

Letter to the Editor

Charmaine Childs*, Eduardo Martínez-Morillo, Aung Phyo Wai, Mya Myint Zu, Anastasia Diamandis and Eleftherios P. Diamandis

Exploring the relationship between serum biomarkers, acute intracerebral changes and outcome after severe traumatic brain injury (TBI)

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***Corresponding author: Charmaine Childs**, Yong Loo Lin School of Medicine, National University of Singapore and National University Health System, Level 2, Clinical Research Centre, Block MD11, 10 Medical Drive, Singapore 117597, E-mail: charmaine_childs@hotmail.co.uk

Eduardo Martínez-Morillo, Anastasia Diamandis and Eleftherios P. Diamandis: Samuel Lunenfeld Research Institute, Joseph and Wolf Lebovic Health Centre, Mount Sinai Hospital, Toronto, ON, Canada
Aung Phyo Wai and Mya Myint Zu: Yong Loo Lin School of Medicine, National University of Singapore, Singapore

To the Editor,

In head injury management, clinicians are familiar with the patient who, with a grossly abnormal computed tomography (CT) scan, does better than expected, whilst others with a normal appearing scan on admission do badly. Efforts to improve prognostic capabilities at the bedside have addressed the possibilities of using serum biomarkers independently or, as in recent investigations [1], in conjunction with clinical and demographic factors. Although the relationship between the extent of cerebral damage and plasma concentration of serum S100B protein has been widely studied after traumatic brain injury (TBI), much less is known of the temporal relationship of this biomarker with ‘key’ intracerebral physiological disturbances during the acute phase after injury.

It is well recognised that the extent and severity of (primary) tissue damage does not stay ‘fixed’ but frequently evolves during the first days after injury [2]. One-off measures of a serum biomarker protein such as S100B may not reflect the net extent of pathology of the evolving lesion. Our earlier (unpublished) data rather supports a link between ‘rebound’ increases in serum S100B in patients with unexpected clinical deterioration. We may

therefore posit a hypothesis that, if temporal changes in serum levels of a brain damage biomarker protein is linked to subsequent (in-hospital) pathology and intracerebral catastrophe, serial measurements of S100B may be of value to further understand the relationship between biomarker concentrations and outcome at longer times after injury.

To this end, a pilot study was undertaken to explore whether serum concentrations of an established (S100B) and a novel (Kallikrein 6, KLK6) biomarker protein are associated with temporal changes of intracranial pressure (ICP), temperature and oxygen content in patients with unfavourable and favourable outcomes.

Adults with severe TBI were admitted as direct referrals from the emergency department. All patients were sedated, intubated, mechanically ventilated and all had an intra- or extra-axial lesion on CT scan. Patients were treated in accordance with local neurointensive care protocols and following international guidelines [3]. Decompressive craniectomy was performed for those patients who, with or without primary surgical craniectomy, required decompression due to refractory ICP.

Measurements of ICP, brain temperature (T_{br}) and brain tissue oxygen partial pressure ($P_{bt}O_2$) (Raumedic, Germany) were measured. Blood sampling began as soon as possible following neurosurgery and on arrival to the intensive care unit (ICU). Subsequent blood samples were obtained every 12 h after the first sampling time and from then onwards for 5 days. At the time of blood sampling, measures of each variable were averaged, spanning a period of 15 min either side of the sampling point. After centrifugation, serum was stored (-70°C) in two aliquots until batch analysis. The concentration of S100B was measured using a fully-automated electrochemoluminometric immunoassay (Roche Diagnostics). Duplicate samples were transported frozen (Singapore to Toronto) for analysis (in-house spectrophotometric ELISA for KLK6) [4].

Follow-up assessments were undertaken at 3 months using the Glasgow outcome scale (GOS) [5]. Statistical

analysis was performed using SPSS software, version 15.0 (SPSS Inc.). Mann-Whitney U-tests were performed for comparison between groups. Associations between variables were reported by Spearman's rank correlation coefficient. A p-value less than 0.05 was considered to be statistically significant.

