Serum biomarkers as predictors of long-term outcome in severe traumatic brain injury: analysis from a randomized placebo-controlled Phase II clinical trial

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OBJECTIVE There has been increased interest in the potential importance of biochemical parameters as predictors of outcome in severe traumatic brain injury (sTBI).

METHODS Of 107 patients with sTBI (age 18–65 years with a Glasgow Coma Scale score of 4–8 presenting within 8 hours after injury) who were randomized for a placebo-controlled Phase II trial of progesterone with or without hypothermia, the authors serially analyzed serum biomarkers (S100-B, glial fibrillary acidic protein [GFAP], neuron-specific enolase [NSE], tumor necrosis factor- α , interleukin-6 [IL-6], estrogen [Eg], and progesterone [Pg]). This analysis was performed using the sandwich enzyme-linked immunosorbent assay technique at admission and 7 days later for 86 patients, irrespective of assigned group. The long-term predictive values of serum biomarkers for dichotomized Glasgow Outcome Scale (GOS) score, functional independence measure, and survival status at 6 and 12 months were analyzed using an adjusted binary logistic regression model and receiver operating characteristic curve.

RESULTS A favorable GOS score (4–5) at 1 year was predicted by higher admission IL-6 (above 108.36 pg/ml; area under the curve [AUC] 0.69, sensitivity 52%, and specificity 78.6%) and Day 7 Pg levels (above 3.15 ng/ml; AUC 0.79, sensitivity 70%, and specificity 92.9%). An unfavorable GOS score (1–3) at 1 year was predicted by higher Day 7 GFAP levels (above 9.50 ng/ml; AUC 0.82, sensitivity 78.6%, and specificity 82.4%). Survivors at 1 year had significantly higher Day 7 Pg levels (above 3.15 ng/ml; AUC 0.78, sensitivity 66.7%, and specificity 90.9%). Nonsurvivors at 1 year had significantly higher Day 7 GFAP serum levels (above 11.14 ng/ml; AUC 0.81, sensitivity 81.8%, and specificity 88.9%) and Day 7 IL-6 serum levels (above 71.26 pg/ml; AUC 0.87, sensitivity 81.8%, and specificity 87%). In multivariate logistic regression analysis, independent predictors of outcome at 1 year were serum levels of Day 7 Pg (favorable GOS—OR 3.24, CI 1.5–7, $p = 0.003$; and favorable survival—OR 2, CI 1.2–3.5, $p = 0.01$); admission IL-6 (favorable GOS—OR 1.04, CI 1.00–1.08, $p = 0.04$); and Day 7 GFAP (unfavorable GOS—OR 0.79, CI 0.65–0.95, $p = 0.01$; and unfavorable survival—OR 0.80, CI 0.66–0.96, $p = 0.01$).

CONCLUSIONS Serial Pg, GFAP, and IL-6 monitoring could aid in prognosticating outcomes in patients with acute sTBI. A cause and effect relationship or a mere association of these biomarkers to outcome needs to be further studied for better understanding of the pathophysiology of sTBI and for choosing potential therapeutic targets.

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KEY WORDS glial fibrillary acidic protein; interleukin-6; progesterone; predictors of outcome; serum biomarkers; severe traumatic brain injury; trauma

ABBREVIATIONS AUC = area under the curve; BBB = blood-brain barrier; DAI = diffuse axonal injury; Eg = estrogen; FIM = functional independence measure; GCS = Glasgow Coma Scale; GFAP = glial fibrillary acidic protein; GOS = Glasgow Outcome Scale; ICP = intracranial pressure; IL = interleukin; IQR = interquartile range; NGF = nerve growth factor; NSE = neuron-specific enolase; Pg = progesterone; ROC = receiver operating characteristic; sTBI = severe traumatic brain injury; TNF- α = tumor necrosis factor- α .

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PATHOPHYSIOLOGY in traumatic brain injury (TBI) is a complex interplay of brain tissue-specific antigens, hormonal imbalance, and cytokine-mediated humoral and cellular immune reactions.^{3,29,32} Due to the limitations of existing clinical and radiological evaluations for assessment and prognostication of TBI severity, there has been a considerable paradigm shift toward development and use of biochemical markers to delineate the extent of brain tissue damage and to independently predict global outcome. Neuronal (ubiquitin C-terminal hydrolase L1, neuron-specific enolase [NSE])^{4,7,19–21} and glial (glial fibrillary acidic protein [GFAP], S100-B)^{10,18,28,31,37} markers have been studied extensively over the past decade in various laboratory and clinical studies, with heterogeneous results. Similarly, the role of proinflammatory (interleukin [IL]-6, IL-1, tumor necrosis factor- α [TNF- α]) and antiinflammatory (IL-4, IL-10, transforming growth factor) cytokines has been validated in recent trials.^{1,2,6,8,9,11–13,22,26,27,30,36}

More recently, posttraumatic suppression of the hypothalamic-pituitary-adrenal axis and an increase in cytokine-mediated peripheral aromatase activity, leading to an imbalance in sex steroid (estrogen [Eg], progesterone [Pg], and testosterone) serum levels, have been areas of active interest for precise understanding of the mechanisms involved.^{5,15,23,25,29} Most of the recent literature deals with these biomarkers in isolation, and there is a paucity of existing literature for establishing a comprehensive model dealing with all 3 domains together. To this end, we serially analyzed serum biomarkers (S100-B, GFAP, NSE, TNF- α , IL-6, Eg, and Pg) as part of a randomized placebo-controlled Phase II trial of progesterone with or without hypothermia in patients with severe TBI (sTBI), and analyzed the long-term predictive value of these biomarkers on Glasgow Outcome Scale (GOS) score, functional independence measure (FIM), and survival status at 6 and 12 months.

