Clinical Study

Serum Levels of Biochemical Markers of Traumatic Brain Injury

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Background. A biomarker would be valuable in the diagnosis, risk stratification and prognosis of patients with traumatic brain injury (TBI).

Methods. We measured serum levels of S-100β, neuron specific enolase (NSE) and myelin basic protein (MBP) in 50 TBI subjects, and 50 age and gender matched controls. Patients were recruited within 6 hours of the initial injury, they had an initial Glasgow Coma Scale (GCS) score of 14 or less, or a GCS score of 15 with witnessed loss of consciousness (LOC) or amnesia.

Results. S-100β, NSE and MBP levels were significantly higher in TBI subjects than in control subjects (P<0.001 for S-100β and NSE; P=0.009 for MBP). Initial S-100β levels were significantly higher in TBI subjects who had not returned to normal activities 2 weeks following their injury than in TBI subjects who had returned to normal activities (P = 0.022). MBP levels were higher in TBI subjects with positive findings on the baseline CT scan than in CT-negative subjects (P = 0.007).

Conclusions. S-100β, NSE and MBP may be present in the sera of TBI subjects in elevated quantities relative to controls. S-100β may aid in predicting short-term outcome in TBI subjects.

1. Introduction

Approximately 1.4 million people sustain a traumatic brain injury (TBI) in the United States each year [1] (CDC ref.) [2, 3]. Computerized axial tomography (CT) scanning is currently accepted as the “gold” standard diagnostic procedure for initial evaluation of TBI, as it identifies pathology associated with primary brain injury [4], is widely available, and can be performed at a relatively low cost [5]; however, the utilization of CT scanning in the diagnosis and management of patients presenting to emergency departments (EDs) with TBI varies among institutions, and CT scan results in isolation have limited fidelity in predicting neuropsychiatric outcome in mild TBI subjects [6]. Additionally, less than 1% of subjects presenting to EDs with mild TBI have acute lesions identified on head CT which require surgical intervention, yet nearly 15% of mild TBI subjects have not returned to normal daily function one year after their injury [7].

There is a compelling clinical need for real-time serum biochemical marker tests to aid in the diagnosis and severity stratification of head injury, particularly when access to neuroimaging techniques is limited [8]. Biochemical marker testing that provides prognostic information about short-term patient outcome, especially among mild TBI cases, would be of immense value as well [9]. There are no commercially available protein marker assays with FDA approval for TBI identification or prediction of severity; however, robust research efforts have identified promising biochemical markers of brain injury.

S-100β is a calcium-binding protein that is found primarily in astrocytes and Schwann’s cell, and serum S-100β levels have been shown to rise immediately following head trauma [10, 11] and are significantly elevated in children with closed head injuries [12]. Neuron-specific enolase (NSE) is a glycolytic enzyme found in neurons and neuroendocrine cells, and serum levels of NSE are significantly elevated in patients with acute ischemic stroke [13]. NSE is significantly
This observational case-control study was designed to compare serum levels of S-100β, NSE, and MBP in patients presenting to the ED of a major urban trauma center with symptoms consistent with isolated TBI to serum marker levels in age and gender-matched non-TBI control subjects. Additionally, the relationships between serum marker levels, CT imaging results, and short-term clinical outcomes were also investigated.