Twelve patients (8 male) aged 21–78 years with a baseline GCS of 3–14 (median 8) were recruited to the study following institutional ethics approval. TBI severity was in the category of severe [6]. Four patients died (GOS 1). Three survivors had an unfavourable outcome; GOS 2 (n=2) and GOS 3 (n=1). Five patients had favourable outcomes (GOS 4). There were no survivors with GOS 5.

Of 107 blood samples obtained, 106 were analysed [between 4 and 11 (median 10) samples per patient]. The first blood samples were obtained within the first 48 h (median 17 h) after TBI.

S100B concentration ranged from 0.02 to 2.42 $\mu\text{g/L}$ (median value for each patient 0.05–0.76 $\mu\text{g/L}$). For KLK6 samples, serum concentration tended to be higher, range 0.55–2.38 $\mu\text{g/L}$ (median for each patient 0.73–1.43 $\mu\text{g/L}$). S100B levels were higher and more variable for patients with GOS 1 and 2 at 3-month follow-up (Figure 1). For KLK6 the range of serum concentration was broadly, 0.5–1.8 $\mu\text{g/L}$ across all GOS categories. KLK6 did not show promise as a prognostic biomarker despite KLK6 concentrations below the lowest reference limit (1.04 $\mu\text{g/L}$) in approximately 50% of serum samples analysed (49 of 106).

S100B concentration was significantly higher ($p < 0.001$) in patients (n=7) with unfavourable outcome, GOS 1, 2, 3 (samples, n=57) compared to those patients (n=5) with favourable outcome, GOS 4 (samples, n=49) (Figure 1). Individual patient median S100B values for GOS 1, 2, 3=0.12–0.76 $\mu\text{g/L}$ vs. 0.02–0.12 $\mu\text{g/L}$ for GOS 4, lending support for the analysis as two separate groups, unfavourable outcome (including death) GOS 1, 2, 3 and favourable outcome (GOS 4).