Methods

Study Design

This study was part of a prospective, outcome-assessor- and statistician-blinded, randomized, placebo-controlled Phase II trial of progesterone with or without hypothermia (Factorial design, unpublished data [S. Sinha et al., *Neurology India*, in press; accepted October 2015]). The study population included adult (age 18–65 years) patients with sTBI (Glasgow Coma Scale [GCS] Score 4–8) who presented at our tertiary care trauma center within 8 hours after injury. The study was registered under the Clinical Trials Registry–India (<http://www.ctri.nic.in>) with trial number CTRI/2009/091/000893. We obtained approval from our institutional ethics committee prior to commencement of the trial. Of 107 patients with sTBI who were randomized for the trial, we serially analyzed 7 serum biomarkers (S100-B, GFAP, NSE, TNF- α , IL-6, Eg, and Pg) in 86, irrespective of assigned group. We analyzed the long-term predictive value of serum biomarkers on dichotomized GOS score (poor recovery, GOS 1–3; good recovery, GOS 4–5), dichotomized FIM score (functionally dependent, FIM $\leq$ 108; functionally independent,

FIM 109–126), and survival status (survivor/nonsurvivor) at 6- and 12-month follow-up.

Biomarker Assessment

The serum levels of S100-B (BioVendor), NSE (DRG International), IL-6 (Diclone), GFAP (BioVendor), TNF- α (Diclone), Pg (DRG), and Eg (DRG) were determined using a commercially available kit based on the principle of the sandwich enzyme-linked immunosorbent assay technique. The samples for the determination of these biomarkers were taken at the time of admission, immediately after randomization (< 8 hours after injury), and then 1 week later (approximately 7 days after injury). A standard curve was prepared using standards supplied with the assay (irrespective of biomarkers), and the readings were analyzed using this standard curve.

Statistical Analysis

Using SPSS software (version 20, IBM Corp.), the baseline parameters, serum biomarkers, and outcome are presented as either number (percentage), mean ($\pm$ SD), or median (interquartile range [IQR]), wherever appropriate. Comparative analysis for admission, Day 7, and average serum biomarkers (stratified according to outcome) was performed using the Wilcoxon rank-sum test. To define independent factors predicting outcome, a binary logistic regression model was used after adjusting for GCS score at admission and treatment group assigned at randomization. The impact of factors on outcome was expressed as the OR (95% CI). A receiver operating characteristic (ROC) curve was made for independent factors predicting outcome, along with estimation of area under the curve (AUC), sensitivity, and specificity of optimal cutoff value (calculated by Youden index). In line with current statistical consensus, an AUC of 0.8–0.9 is considered very good, 0.7–0.8 is considered adequate, and < 0.7 is considered poor in terms of accuracy of the test under consideration. A *p* value of < 0.05 was considered significant.

Results

Demographic Profile

A total of 86 patients (84.9% of them male) with mean age 32.3 years were analyzed for the current study (Table 1). The average intracranial pressure (ICP) in the present cohort (measured serially for 5 days) was 11.8 mm Hg. Associated injuries (polytrauma) were present in 62% of analyzed patients. Contusion (59.3%) was the most common radiological presentation. Decompressive craniectomy was performed for refractory intracranial hypertension in 32.6% of patients. The median hospitalization period was 13 days. Sixteen (18.6%) and 21 (24.4%) patients were lost to follow-up at 6 and 12 months, respectively. Favorable GOS scores were found in 71.4% of patients at 6 months and in 78.5% of patients at 12 months. Also, 63.3% and 81.5% of patients were functionally independent at 6 and 12 months, respectively. The mortality rate was 14.3% and 16.9% at the 6- and 12-month follow-up, respectively.

Comparative Analysis of Serum Biomarkers

Stratified by GOS

The serum levels of Eg, S100-B, and TNF- α were similar across both dichotomized GOS groups at 6 and 12 months ($p > 0.05$) (Tables 2 and 3). At 6 and 12 months, the serum levels of Day 7 Pg, NSE levels at admission, and IL-6 levels at admission were significantly higher in favorable outcome groups (Day 7 Pg: 5.1 ng/ml vs 2.1 ng/ml [$p = 0.002$] and 5.1 ng/ml vs 2 ng/ml [$p = 0.001$]; admission NSE: 49.2 ng/ml vs 21.1 ng/ml [$p = 0.02$] and 49.2 ng/ml vs 25.1 ng/ml [$p = 0.04$]; and admission IL-6: 109.3 pg/ml vs 83.1 pg/ml [$p = 0.005$] and 105.7 pg/ml vs 89.8 pg/ml [$p = 0.04$] for favorable vs unfavorable outcome at 6 and 12 months, respectively). Serum Day 7 GFAP and Day 7 IL-6 levels were significantly higher in unfavorable outcome groups at 6 and 12 months (Day 7 GFAP: 11.9 ng/ml vs 6.4 ng/ml [$p = 0.001$] and 16.5 ng/ml vs 6.7 ng/ml [$p < 0.001$]; and Day 7 IL-6: 75.8 pg/ml vs 41.1 pg/ml [$p = 0.004$]; and 90.2 pg/ml vs 41.3 pg/ml [$p = 0.001$] for unfavorable vs favorable outcome at 6 and 12 months, respectively).

Stratified by FIM

The Eg, Pg, S100-B, IL-6, and TNF- α serum levels were similar across both dichotomized FIM groups at 6 and 12 months ($p > 0.05$) (Tables 4 and 5). Serum Day 7 NSE levels were significantly higher in the functionally independent group at 6 months (20.1 ng/ml vs 10.5 ng/ml, $p = 0.03$). Serum Day 7 GFAP levels were significantly higher in the functionally dependent group at 12 months (9.7 ng/ml vs 6.3 ng/ml, $p = 0.02$).

Stratified by Survival Status

The S100-B, NSE, and TNF- α serum levels were similar across both dichotomized survival status groups at 6 and 12 months ($p > 0.05$) (Tables 6 and 7). At 6 and 12 months, the serum Day 7 Pg and Eg levels at admission were significantly higher in survivors (Day 7 Pg: 5 ng/ml vs 1.9 ng/ml [$p = 0.007$] and 5.1 ng/ml vs 1.9 ng/ml [$p = 0.003$]; and admission Eg: 0.38 pg/ml vs 0.14 pg/ml [$p = 0.03$] and 0.51 pg/ml vs 0.14 pg/ml [$p = 0.05$] for survivors vs nonsurvivors at 6 and 12 months, respectively). Serum Day 7 GFAP and Day 7 IL-6 levels were significantly higher in nonsurvivors at 6 and 12 months (Day 7 GFAP: 18 ng/ml vs 6.5 ng/ml [$p < 0.001$] and 17.8 ng/ml vs 6.7 ng/ml [$p = 0.001$]; Day 7 IL-6: 98.1 pg/ml vs 41.3 pg/ml [$p < 0.001$] and 95.5 pg/ml vs 41.3 pg/ml [$p < 0.001$] for nonsurvivors and survivors at 6 and 12 months, respectively).