2. Methods

2.1. Study Design. This observational case-control study was conducted at the Emergency Department of the Sunnybrook and Women’s College Health Sciences Centre in Toronto, Ontario, between September, 2001, and December, 2002. Approval of the study protocol was obtained by the hospital’s research ethics board prior to commencement of the study, and subjects or their legally authorized representatives were required to provide written informed consent prior to participation in the study. A total of 50 TBI subjects and 50 age and gender-matched non-TBI control subjects were enrolled. To be included, TBI subjects must have been at least 16 years of age at presentation, admitted to the emergency department within 6 hours of the initial injury, have had an initial ED Glasgow Coma Scale (GCS) score of 14 or less, a GCS score of 15 with witnessed loss of consciousness (LOC), or amnesia of the traumatic event. Subjects meeting inclusion criteria were enrolled until the target enrollment of 50 was reached. Control subjects were patients who presented to the ED with a condition unrelated to head trauma, a GCS score of 15 without prepresentation witnessed LOC, or amnesia, were of the same gender and within 3 years of age of an enrolled TBI subject. Control subjects provided written informed consent for project inclusion as well and were then case-matched to a TBI subject. Subjects excluded from the study if they had a known history of neurological disease, neuropsychiatric disorders, or known malignant melanomas or were undergoing brain or spinal cord surgery within one month prior to the injury.

2.2. Methods of Measurement. Serum samples were collected from all enrolled subjects during the baseline evaluation. Samples were initially collected in EDTA-containing blood collection tubes and promptly centrifuged at 10,000 g. Supernatant was aliquoted and immediately frozen at −70°C until later analysis. They were then shipped on dry ice to the Nanogen Point-of-Care Diagnostics Division (Toronto, ON, Canada) for subsequent evaluation of marker levels. S-100β levels were determined using an enzyme-linked immunosorbent assay (ELISA) with a monoclonal anti-S-100 capture antibody and a polyclonal rabbit anti-S-100 detector antibody [17]. NSE levels were determined using an ELISA with a monoclonal anti-NSE capture antibody and a monoclonal anti-NSE detector antibody. MBP levels were determined using an ELISA with a goat polyclonal anti-MBP capture antibody and a monoclonal anti-MBP detector antibody. The detection limits for the respective assays were 10 pg/mL for S-100β, 1 ng/mL for NSE, and 20 pg/mL for MBP. The personnel responsible for completion of the assays were blinded to any clinical data related to the subjects.

2.3. Imaging and Clinical Followup. Board-certified radiologist CT scan reports were made available to the primary investigator for the subset of TBI subjects for whom CT scans were available. TBI subjects enrolled in the study were contacted by phone a minimum of 2 weeks following injury. Telephone followup was conducted via structured phone interview, using the Canadian CT Head and Cervical Spine Radiography Study Telephone Followup survey tool. This assessment tool has been previously validated in a large clinical study of mild TBI subjects [18].

2.4. Data Collection and Processing. Data were retrospectively collected by the research personnel at the investigative site. To ensure the integrity of data prior to analysis, CRFs were reviewed for accuracy against the source documents by a study investigator, and the data were entered (single entry) into the clinical database (Microsoft Access 2000 and SyMetric). Logical and integrity checks were performed, and all generated queries were resolved by the site. Biomarker data were also entered in the databases by single entry, and subsequent integrity checks were performed.

2.5. Outcome Measures. The primary outcome measures were the baseline serum concentrations of biomarkers drawn from each enrolled subject. A secondary outcome measure was the presence or absence of radiographic abnormality on initial CT scan. In particular, subjects were classified as CT positive by the primary investigator if evidence of at least one of the following was demonstrated on the CT scan:

(i) subdural hematoma (SDH),
(ii) epidural hematoma (EDH),
(iii) subarachnoid hemorrhage (SAH),
(iv) cerebral contusion,
(v) diffuse axonal injury (DAI).

Subjects with signs of orbital or sinus fracture, scalp lacerations, or soft tissue injury but with none of the above signs of intracranial injury were classified as CT negative. The primary investigator was blinded to the results of the biomarker measurements when classifying the CT scan results.

Another secondary outcome measure was short-term prognosis in the TBI cohort, determined via the telephone followup survey and dichotomized into good versus poor prognosis depending on whether the TBI subject had
returned to normal daily activities 2 weeks after injury. Personnel conducting the survey were blinded to the biomarker measurements.

