Predictors of recovery in moderate to severe traumatic brain injury

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OBJECTIVE Traumatic brain injury (TBI) is a significant cause of morbidity and mortality worldwide. Clinical outcomes in TBI are determined by the severity of injury, which is dependent on the primary and secondary brain injury processes. Whereas primary brain injury lesions are related to the site of impact, secondary brain injury results from physiological changes caused by oxidative stress and inflammatory responses that occur after the primary insult. The aim of this study was to identify important clinical and biomarker profiles that were predictive of recovery after moderate to severe TBI. A good functional outcome was defined as a Glasgow Outcome Scale (GOS) score of ≥ 4 .

METHODS This was a prospective study of patients with moderate to severe TBI managed at the Nelson Mandela Academic Hospital during the period between March 2014 and March 2016. Following admission and initial management, the patient demographic data (sex, age) and admission Glasgow Coma Scale score were recorded. Oxidative stress and inflammatory biomarkers in blood and CSF were sampled on days 1–7. On day 14, only blood was sampled for the same biomarkers. The primary outcome was the GOS score—due to its simplicity, the GOS was used to assess clinical outcomes at day 90. Because of difficulty in performing regular follow-up due to the vastness of the region, difficult terrain, and long travel distances, a 3-month follow-up period was used to avoid default.

RESULTS Sixty-four patients with Glasgow Coma Scale scores of ≤ 12 were seen and managed. Among the 56 patients who survived, 42 showed significant recovery (GOS score ≥ 4) at 3 months. Important predictors of recovery included antioxidant activity in the CSF (superoxide dismutase and total antioxidant capacity).

CONCLUSIONS Recovery after TBI was dependent on the resolution of oxidative stress imbalance.

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KEYWORDS trauma; traumatic brain injury; oxidative stress; inflammatory changes; recovery

TRAUMATIC brain injury (TBI) is a significant cause of death and disabling neurological deficits.^{2,32} Although the Glasgow Coma Scale (GCS) score is important in assessing the state of consciousness,⁴⁸ it has been noted to have great limitations and inconsistencies when used in assessing survival and functional outcomes among patients with TBI.^{7,15,51} Clinical outcomes in patients with TBI can be assessed using the Glasgow Outcome Scale (GOS) score.²² Although current management strategies take into account factors such as the admission GCS score, pupillary reactivity, age of the patient, mecha-

nism of injury,²⁰ CT scan findings,³⁰ intracranial pressure (ICP), and brain tissue oxygen tension (PBO₂), little or no information regarding the degree of ischemic cell damage, the level of oxidative stress imbalance, and inflammatory changes can be deduced from these parameters.

Oxidative stress is a disturbance in the equilibrium status of prooxidant/antioxidant systems and accounts for most of the negative consequences of secondary brain injury. The process of oxidative stress involves enhancement in the production of free radicals and strong oxidants as well as depletion of body stores of antioxidants following

ABBREVIATIONS AUC = area under the curve; BBB = blood-brain barrier; CI = confidence interval; GCS = Glasgow Coma Scale; GOS = Glasgow Outcome Scale; HR = hazard ratio; ICP = intracranial pressure; IL = interleukin; MDA = malondialdehyde; PBO₂ = brain tissue oxygen tension; ROC = receiver operating characteristic; SOD = superoxide dismutase; TAC = total antioxidant capacity; TBARS = thiobarbituric acid reactive substances; TBI = traumatic brain injury; TNF α = tumor necrosis factor- α .

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TABLE 1. Inclusion and exclusion criteria

Inclusion Criteria	Exclusion Criteria
Patients w/ moderate to severe TBI (GCS ≤ 12) in whom neuromonitoring & surgical intervention were indicated	Patients who died while still in the accident & emergency department before admission
Patients w/ intracranial pathology requiring surgical intervention &/or temporary CSF diversion to reduce ICP	Patients in whom neuromonitoring was not done due to logistical problems
Patients w/ informed consent (from relatives) to participate in the study	Patients w/ no informed consent due to refusal by relatives to participate in the study

trauma or inflammation. Early after TBI, oxidative stress changes occur, leading to increased lipid peroxidation and decreased enzymatic antioxidant activities, which can affect clinical outcomes.^{16,31}

The reactive oxygen species generated during oxidative stress imbalance cause neuronal damage, dysfunction of the blood-brain barrier (BBB), and brain edema in TBI.¹⁹ These reactive oxygen intermediates, including superoxide anions, hydroxyl radicals, hydrogen peroxide, and hypochlorous acid, cause oxidative damage to cellular proteins and nucleic acids in addition to lipid peroxidation.²⁶ Lipid peroxidation results in the generation of free radicals including malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE) or 2-propenol (acrolein), which can cause increased cell membrane permeability, brain edema, and neuronal damage.^{49,55} Termination of lipid peroxidation occurs in reactions involving lipid radicals with endogenous antioxidants.