Adjusted Binary Logistic Regression Model Analysis

After adjusting for GCS score and the randomization arm, we observed that Day 7 Pg serum levels had the highest positive odds of predicting favorable GOS score at 6 and 12 months (OR 2.37, CI 1.29–4.36, $p = 0.005$; and OR 3.24, CI 1.5–7, $p = 0.003$), respectively, and survival at 6 and 12 months (OR 1.9, CI 1.14–3.23, $p = 0.01$; and OR 2, CI 1.2–3.5, $p = 0.01$), respectively (Table 8). Although the impact occurred to a lesser extent, IL-6 serum levels at admission also positively predicted GOS score at 6 and 12 months (OR 1.03, CI 1.00–1.06, $p = 0.02$; and OR 1.04, CI 1.00–1.08, $p = 0.04$), respectively. In contrast, Day 7

TABLE 1. Demographic profile and outcome in study population of patients with sTBI

Variable & Subcategory*	All (n = 86)
Age†	32.27 $\pm$ 11.37
Sex (male)	73 (84.9%)
Study group	
Placebo	19 (22.1%)
Pg	20 (23.3%)
Hypothermia	24 (27.9%)
Hypothermia + Pg	23 (26.7%)
Average ICP (mm Hg)†	11.81 $\pm$ 4.1
GCS score at admission‡	7 (6–7)
Associated injury (polytrauma)	53 (61.6%)
CT findings	
Extradural hematoma	6 (7%)
Subdural hematoma	30 (34.9%)
Fracture	24 (27.9%)
Contusions	51 (59.3%)
SAH	24 (27.9%)
Infarct	1 (1.2%)
DAI	11 (12.8%)
Cerebral edema	3 (3.5%)
Decompressive craniectomy	28 (32.6%)
Length of hospital stay in days‡	13 (10–18)
GOS score at 6 mos—dichotomous (n = 70)	
Unfavorable (1–3)	20 (28.6%)
Favorable (4–5)	50 (71.4%)
GOS score at 6 mos‡	5 (3–5)
FIM at 6 mos—dichotomous (n = 60)	
Dependent (18–108)	22 (36.7%)
Independent (109–126)	38 (63.3%)
FIM at 6 mos†	102.72 $\pm$ 35.41
GOS score at 12 mos—dichotomous (n = 65)	
Unfavorable (1–3)	14 (21.5%)
Favorable (4–5)	51 (78.5%)
GOS score at 12 mos‡	5 (4–5)
FIM at 12 mos—dichotomous (n = 54)	
Dependent (18–108)	10 (18.5%)
Independent (109–126)	44 (81.5%)
FIM at 12 mos†	115.48 $\pm$ 25.25
Mortality rate at 6 mos (n = 70)	10 (14.3%)
Mortality rate at 12 mos (n = 65)	11 (16.9%)

SAH = subarachnoid hemorrhage.

* The number of patients in each category varies according to the number who were either lost to follow-up or dead (in the case of FIM) when the categories were stratified.

† Values are expressed as the mean $\pm$ SD.

‡ Values are expressed as the median (IQR).

GFAP serum levels had the highest negative odds of predicting favorable GOS score at 6 and 12 months (OR 0.85, CI 0.73–0.99, $p = 0.04$; and OR 0.79, CI 0.65–0.95, $p = 0.01$), respectively, and survival at 6 and 12 months (OR

TABLE 2. Comparative serum biomarker values stratified by dichotomized GOS score in 70 patients at 6 months*

Biomarkers	Admission Serum Values			Day 7 Serum Values			Average Serum Values		
	Good Outcome, n = 50	Poor Outcome, n = 20	p Value	Good Outcome, n = 50	Poor Outcome, n = 20	p Value	Good Outcome, n = 50	Poor Outcome, n = 20	p Value
S100-B, pg/ml	726.3 (534.2–1484.9)	758.4 (541.9–1719.9)	0.66	276.5 (81.8–614.6)	207.3 (87.1–1266.6)	0.60	543.3 (326.7–991.9)	494.7 (362.5–1366.9)	0.49
Pg, ng/ml	3.2 (2.1–4.1)	3.8 (2.1–5.7)	0.20	5.1 (2.7–6.6)	2.1 (1.6–3.1)	0.002	3.8 (3–5.4)	3.4 (2.1–4.3)	0.13
Eg, pg/ml	0.28 (0.15–0.92)	0.23 (0.14–0.86)	0.57	0.18 (0.09–0.65)	0.25 (0.12–0.89)	0.24	0.27 (0.11–0.76)	0.24 (0.13–0.80)	1.00
NSE, ng/ml	49.2 (22–71.5)	21.1 (11.1–48.4)	0.02	20 (9–42.5)	10.9 (5.6–29.6)	0.07	34.8 (17–59.1)	18.4 (8.1–37.3)	0.03
GFAP, ng/ml	11.9 (9.2–13.3)	12.2 (7.8–15.9)	0.99	6.4 (4.2–9.1)	11.9 (6.6–18.1)	0.001	9.4 (7.7–10.9)	11.2 (7.9–15.2)	0.03
IL-6, pg/ml	109.3 (92.5–129.5)	83.1 (40.9–106.6)	0.005	41.1 (25.9–63.9)	75.8 (41.9–100.6)	0.004	77.9 (58.8–95.9)	76.9 (51.1–96.7)	0.89
TNF- α , pg/ml	11.8 (8.3–24.7)	8.4 (6.5–25)	0.19	6.4 (3–11.6)	8.5 (4.3–14.4)	0.20	9.8 (5.6–18.6)	7.8 (6.5–18.5)	0.86

* Values are expressed as the median (IQR). Of 86 patients stratified by biomarkers, 16 were lost to follow-up at 6 months.