2.6. Data Analysis. Summary statistics for baseline marker levels were computed for both TBI subjects and non-TBI control subjects. For each biomarker, comparisons between TBI and control groups were made using nonparametric Mann-Whitney tests. Receiver operating characteristic (ROC) curves were generated, and areas under the curve (AUC) were computed to provide a basis for comparison of each of the markers to discriminate between TBI subjects and controls. An optimal cutoff (defined in terms of the largest sum of sensitivity and specificity) was identified from the ROC curve for each marker. Pairwise comparisons of AUC values between markers were conducted following the procedure of Hanley and McNeil [19].

With respect to the subgroup of TBI subjects for whom CT scans were clinically indicated, summary statistics for baseline marker levels were computed for both CT-positive and CT-negative subjects. For each biomarker, comparisons between CT-positive and CT-negative subjects were made using the Mann-Whitney tests.

In the subgroup of TBI subjects who were reached for followup telephone surveys, summary statistics for baseline marker levels were computed for subjects who returned to normal activities 2 weeks following their injury and for those who did not. For each biomarker, comparisons between TBI subjects returning versus not returning to normal activities after 2 weeks were made using the Mann-Whitney tests.

Receiver operating characteristic (ROC) curve analyses were performed using MedCalc Version 7.1 (MedCalc Software, Mariakerke, Belgium); all other statistical analyses were conducted using S-Plus Version 6 for Windows (Insightful Corporation, Seattle, WA, USA).

3. Results

3.1. Characteristics of Study Subjects. Blood samples were not available for one TBI subject, who died shortly after admission. Of the 49 remaining subjects, 32 (65%) were male. A total of 34 (69%) of the TBI subjects were Caucasian, 3 were Black, 7 were Asian, and 5 were of other races. The median age of TBI subjects was 42, with a range of 16 to 89. A total of 27 (55%) of the TBI subjects had baseline GCS scores of 14 or 15 (22 of these being GCS 15), and 22 had baseline GCS scores of 13 or less. The majority of the injuries were motor vehicle related, with 21 (43%) occurring to drivers or passengers in vehicles involved in collisions and another 11 (22%) occurring to pedestrians or cyclists struck by motor vehicles. Of the remaining injuries, 12 (24%) were caused by falls of various types, 3 were caused by industrial accidents, one was sports related, and one was the result of an assault.

Of the 49 matched non-TBI control subjects, 11 (22%) presented with a primary complaint of abdominal pain, 6 (12%) presented with an arm, wrist, or hand injury, 5 (10%) presented with shortness of breath, 5 (10%) presented with chest pain, 4 (8%) presented with a sore throat or cough, 4 (8%) presented with a leg or knee injury, and 14 (29%) presented with other complaints.

3.2. Baseline Marker Levels. In 2 cases, the amount of serum obtained from the TBI subject was deemed insufficient for testing of all three biomarkers, and in another 2 cases sample hemolysis compromised the NSE result; therefore, baseline levels of all three TBI markers were obtained for 45 of the 49 matched pairs. Table 1 displays summary statistics for baseline levels of all three markers, and Figure 1 displays dot plots of baseline marker levels, stratified by subgroup (TBI versus control). S-100β displayed a very high clinical specificity, with 42 of the 45 control samples (93%) having S-100β levels at or below 10 pg/mL, the detection limit of the assay; the corresponding sensitivity of S-100β at this cutoff level with respect to the matching TBI subjects was 78% (35/45). TBI subjects had a median NSE level of 12.5 ng/mL (interquartile range = [6.9, 19.5]), whereas control subjects had a median NSE level of 4.6 ng/mL (interquartile range = [3.5, 7.3]). TBI subjects had a median MBP level of 76 pg/mL (interquartile range = [60, 146]), whereas control subjects had a median MBP level of 60 pg/mL (interquartile range = [49, 82]). The Mann-Whitney tests revealed that S-100β (P < 0.001), NSE (P < 0.001), and MBP (=0.009) levels were significantly higher in TBI subjects than in control subjects.

Table 1: Summary statistics with respect to baseline marker levels in TBI and matching control subjects. (Concentrations are given in ng/mL for NSE and in pg/mL for MBP and S-100β).