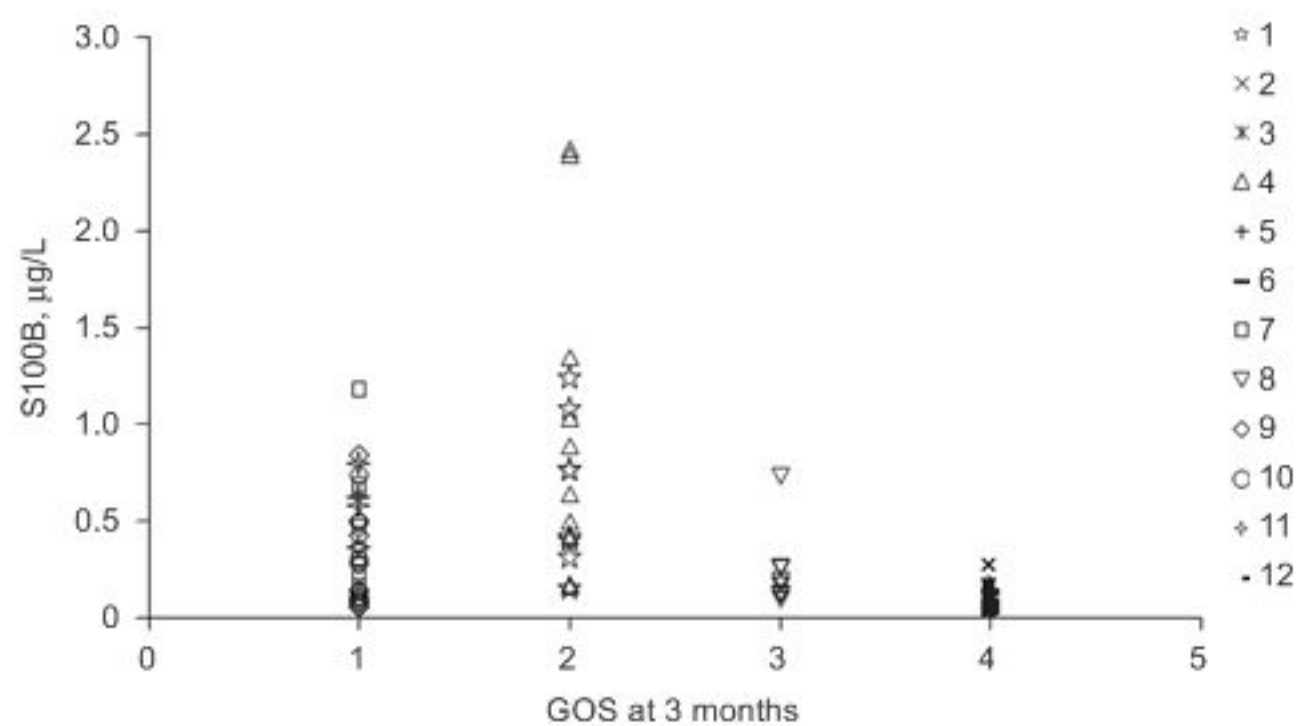


Figure 1 S100B protein levels and Glasgow outcome scale (GOS) scores at 3 months for 12 patients.

Further analysis for associations amongst S100B, KLK6, ICP, $P_{\text{bt}}\text{O}_2$ and T_{br} were explored. Significant associations for biomarkers and outcome were noted only for S100B. Here, an inverse correlation was observed for S100B and $P_{\text{bt}}\text{O}_2$ ($\rho = -0.61$, $p < 0.001$) and also for S100B and ICP ($\rho = -0.43$, $p = 0.001$) but only for those who died or had unfavourable outcomes (Figure 2). There were no significant associations between serum concentration of S100B and the intracerebral variables tested for patients with favourable outcome (GOS 4, n=5).

Multivariate regression analysis (stepwise method) between S100B and $P_{\text{bt}}\text{O}_2$, ICP (GOS 1–3) was performed after transformation of variables (logarithmic transformation for S100B and ICP and square-root transformation for $P_{\text{bt}}\text{O}_2$) and for a normal distribution of data. Significant associations were found for S100B concentration and variables, $P_{\text{bt}}\text{O}_2$ and ICP. The coefficient of determination between S100B and $P_{\text{bt}}\text{O}_2$ was 0.35 ($p < 0.001$). By inclusion of ICP to the regression analysis, the coefficient increased significantly to 0.40 ($p = 0.03$) indicating that 40% of the total variation of S100B concentration is explained by $P_{\text{bt}}\text{O}_2$ and, to a lesser extent ICP.

In the setting of South East Asia, serum concentrations of S100B protein were significantly elevated in patients with GOS 1–3 compared with those patients with GOS 4. Furthermore, S100B was below the cut-off, 0.53 $\mu\text{g/L}$ [7] for patients with favourable outcome at 3 months.

Two observations are of note (see Figure 2). S100B above the cut-off (0.53 $\mu\text{g/L}$) was associated with low $P_{\text{bt}}\text{O}_2$ (<15 mm Hg, i.e., commensurate with cerebral hypoxia) but *not* raised (>20 mm Hg) ICP. Whilst decompression of the brain may ameliorate ICP, measurement of brain tissue oxygen (and serum levels of S100B) provide