TABLE 3. Comparative serum biomarker values stratified by dichotomized GOS score in 65 patients at 12 months*

Biomarkers	Admission Serum Values			Day 7 Serum Values			Average Serum Values		
	Good Outcome, n = 51	Poor Outcome, n = 14	p Value	Good Outcome, n = 51	Poor Outcome, n = 14	p Value	Good Outcome, n = 51	Poor Outcome, n = 14	p Value
S100-B, pg/ml	758.2 (536.9–1496.1)	1212.5 (507.2–1934.9)	0.25	288.3 (81.8–616.7)	297 (89.5–1466.2)	0.20	589.6 (327.3–1013.2)	868.1 (371.4–1588.3)	0.12
Pg, ng/ml	3.4 (2.2–4.4)	3.8 (2.4–5.1)	0.29	5.1 (2.3–6.6)	2 (1.7–2.5)	0.001	3.8 (3–5.4)	3.1 (1.9–3.8)	0.03
Eg, pg/ml	0.51 (0.15–0.95)	0.24 (0.13–0.95)	0.50	0.19 (0.10–0.70)	0.23 (0.11–0.92)	0.42	0.39 (0.12–0.84)	0.31 (0.12–0.84)	0.87
NSE, ng/ml	49.2 (22–69.8)	25.1 (10.8–42.8)	0.04	18.4 (8.6–40.7)	16.1 (6–34.7)	0.41	34.1 (16.6–55.1)	20.1 (7.4–37.2)	0.10
GFAP, ng/ml	11.9 (9.1–13.3)	12.3 (10.1–16)	0.44	6.7 (4.2–9.2)	16.5 (9–18.4)	<0.001	9.4 (7.7–10.9)	14.3 (9.4–16.7)	0.005
IL-6, pg/ml	105.7 (91.1–122.7)	89.8 (49.6–108.6)	0.04	41.3 (27.8–62.9)	90.2 (44.8–105.4)	0.001	74.2 (56.7–94.1)	81.2 (68.2–98)	0.57
TNF- α , pg/ml	11.3 (8–24.7)	8.7 (5.5–27)	0.49	6.4 (3–11.6)	8.6 (4.5–15.9)	0.27	9.3 (5.6–18.6)	9.2 (6.4–21.1)	0.94

* Values are expressed as the median (IQR). Of 86 patients stratified by biomarkers, 21 were lost to follow-up at 12 months.

TABLE 4. Comparative serum biomarker values stratified by FIM score in 60 patients at 6 months*

Biomarkers	Admission Serum Values			Day 7 Serum Values			Average Serum Values		
	Good Outcome, n = 38	Poor Outcome, n = 22	p Value	Good Outcome, n = 38	Poor Outcome, n = 22	p Value	Good Outcome, n = 38	Poor Outcome, n = 22	p Value
S100-B, pg/ml	758.2 (536.9–1531.4)	697.6 (551.7–1484.9)	0.68	311.8 (125.2–614.6)	110.7 (70.1–780.8)	0.37	649.5 (347.3–991.9)	447.9 (301.9–1054.6)	0.59
Pg, ng/ml	3.2 (2.1–4.1)	3.5 (2.1–5.1)	0.59	5.1 (2.3–6.6)	4 (2–5.9)	0.39	3.9 (2.8–5.4)	3.7 (3.1–4.7)	0.73
Eg, pg/ml	0.38 (0.13–0.95)	0.40 (0.16–0.96)	0.61	0.18 (0.10–0.69)	0.23 (0.11–0.92)	0.29	0.33 (0.11–0.76)	0.32 (0.19–0.90)	0.49
NSE, ng/ml	49.2 (19.6–73.3)	29.7 (15.9–65.7)	0.26	20.1 (8.7–44)	10.5 (6.3–20.3)	0.03	34.8 (14.5–60.7)	21.2 (11.1–44.7)	0.09
GFAP, ng/ml	11.9 (9.2–12.9)	11.1 (8.2–14.4)	0.94	6.2 (3.7–9.1)	6.8 (6–10.2)	0.09	9.2 (7.5–10.7)	9.5 (7.9–12)	0.35
IL-6, pg/ml	109.3 (92.5–129.5)	97.2 (53.5–113.3)	0.12	38 (20.9–67.4)	44.2 (34.9–63.3)	0.41	82.4 (58.8–95.9)	71.1 (49.7–83.4)	0.20
TNF- α , pg/ml	10.5 (8–24.7)	12 (8.2–26.8)	0.45	6.6 (2.7–12)	6.4 (3.9–12.3)	0.72	9.3 (4.6–18.7)	10 (6.5–17.9)	0.41

* Values are expressed as the median (IQR). Of 86 patients stratified by FIM, 16 were lost to follow-up at 6 months and another 10 had died.

TABLE 5. Comparative serum biomarker values stratified by FIM score in 54 patients at 12 months*