<table>
<thead>
<tr>
<th>Marker</th>
<th>TBI (n = 45)</th>
<th>Control (n = 45)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-100β</td>
<td>Min. 0 Q1 12 Median 48 Q3 159 Max. 421</td>
<td>Min. 0 Q1 0 Median 0 Q3 0 Max. 55</td>
</tr>
<tr>
<td>NSE</td>
<td>Min. 3.2 Q1 6.9 Median 12.5 Q3 19.5 Max. 85.0</td>
<td>Min. 2.4 Q1 3.5 Median 4.6 Q3 7.3 Max. 39.0</td>
</tr>
<tr>
<td>MBP</td>
<td>Min. 40 Q1 60 Median 76 Q3 146 Max. 2010</td>
<td>Min. 49 Q1 60 Median 60 Q3 82 Max. 336</td>
</tr>
</tbody>
</table>

Figure 2 displays receiver operator characteristic (ROC) curves for each of the three markers; S-100β displayed the highest overall ability to discriminate between TBI and control subjects, with an area under the curve (AUC) of 0.868, as compared with an AUC of 0.820 for NSE and 0.659 for MBP. Pairwise comparisons revealed that both S-100β (P = 0.001) and NSE (P = 0.018) showed significantly higher discriminatory ability than MBP in this respect, whereas the discriminatory ability of S-100β was not significantly higher than that of NSE. Optimal cutoffs, sensitivity, and specificity estimates, ROC, AUC estimates and corresponding 95% confidence intervals are shown in Table 2.
Table 2: Summary of overall ROC curve analyses, estimates, and associated 95% confidence intervals (95% C.I.’s).

<table>
<thead>
<tr>
<th>Marker</th>
<th>AUC [95% C.I.]</th>
<th>Optimal cutoff</th>
<th>Sensitivity at cutoff [95% C.I.]</th>
<th>Specificity at cutoff [95% C.I.]</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-100β</td>
<td>0.868 [0.780, 0.930]</td>
<td>10 pg/mL</td>
<td>77.8% [62.9%, 88.8%]</td>
<td>93.3% [81.7%, 98.5%]</td>
</tr>
<tr>
<td>NSE</td>
<td>0.820 [0.725, 0.893]</td>
<td>8.15 ng/mL</td>
<td>71.1% [55.7%, 83.6%]</td>
<td>82.2% [67.9%, 92.0%]</td>
</tr>
<tr>
<td>MBP</td>
<td>0.659 [0.552, 0.756]</td>
<td>65 pg/mL</td>
<td>71.1% [55.7%, 83.6%]</td>
<td>55.6% [40.0%, 70.3%]</td>
</tr>
</tbody>
</table>

3.3. Correlation with CT Scan Results. CT scans were performed on 39 of the 45 TBI subjects for whom baseline levels of all three markers were available. All TBI subjects with baseline GCS scores of less than 15 obtained CT scans; the 6 who were discharged without having a CT scan performed (5 falls and 1 assault victim); all had baseline GCS scores of 15 and experienced witnessed loss of consciousness for a period of 5 minutes or less and/or posttraumatic amnesia for a period of 10 minutes or less.

