of impaired consciousness in surviving patients. Patients in this study were divided into two main cohorts, for retrospective analysis, based on a dichotomized clinical outcome at 72 hours after admission. The poor clinical status cohort (death or GCS that remained $\leq$8) consisted of 30 cases (68%), and the improved clinical status cohort (GCS improved to >8) consisted of 14 cases (32%). As a result of the relatively small sample size, no independent analysis was performed solely on patients who died within the first 72 hours after admission (i.e., seven cases; 16%).

Statistical Analysis

A comparison of median S-100B, MMP-9, and D-dimer values, both on study entry and at 24 hours after study entry, in the different cohorts under investigation was made with the standard Mann-Whitney U-test. The correlation between serum concentrations of S-100B, MMP-9, and D-dimer levels on arrival and poor clinical status (death or GCS $\leq$8) at 72 hours was assessed with the Spearman rank-order coefficient. In addition, the extent to which each biomarker at a given time point differed between individuals who demonstrated improved (GCS >8 at 72 hours) versus poor (death or GCS $\leq$8 at 72 hours) clinical trends was assessed by the use of receiver operating characteristic (ROC) analyses. The ROC plots were estimated by using the algorithm built into the Statistical Package for the Social Sciences (SPSS; SPSS Institute, Cary, North Carolina, USA). Each biomarker was evaluated at its optimal cutoff value on the basis of the standard sensitivity versus 1-specificity and positive predictive value (PPV) and negative predictive value (NPV) measures, both at study entry and 24 hours after study entry, for prediction of unfavorable (poor) clinical status. A cut-off point on the curves was chosen to attain the best compromise between sensitivity and specificity for poor outcome at 72 hours after study entry.

To determine the best combination of biomarkers for early outcome prediction, a multivariate logistic regression model was used. A stepwise procedure was implemented to find the optimal biomarker combination within the first 24 hours after study entry. Various combinations of biomarkers were explored in multiple data analysis iterations, including a wide variety of alternative models and analysis techniques. Measures of predictive performance for the multivariate model were derived from ROC analysis.

RESULTS

Table 1 depicts the characteristics of the TBI population stratified by outcome measure (poor vs. improved) at 72 hours after severe TBI. Retrospective analysis was performed on 44 patients (31 male and 13 female), ages 16–64 years (median, 28 years), during a period of 20 months. All patients had sustained severe brain trauma (GCS $\leq$8 on admission) and were subsequently admitted within 3 hours of injury. A total of 30 patients (68%) from this study either died (7 patients) or remained at a GCS $\leq$8 (23 patients) at 72 hours after admission, whereas 14 patients (32%) demonstrated significant improvement in GCS to >8 during this period.

Concentrations of the various biomarkers were determined upon admission with subsequent measurements obtained at 24, 48, and 72 hours after admission in surviving patients. The concentrations of serum S-100B ranged from 0.00 ng/mL to 6.11 ng/mL upon admission after injury and from 0.00 ng/mL to 6.07 ng/mL at 24 hours after the inciting trauma. Median S-100B concentrations were found to be statistically greater, both on admission ($P = 0.007$) and at 24 hours ($P = 0.003$), in patients who either died or failed to