Biomarkers	Admission Serum Values			Day 7 Serum Values			Average Serum Values		
	Good Outcome, n = 44	Poor Outcome, n = 10	p Value	Good Outcome, n = 44	Poor Outcome, n = 10	p Value	Good Outcome, n = 44	Poor Outcome, n = 10	p Value
S100-B, pg/ml	726.3 (537.6–1452.4)	804.4 (495.1–1578.6)	0.80	288.3 (119.8–615.5)	227.6 (61.3–1174.1)	0.77	589.6 (333.4–997.5)	724.6 (261.8–1143.8)	0.75
Pg, ng/ml	3.2 (2.1–4)	4.6 (2.2–5.5)	0.09	5.1 (2.4–6.5)	4.2 (2–7.8)	0.97	3.8 (3–5.4)	3.9 (3.2–6.5)	0.68
Eg, pg/ml	0.53 (0.15–0.96)	0.40 (0.14–0.96)	0.91	0.18 (0.09–0.67)	0.31 (0.14–0.92)	0.20	0.39 (0.11–0.81)	0.35 (0.16–0.92)	0.77
NSE, ng/ml	49.2 (21.7–74.6)	41.5 (20.9–62.4)	0.41	20 (8.4–42.9)	11.2 (8.1–23.5)	0.20	34.8 (15.2–60.5)	26.8 (16.3–40.4)	0.25
GFAP, ng/ml	11.9 (9.3–12.9)	12 (7.8–14.4)	0.95	6.3 (3.9–9.1)	9.7 (6.4–11.7)	0.02	8.8 (7.7–10.6)	10 (8.7–12.4)	0.16
IL-6, pg/ml	109.3 (92.3–126.8)	97.8 (45–127.6)	0.29	38.2 (21.7–66)	45.3 (40.9–52.4)	0.23	77.9 (58.3–95.1)	69 (45.8–90.5)	0.40
TNF- α , pg/ml	11.3 (8–25)	11 (6.4–21.5)	0.62	6.6 (2.9–12)	5.8 (2.8–10.7)	0.93	9.8 (5.4–19.1)	9 (6.4–15.9)	0.71

* Values are expressed as the median (IQR). Of 86 patients stratified by FIM, 21 were lost to follow-up at 12 months and another 11 had died.

TABLE 6. Comparative serum biomarker values stratified by mortality in 70 patients at 6 months*

Biomarkers	Admission Serum Values			Day 7 Serum Values			Average Serum Values		
	Alive, n = 60	Dead, n = 10	p Value	Alive, n = 60	Dead, n = 10	p Value	Alive, n = 60	Dead, n = 10	p Value
S100-B, pg/ml	726.3 (537.6–1489.7)	897.8 (420.5–1871.7)	0.92	276.5 (83–615.5)	207.3 (89.5–1340.4)	0.66	516.2 (333.4–1002.3)	522.6 (315.1–1607.3)	0.76
Pg, ng/ml	3.3 (2.1–4.5)	3.8 (2.3–5.1)	0.56	5 (2.1–6.3)	1.9 (1.6–3)	0.007	3.8 (3–5.1)	3 (1.6–3.9)	0.06
Eg, pg/ml	0.38 (0.15–0.94)	0.14 (0.10–0.34)	0.03	0.22 (0.11–0.71)	0.13 (0.09–0.34)	0.35	0.33 (0.12–0.83)	0.16 (0.09–0.47)	0.06
NSE, ng/ml	41.6 (18–68.6)	32.3 (14.2–59.8)	0.46	17.7 (7.8–35)	29.6 (10.6–39.3)	0.33	29.9 (12.9–49.7)	31.9 (12.3–49.5)	0.89
GFAP, ng/ml	11.9 (8.7–13.4)	12.3 (9.9–15.7)	0.63	6.5 (4.2–9.2)	18 (12.3–18.8)	<0.001	9.4 (7.7–10.9)	14.9 (10.2–16.7)	0.004
IL-6, pg/ml	103 (84.6–120)	89.8 (64.8–105.2)	0.14	41.3 (27.1–64.1)	98.1 (82.1–120.2)	<0.001	73.6 (56.1–93.2)	90 (77.6–101)	0.08
TNF- α , pg/ml	10.8 (8–25)	8.7 (5.5–23.3)	0.32	6.4 (3–12)	8.6 (6–15.9)	0.30	9.3 (5.8–18.6)	9.2 (7.1–15.1)	1.00

* Values are expressed as the median (IQR). Of 86 patients stratified by survival status, 16 were lost to follow-up at 6 months.

TABLE 7. Comparative serum biomarker values stratified by mortality in 65 patients at 12 months*

Biomarkers	Admission Serum Values			Day 7 Serum Values			Average Serum Values		
	Alive, n = 54	Dead, n = 11	p Value	Alive, n = 54	Dead, n = 11	p Value	Alive, n = 54	Dead, n = 11	p Value
S100-B, pg/ml	758.2 (536.9–1496.1)	1097.1 (444.1–1949.3)	0.64	288.3 (81.8–616.7)	211.9 (91.7–1586.2)	0.42	589.6 (327.3–1013.2)	590 (328–1787.1)	0.48
Pg, ng/ml	3.4 (2.2–4.4)	3.6 (1.9–5)	0.81	5.1 (2.3–6.6)	1.9 (1.6–3)	0.003	3.8 (3–5.4)	2.7 (1.6–3.7)	0.03
Eg, pg/ml	0.51 (0.15–0.95)	0.14 (0.10–0.56)	0.05	0.19 (0.10–0.70)	0.13 (0.09–0.49)	0.74	0.39 (0.12–0.84)	0.19 (0.10–0.60)	0.12
NSE, ng/ml	49.2 (22–69.8)	24.6 (11.4–51.2)	0.17	18.4 (8.6–40.7)	29.6 (6.2–37.8)	0.89	34.1 (16.6–55.1)	29.1 (7.4–44.5)	0.41
GFAP, ng/ml	11.9 (9.1–13.3)	12.3 (10.8–16)	0.35	6.7 (4.2–9.2)	17.8 (11.2–18.2)	0.001	9.4 (7.7–10.9)	14.5 (10–16.7)	0.005
IL-6, pg/ml	105.7 (91.1–122.7)	97 (74.3–109.3)	0.15	41.3 (27.8–62.9)	95.5 (71.3–119.6)	<0.001	74.2 (56.7–94.1)	84.2 (73–101)	0.11
TNF- α , pg/ml	11.3 (8–24.7)	9.2 (6–27)	0.63	6.4 (3–11.6)	9.1 (7–19)	0.12	9.3 (5.6–18.6)	10.2 (7.5–23)	0.64

* Values are expressed as the median (IQR). Of 86 patients stratified by survival status, 21 were lost to follow-up at 12 months.