All of the 39 subjects for whom CT scans were clinically indicated had baseline GCS scores of less than 15. 21 of the subjects were classified as CT positive, and 18 were classified as CT negative. The following frequencies were observed with respect to specific abnormalities as detected on the CT scan: 10 subjects with SDH, 15 with SAH, 14 with cerebral contusions, 2 with EDH, and 1 with DAI. There was a significant association between severity of TBI and CT result; 15 out of 19 subjects (79%) with a baseline GCS score of 13 or less were CT positive, whereas only 6 out of 20 subjects (30%) with a baseline GCS score higher than 13 were CT positive (Fisher’s exact test P = 0.004). Table 3 displays summary statistics for baseline levels of all three markers, stratified by CT result. Of the three markers, MBP provided the best discrimination between CT-positive and CT-negative TBI cases. CT-positive subjects had a median MBP level of 97 pg/mL (interquartile range = 76, 177), whereas control subjects had a median MBP level of 67 pg/mL (interquartile range = 52, 76). The Mann-Whitney tests revealed that MBP levels were higher in CT-positive subjects than in CT-negative subjects (P = 0.007), but S-100β (P = 0.921) and NSE (P = 0.632) could not discriminate between CT-positive and CT-negative subjects. With respect to the subset of mild TBI subjects alone, median baseline MBP levels showed a more than twofold increase when comparing CT-positive subjects and CT-negative subjects (148 pg/mL versus 69 pg/mL).

3.4. Correlation with Outcome Status after 2 Weeks. Of the TBI subjects who were discharged from the emergency department, a total of 29 were followed up after a minimum 2-week period with the structured telephone interview. The median elapsed time from injury to followup contact was 17 days, with an interquartile range of 14 to 21 days. Of the 21 subjects for whom followup survey data was not available, 3 had died as a result of their injuries, 6 had been admitted
Normal activities after 2 weeks to their TBI (Fisher’s exact test) with a baseline GCS score of 13 or less had returned to normal activities after 2 weeks, whereas none of the 7 subjects with a baseline GCS score higher than 13 had returned to normal activities after 2 weeks, whereas 19 had not returned to normal daily activities as a direct result of their injury. A total of 10 out of 22 subjects (45%) ing returned to normal daily activities after 2 weeks, whereas 6 were lost to followup.

Ten of the 29 subjects who were contacted having returned to normal daily activities after 2 weeks, whereas 19 had not returned to normal daily activities as a direct result of their injury. A total of 10 out of 22 subjects (45%) with a baseline GCS score higher than 13 had returned to normal activities after 2 weeks, whereas none of the 7 subjects with a baseline GCS score of 13 or less had returned to normal activities after 2 weeks to their TBI (Fisher’s exact test). Table 3 displays summary statistics with respect to baseline marker levels in TBI subjects, stratified by CT result. (Concentrations are given in ng/mL for NSE and in pg/mL for MBP and S-100β).

### Table 3: Summary statistics with respect to baseline marker levels in TBI subjects, stratified by CT result. (Concentrations are given in ng/mL for NSE and in pg/mL for MBP and S-100β).

<table>
<thead>
<tr>
<th>Marker</th>
<th>CT positive (n = 21)</th>
<th>CT negative (n = 18)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Min.</td>
<td>Q1</td>
</tr>
<tr>
<td>S-100β</td>
<td>0</td>
<td>21</td>
</tr>
<tr>
<td>NSE</td>
<td>4.9</td>
<td>8.5</td>
</tr>
<tr>
<td>MBP</td>
<td>51</td>
<td>76</td>
</tr>
</tbody>
</table>

To hospital or transferred to a rehabilitation facility, 6 were verbally unresponsive or suffering from cognitive impairment, and 6 were lost to followup.

Within the subset of mild TBI subjects with negative CT scans (n = 11; 4 of whom returned to normal activities after 2 weeks), baseline S-100β level remained a significant predictor of short-term outcome status (Mann-Whitney P = 0.047; Figure 3).