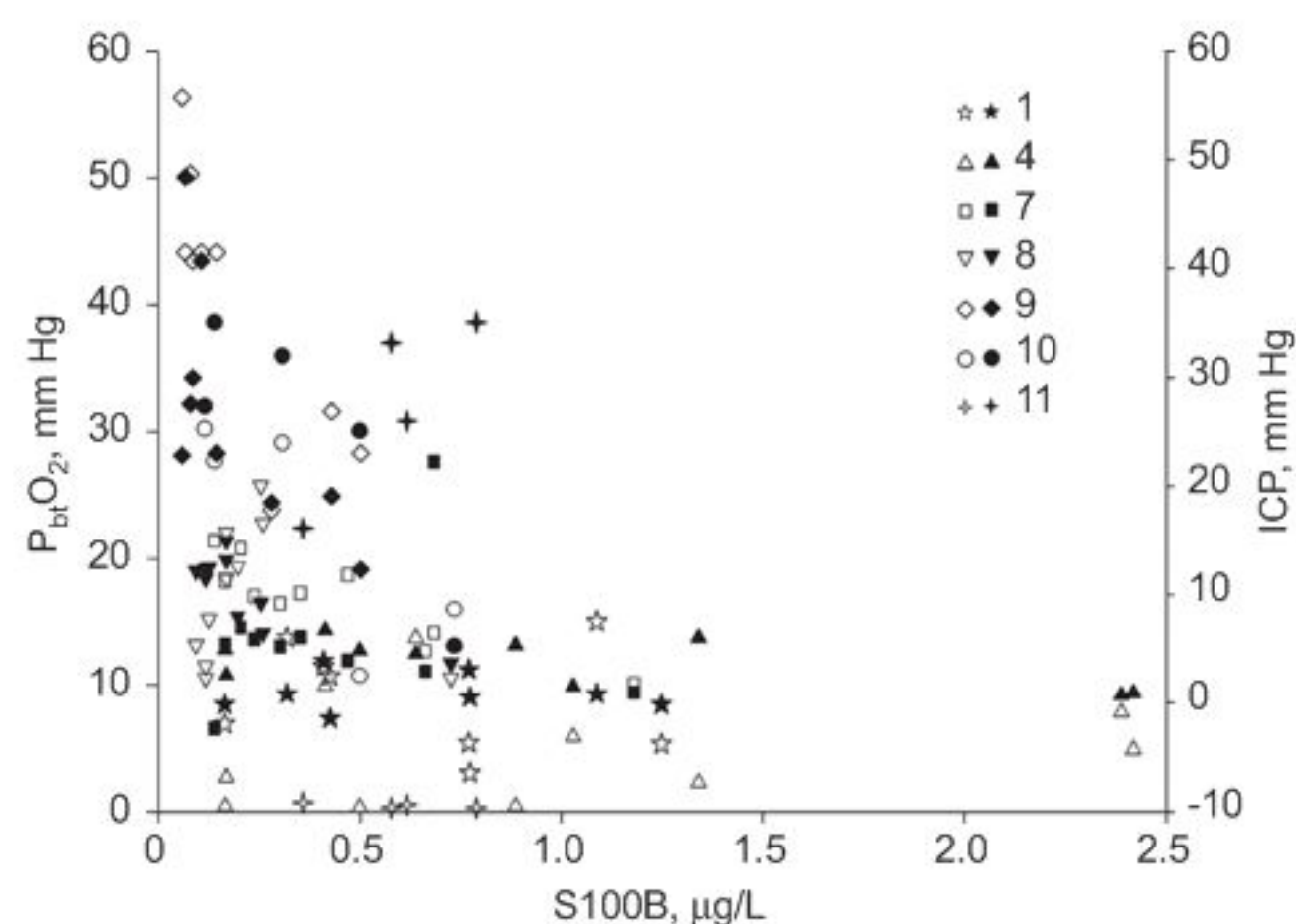


Figure 2 Correlation between S100B protein and $P_{\text{bt}}\text{O}_2$ (open symbols) and ICP (filled symbols), respectively, in patients with unfavourable outcomes, GOS score 1, 2 and 3.

additional parameters with which to verify adverse intracranial events even in the presence of acceptable ICP readings. This has potential clinical utility. The second note is the elevated $P_{bt}O_2$ readings (above the hypoxia critical threshold of 15 mm Hg) and elevation in ICP values (>20 mm Hg) but with low S100B (0.53 µg/L) concentration. Remembering that these data are from patients with unfavourable outcomes, it is worthy of note that the data presented reveal potential ‘weaknesses’ in the prognostic performance of commonly used laboratory *and* clinical biomarkers.

Whilst we acknowledge the limitations of this study due to sample size, we can confirm that our results support the findings of previous studies in that S100B appears to have reasonable prognostic performance for distinguishing unfavourable from favourable outcome at the 3-month time-point. Furthermore, there is a suggestion that S100B above the threshold (0.53 µg/L) may be a clinically valuable marker of brain tissue hypoxia even in the presence of a concomitant ‘normal’ ICP. Further investigations are required to investigate the role of S100B as a marker of brain tissue hypoxia in the absence of demonstrable intracranial hypertension.

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Conflict of interest statement

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