TABLE 8. Adjusted OR for predicting better outcome using a binary logistic multivariate regression model

Outcome	Follow-Up (mos)	Prediction	Parameters	p Value*	OR (95% CI)
GOS score (dichotomous)†	6	Favorable	Pg-D7	0.005	2.37 (1.29–4.36)
			NSE-D1	0.86	1.00 (0.97–1.03)
			IL-6-D1	0.02	1.03 (1.00–1.06)
			GCS score	0.06	1.93 (0.98–3.79)
			Pg	0.03	30.5 (1.41–658.4)
			Hypo	0.08	10.8 (0.76–154.0)
			Hypo + Pg	0.06	19.4 (0.84–450.5)
		Unfavorable	GFAP-D7	0.04	0.85 (0.73–0.99)
			IL-6-D7	0.04	0.98 (0.96–1.00)
			GCS score	0.03	2.01 (1.08–3.75)
			Pg	0.13	4.49 (0.63–31.91)
			Hypo	0.03	11.34 (1.26–102.3)
			Hypo + Pg	0.45	2.05 (0.32–13.06)
	12	Favorable	Pg-D7	0.003	3.24 (1.50–6.96)
			NSE-D1	0.79	1.00 (0.97–1.04)
			IL-6-D1	0.04	1.04 (1.00–1.08)
			GCS score	0.03	2.41 (1.08–5.41)
			Pg	0.25	8.64 (0.21–349.9)
			Hypo	0.73	1.88 (0.05–68.45)
			Hypo + Pg	0.21	9.71 (0.27–349.2)
		Unfavorable	GFAP-D7	0.01	0.79 (0.65–0.95)
			IL-6-D7	0.06	0.98 (0.95–1.00)
			GCS score	0.07	1.94 (0.93–4.03)
			Pg	0.78	0.69 (0.05–8.83)
			Hypo	0.62	1.99 (0.13–30.8)
			Hypo + Pg	0.76	0.68 (0.06–7.86)
FIM†	6	Favorable	NSE-D7	0.05	1.04 (1.00–1.08)
			GCS score	0.003	2.39 (1.34–4.27)
			Pg	0.69	1.42 (0.26–7.81)
			Hypo	0.39	2.15 (0.38–12.07)
			Hypo + Pg	0.40	2.41 (0.32–18.42)
	12	Unfavorable	GFAP-D7	0.15	0.85 (0.68–1.06)
			GCS score	0.008	3.02 (1.33–6.85)
			Pg	0.21	6.62 (0.36–122.9)
			Hypo	0.28	3.66 (0.35–38.35)
			Hypo + Pg	0.30	4.08 (0.28–59.09)
Survival advantage‡	6	Favorable	Pg-D7	0.01	1.9 (1.14–3.23)
			Eg-D1	0.09	11.78 (0.68–205.5)
			GCS score	0.82	1.08 (0.56–2.09)
		Unfavorable	GFAP-D7	0.01	0.64 (0.45–0.90)
			IL-6-D7	0.006	0.96 (0.93–0.99)
			GCS score	0.23	0.30 (0.04–2.14)
	12	Favorable	Pg-D7	0.01	2 (1.17–3.45)
			Eg-D1	0.07	12.48 (0.82–189.65)
			GCS score	0.19	1.57 (0.79–3.12)
		Unfavorable	GFAP-D7	0.01	0.80 (0.66–0.96)
			IL-6-D7	0.01	0.97 (0.94–0.99)
			GCS score	0.81	1.11 (0.47–2.63)

D1 = admission value; D7 = Day 7 value; Hypo = hypothermia; Hypo + Pg = combined hypothermia and Pg group.

* A p value < 0.05 is considered significant.

† Adjusted for both GCS score and randomized treatment group.

‡ Adjusted only for GCS score.

0.64, CI 0.45–0.90, $p = 0.01$; and OR 0.80, CI 0.66–0.96, $p = 0.01$), respectively. Similarly, Day 7 IL-6 serum levels had the highest negative odds of predicting favorable GOS score at 6 months (OR 0.98, CI 0.96–1.00, $p = 0.04$) and survival at 6 and 12 months (OR 0.96, CI 0.93–0.99, $p = 0.006$; and OR 0.97, CI 0.94–0.99, $p = 0.01$), respectively. Although not statistically significant, there was weak evidence for admission Eg predicting survival ($p = 0.07$), Day 7 NSE predicting functional independence ($p = 0.05$), and Day 7 GFAP predicting functional dependence ($p = 0.15$).

The ROC Curve Analysis

A favorable GOS score (4–5) at 6 and 12 months was predicted by higher admission IL-6 above 108.36 pg/ml (AUC 0.72, CI 0.59–0.85, sensitivity 53.1%, and specificity 80% at 6 months; AUC 0.69, CI 0.54–0.83, sensitivity 52%, and specificity 78.6% at 12 months) and Day 7 Pg levels above 3.15 ng/ml (AUC 0.73, CI 0.60–0.86, sensitivity 69.4%, and specificity 80% at 6 months; AUC 0.79, CI 0.68–0.90, sensitivity 70%, and specificity 92.9% at 12 months) (Table 9, Figs. 1 and 2). An unfavorable GOS score (1–3) was predicted at 6 and 12 months by higher Day 7 GFAP levels above 9.50 ng/ml (AUC 0.76, CI 0.61–0.90, sensitivity 60%, and specificity 84% at 6 months; AUC 0.82, CI 0.65–0.98, sensitivity 78.6%, and specificity 82.4% at 12 months) and by higher Day 7 IL-6 levels above 71.26 pg/ml at 6 months (AUC 0.72, CI 0.58–0.86, sensitivity 55%, and specificity 86%). Survivors at 6 and 12 months had significantly higher Day 7 Pg levels above 3.15 ng/ml (AUC 0.77, CI 0.65–0.89, sensitivity 63.3%, and specificity 90% at 6 months; AUC 0.78, CI 0.67–0.90, sensitivity 66.7%, and specificity 90.9% at 12 months). Nonsurvivors at 6 and 12 months had significantly higher Day 7 GFAP serum levels above 11.14 ng/ml (AUC 0.88, CI 0.71–1.06,

sensitivity 90%, and specificity 90% at 6 months; AUC 0.81, CI 0.61–1.01, sensitivity 81.8%, and specificity 88.9% at 12 months) and Day 7 IL-6 serum levels above 71.26 pg/ml (AUC 0.91, CI 0.83–0.98, sensitivity 90%, and specificity 85% at 6 months; AUC 0.87, CI 0.75–0.98, sensitivity 81.8%, and specificity 87% at 12 months).