### 4. Discussion

Previous research has demonstrated that S-100β is superior to NSE in predicting the outcome status of both mild [20, 21] and severe TBI subjects [22]. Wunderlich et al. [23] showed that serum S-100β levels in acute stroke patients were predictive of neurological outcome at discharge and that serum NSE levels or lesion volumes obtained from CT scans did not add predictive value after adjusting for S-100β concentrations. Herrmann et al. [24] found that the initial S-100β level obtained from TBI subjects presenting with predominantly minor head injuries predicted adverse neuropsychological outcomes after 2 weeks and after 6 months and that S-100β was a better predictor of both short-term and long-term outcome than NSE or intracranial pathology as detected on the CT scan. The current analysis reveals similar trends among mild TBI subjects, namely, that S-100β levels predict short-term outcome status, while NSE levels and CT scan results do not. It should also be noted that NSE is also present in erythrocytes, and serum NSE levels can be markedly affected by hemolysis, whereas serum S-100β levels are not [25].
The S-100β assay used in this investigation has a detection limit of 10 pg/mL, which is lower than that for other commercially available S-100β assays [26]; furthermore, the 98th percentile reference limit for this assay in a healthy adult control population is 21 pg/mL [17], whereas other commercial assays have normal reference limits exceeding 100 pg/mL [27]. Apparent differences in assay specificities for the brain-specific isoform of the S-100 protein would account for the fact that the serum S-100β levels observed in the current investigation are generally lower than those reported in previous studies of S-100β in TBI [26].

Prior studies suggest that MBP is released into the CSF and subsequently into the general circulation following acute neurological events. MBP is well established as a marker of clinical activity in multiple sclerosis patients [28] and has also been shown to correlate with cerebral damage in acute stroke patients [29]. Yamazaki et al. found a correlation between serum MBP levels and severity of TBI in acute head injury patients [16]. Ng et al. performed comprehensive histological postmortem examinations of brains of 22 victims of blunt nonpenetrating head trauma and found that 17 of these cases exhibited myelin damage as detected by MBP immunostaining [30].

It may appear that the estimated prevalence of CT abnormalities in mild TBI subjects observed in this study (6 out of 20 cases, or 30%) is very high; previous studies have reported rates of occurrence of abnormalities on CT scans between 3% and 13% in mild TBI subjects [7]. However, a study performed at the same trauma center as the current study revealed that 26% of mild TBI subjects had associated CT abnormalities [6], a figure consistent with that reported in the current study. One possible explanation for this apparent discrepancy could be due to the different rates of utilization of CT scanning in different geographical regions. Future studies would need to incorporate trauma units from various regions in order to mitigate the possibility of site selection bias.

Researchers have established a link between polymorphism of the apolipoprotein E gene and adverse outcome following TBI, specifically with respect to carriers of the ε4 allele of the gene [31]. Future clinical studies should incorporate genetic testing along with measurements of serum proteins, in order to provide a more comprehensive assessment of the risk profile of a subject presenting to the emergency department with symptoms consistent with TBI.

### 5. Limitations

The small sample size of the present study did not allow for detailed subgroup analyses by severity of TBI and CT result. Other limitations of the current study included the lack of CT imaging results and/or short-term followup data for a small number of enrolled TBI subjects and the lack of longer-term followup data (90 days or more after trauma) for all enrolled TBI subjects. Nevertheless, the current data is useful in generating hypotheses regarding the diagnostic utility of MBP and the prognostic utility of S-100β, especially among the cohort of mild TBI subjects. The trends that were observed here would need to be confirmed in a larger prospective study.

### 6. Conclusions

The current study demonstrates that S-100β, NSE, and MBP are present and elevated in the sera of acutely injured TBI subjects relative to non-TBI subjects. S-100β may also serve as an aid in predicting short-term outcome status among TBI subjects, and MBP may predict the presence of brain injury seen on CT scanning. The clinical utility of these biomarkers needs to be confirmed in a larger prospective study.

### References


### Table 4: Summary statistics with respect to baseline marker levels in TBI subjects, stratified by short-term outcome status. (Concentrations are given in ng/mL for NSE and in pg/mL for MBP and S-100β).  

<table>
<thead>
<tr>
<th>Marker</th>
<th>Not back to normal activities</th>
<th>Back to normal activities</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Min.</td>
<td>Q1</td>
</tr>
<tr>
<td>S-100β</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>NSE</td>
<td>3.8</td>
<td>4.4</td>
</tr>
<tr>
<td>MBP</td>
<td>55</td>
<td>73</td>
</tr>
</tbody>
</table>


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