Discussion

Brain Tissue Antigens

The neuronal injury marker NSE is an isoenzyme of enolase ($t_{1/2}$ approximately 48 hours) that is present predominantly in the cytoplasm of neurons, has poor correlation with contusion volume, and is essentially a marker of diffuse axonal injury (DAI).^{7,16,17,19,20} However, its specificity for neurons is limited by confounding factors like sepsis, hypoperfusion, extracranial trauma, bleeding, and liver or kidney damage, in which serum NSE level also increases, making it of less clinical relevance in patients with polytrauma.^{7,20} The NSE levels in the first 72 hours postinjury have been more closely related with outcome,²¹ probably because of its short half-life. However, in the present cohort with the high incidence of polytrauma (62%) and low incidence of DAI (13%), the relevance of serum NSE levels in the present context is miniscule. We also failed to observe any statistically significant impact of NSE levels on outcome in the adjusted binary logistic model. More specific neuronal markers like ubiquitin C-terminal hydrolase L1 have shown more consistent results in recent trials.^{3,4}

A Ca^{2+} -binding protein ($t_{1/2}$ approximately 2 hours) predominantly secreted by astrocytes, S100-B is correlated with contusion volume. Despite a few studies showing serum S100-B as a predictor of outcome, its serum assay is not ideal because of its limited blood-brain barrier (BBB)

TABLE 9. The ROC curve analysis

Outcome	Serum Biomarker	Cutoff Value*	AUC (95% CI)†	Sensitivity (%)	Specificity (%)
GOS score (dichotomous)					
Favorable (6 mos)	Pg-D7, ng/ml	3.15	0.73 (0.60–0.86)	69.4	80
	IL-6-D1, pg/ml	108.36	0.72 (0.59–0.85)	53.1	80
Favorable (12 mos)	Pg-D7, ng/ml	3.15	0.79 (0.68–0.90)	70	92.9
	IL-6-D1, pg/ml	108.36	0.69 (0.54–0.83)	52	78.6
GOS score (dichotomous)					
Unfavorable (6 mos)	GFAP-D7, ng/ml	9.50	0.76 (0.61–0.90)	60	84
	IL-6-D7, pg/ml	71.26	0.72 (0.58–0.86)	55	86
Unfavorable (12 mos)	GFAP-D7, ng/ml	9.50	0.82 (0.65–0.98)	78.6	82.4
Survivors					
6 mos	Pg-D7, ng/ml	3.15	0.77 (0.65–0.89)	63.3	90
12 mos	Pg-D7, ng/ml	3.15	0.78 (0.67–0.90)	66.7	90.9
Nonsurvivors					
6 mos	GFAP-D7, ng/ml	11.14	0.88 (0.71–1.06)	90	90
	IL-6-D7, pg/ml	71.26	0.91 (0.83–0.98)	90	85
12 mos	GFAP-D7, ng/ml	11.14	0.81 (0.61–1.01)	81.8	88.9
	IL-6-D7, pg/ml	71.26	0.87 (0.75–0.98)	81.8	87

* Cutoff values were calculated using the Youden index.

† Area under the curve (95% CI) representing accuracy of the test performed.

FIG. 1. The ROC curve for truly independent factors predicting favorable and unfavorable GOS score at 6 and 12 months. Figure is available in color online only.

permeability, short half-life (small window for sampling), and peripheral source of production (besides CNS).^{10,18,28} Cerebrospinal fluid or brain tissue S100-B estimation is a better predictor of outcome. We failed to observe any association between serum S100-B and any of the outcome parameters, probably because of the above-mentioned factors.

Glial fibrillary acidic protein, an intermediate filament in astrocyte cytoskeleton ($t_{1/2}$ approximately 48 hours), is a much more specific marker of glial injury and BBB disruption with good discriminative value for focal mass lesions (contusion), and is an independent predictor of poor outcome.^{3,4,16,17,28} We observed statistically significant odds of predicting poor outcome at long-term follow-up (1 year) by using serum Day 7 GFAP levels above 9.50 ng/ml with high accuracy (AUC 0.82) and specificity (82.4%). Serum Day 7 GFAP levels above 11.14 ng/ml predicted long-term mortality (1 year) with high accuracy (AUC 0.81) and specificity (88.9%). Because GFAP has a short half-life, we hypothesize that delayed (Day 7) estimation of serum

GFAP levels could be more beneficial than the initial estimation at admission for detecting patients at long-term risk; it is an estimation of ongoing secondary neuronal injury occurring after 5–7 days, thereby causing elevation of GFAP levels again and emphasizing the role of additional measures to optimize the estimation. Because all of these antigens have relatively short half-lives, identification and prognostication in subacute and chronic TBI models is not possible. Recently, a trial assessing the role of IgG anti-GFAP antibody, which can be estimated from Day 7 until 6 months postinjury, has shown it to be a marker of BBB disruption and inversely correlated with outcome.³⁷

Cytokines

Tumor necrosis factor- α is a potent proinflammatory cytokine, produced primarily by microglia and astrocytes in CNS as a result of combined DAI and hypoxia, with a conflicting dual profile of its impact on outcome data.^{13,22,32} Its concentration in brain parenchyma increases within 1 hour after TBI and peaks between 4 and 8 hours.³² For

FIG. 2. The ROC curve for truly independent factors predicting survivors and nonsurvivors at 6 and 12 months. Figure is available in color online only.

clinical use, its concentration in CSF is a more reliable indicator (peaks at approximately 24 hours) than serum, because it excludes elevations due to polytrauma.³² Serum TNF- α concentration correlates moderately with rise in ICP, providing a window of opportunity to intervene before secondary brain damage.²⁶ We failed to identify any significant association between the serum levels of TNF- α and either of the outcome variables, probably because of confounding by a high incidence of polytrauma (62%) and a low incidence of DAI (13%), making an appropriate inference difficult.

Interleukin-6 is another cytokine expressed in CNS (microglia, astrocytes, and neurons), again with conflicting data in the existing literature regarding its role as an independent predictor of ICP, mortality, and outcome.^{1,2,6,8,9,11–13,30,32} Similar to TNF- α , it is detectable by 1 hour postinjury and peaks in brain parenchyma at approximately 2–8 hours.³² However, unlike TNF- α , particularly related to the DAI model, IL-6 levels do not depend on the injury model, and this is a more sensitive marker.³² It

inhibits TNF- α synthesis and *N*-methyl-D-aspartate-mediated toxicity, and it induces nerve growth factor (NGF) and promotes neural differentiation and survival.³² Despite representing BBB dysfunction and more reliable serum concentrations than TNF- α , it is still less specific and partly affected by the addition of polytrauma to the TBI model. For increasing specificity in the TBI model, recent studies have incorporated the NGF:IL-6 ratio as a more specific predictor and have also included cerebral microdialysis for the most accurate estimation of ongoing variation of cytokines in close vicinity to the actual lesion.³²

We observed statistically significant odds of predicting poor outcome at long-term follow-up (6 months) by using serum Day 7 IL-6 levels above 71.26 pg/ml, with adequate accuracy (AUC 0.72) and specificity (86%). Serum Day 7 IL-6 levels above 71.26 pg/ml predicted long-term mortality (1 year) with high accuracy (AUC 0.87) and specificity (87%). These observations were in line with the delayed rise in serum GFAP levels, predicting long-term outcome and mortality, representing a dismal outcome due to sec-

ondary brain damage. Paradoxically, an elevated serum IL-6 concentration above 108.36 pg/ml at admission (< 8 hours postinjury) was found to be predictive of long-term (1 year) favorable outcome, although with a poor accuracy (AUC 0.69) and lower specificity (78.6%). It is presumed that elevation in these proinflammatory cytokines is an adaptive response of the brain to injury, which causes transient destruction and apoptosis of damaged neural cells, paving the way for the reparative process. Therefore we hypothesize that an initial elevation of IL-6 levels could be protective in the long run, whereas a delayed rise could be catastrophic because it usually represents more grave conditions like elevated ICP, sepsis, multiorgan dysfunction syndrome, and shock.

Gonadal Hormones

Estradiol offers neuroprotection by ameliorating excitotoxicity, promoting neuronal lactate utilization, maintaining cerebral blood flow, and decreasing apoptosis in TBI models.^{5,15,23,25,29} However, this presumed benefit has not been consistently translated from animal (rodent) models to patients in clinical trials, partly due to a difference between the two groups in production of Eg via peripheral aromatization, which is selectively present in primates only.^{5,15,23,25,29} Up to 80% of patients with sTBI have suppression of the hypothalamic-pituitary-gonadal axis and activation of stress hormones produced by the adrenal gland; therefore, Eg production is predominantly under the control of peripheral aromatization in adipose tissue, which in turn is positively affected by proinflammatory cytokines like TNF- α .²⁹ Because there is a surge of systemic proinflammatory chemokines immediately following sTBI, the injury increases Eg levels as a byproduct of ongoing inflammatory response, thereby reflecting a global illness rather than a causal role.^{5,29} Few studies have shown a capacity of serum Eg levels at 48 hours to predict mortality,^{5,15} but their model failed to incorporate proinflammatory cytokines, a major confounder in the primate TBI model. As expected, we failed to observe any statistically significant impact of admission Eg levels on mortality in the adjusted binary logistic model, despite a weak association evident with survival status ($p = 0.09$ and $p = 0.07$ at 6 and 12 months, respectively).

Progesterone mediates its neuroprotection by reducing cerebral edema, lipid peroxidation, isoprostanes, and expression of proinflammatory genes; generating metabolites that reduce proapoptotic and increase antiapoptotic enzymes; and modifying the expression of vascular endothelial growth factor, brain-derived neurotrophic growth factor, and aquaporins responsible for development of edema.¹⁴ Besides these direct effects, it also indirectly affects the TBI model by modulating Eg, testosterone, and cortisol levels.²⁹ Although previous Phase II trials confirmed a beneficial role of Pg therapy in an sTBI model for optimal outcome,^{33,35} more recent Phase III clinical trials failed to do so.^{24,34} We observed statistically significant odds of predicting favorable outcome at long-term follow-up (1 year) using serum Day 7 Pg levels above 3.15 ng/ml with adequate accuracy (AUC 0.79) and high specificity (92.9%). Serum Day 7 Pg levels above 3.15 ng/ml also predicted survivors with adequate accuracy (AUC 0.78) and high

specificity (90.9%). These results imply the importance of maintaining serum levels of Pg over 3.15 ng/ml until 7 days postinjury for optimal outcome.

Limitations of the Study

Small sample size, lack of sampling data between admission and Day 7, and a higher percentage of patients lost to follow-up are the primary limitations of this study. Inclusion of only adults with sTBI might not allow generalization of our results to all TBI models. Lack of data on body mass index and menopausal status may have affected the analysis of effects of gonadal hormones.

Conclusions

Serial monitoring and optimization of serum Pg, GFAP, and IL-6 levels could aid in prognostication in patients with sTBI and guide us to direct more resources toward such patients for optimal outcome. A cause and effect relationship or a mere association of these biomarkers to outcome needs to be further studied for better understanding of the pathophysiology in sTBI and for choosing potential therapeutic targets. Ultimately, a multidimensional prognostic model that incorporates the biochemical profile along with clinical and radiological parameters will be useful for clinical practice as well as clinical research.

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Disclosures

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Author Contributions

Conception and design: Sinha. Acquisition of data: Samson. Analysis and interpretation of data: Raheja. Drafting the article: Raheja, Samson. Critically revising the article: Sinha, Bhoi, Subramanian. Reviewed submitted version of manuscript: all authors. Approved the final version of the manuscript on behalf of all authors: Sinha. Statistical analysis: Raheja. Administrative/technical/material support: Sinha, Bhoi, Subramanian, P Sharma. Study supervision: Sinha, BS Sharma.

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