TBI also induces an inflammatory response, with subsequent release of inflammatory mediators. Delayed or inadequate management of TBI may propagate an unregulated acute inflammatory response and put in motion a vicious cycle of inflammatory damage^{8,19,21,25,33,38,42,45,52,54,60} caused by inflammatory factors such as tumor necrosis factor- α (TNF α), interleukin (IL)-1 β , and IL-18, which are proinflammatory cytokines. The secretion of inflammatory cytokines, including IL-1 β , TNF α , IL-6, and IL-8, which occurs early in TBI, causes the recruitment of leukocytes and increased secretion of nitric oxide during inflammation.^{9,39} These proinflammatory responses lead to a disruption of the BBB, neuronal tissue damage, and worsening of brain edema.^{1,17,41}

The inflammatory process also involves secretion of antiinflammatory factors, including IL-6 and IL-10. IL-6 has a dual inflammatory role, with both antiinflammatory and proinflammatory effects. The antiinflammatory effect of IL-6 is through inhibition of the TNF α synthesis, promotion of release of soluble TNF α receptors, induction of the IL-1 receptor antagonist, and attenuation of oxidative stress.^{37,57} IL-6 also promotes the release of prostaglandin E2, which stimulates IL-10 secretion.²⁴ The neuroprotective effects of IL-6 occur through neuronal apoptosis, regulation of cytokine secretion, and maintenance of BBB integrity by inhibiting the expression of matrix metalloproteinase-9 in ischemic cells.¹⁴ IL-10 is an immunoregulatory

protein produced by macrophages, dendritic cells, and B-lymphocyte cells—it exerts its antiinflammatory effect by limiting or inhibiting adaptive and innate immunity. Actions of IL-10 prevent downregulation of antiapoptotic protein Bcl-2 in ischemic brain tissue and limit excitotoxic brain injury.³ Effects of IL-10 in cerebral ischemia lead to a reduction in volume of infarcted tissue and improvement in clinical outcomes. Low levels of IL-10 are associated with impaired antiinflammatory response and poor recovery in stroke patients.⁴ The antiinflammatory effect of IL-10 attained by limiting the production of TNF α and interferon- γ may lead to a reduction in tissue destruction and limitation of tissue necrosis.^{28,58,59}

Methods

This was a prospective study of patients with moderate to severe TBI managed at the Nelson Mandela Academic Hospital in the Eastern Cape Province of South Africa during the period between March 2014 and March 2016.

At the accident and emergency department, neurological evaluation and radiological investigations including CT scans of the brain were performed. The patients requiring surgical intervention were then taken to the operating room, where surgical procedures such as burr holes for the insertion of external ventricular drain catheters, ICP probes, PBO₂ probes (Licox), and craniotomy or craniectomy were performed in patients with intracranial mass lesions for decompression or evacuation of intracranial hematoma. Criteria for inclusion or exclusion from the study were defined (Table 1).

Postoperatively, the patients were admitted to the ICU, where ventilation, sedation, analgesia, and monitoring of the ICP, PBO₂, and brain tissue temperatures were done. Daily blood and CSF samples were taken at 9:00 AM for evaluation of inflammatory and oxidative stress biomarkers from day 1 to day 7. The blood was again sampled for evaluation of the biomarkers on day 14 of follow-up. The serum and CSF total antioxidant capacity (TAC) and superoxide dismutase (SOD) levels were analyzed. The serum MDA levels were assessed using the thiobarbituric acid reactive substances (TBARS) assay, whereas serum inflammatory biomarkers, including IL-1 β , IL-6, and IL-10, were assayed using the BioLegend enzyme-linked immunosorbent assay (ELISA) method. The patients were reviewed at 2 weeks and 12 weeks, during which time the GOS score was assessed.

Sample Collection and Storage

The blood samples were collected by venipuncture into sterile vacutainer non-EDTA-containing tubes (Becton Dickinson). Following centrifugation of blood samples for 13 minutes at 3000 rpm, the supernatant (serum) was pipetted into vacutainer tubes. Aliquots were then placed into cryovials bearing the patient's name, date, and time of collection before storage at -80°C . The CSF samples were also pipetted into cryovials and stored at -80°C .

MDA Assay by Evaluation of TBARS

The OxiSelect TBARS assay kit for MDA quantitation (Cell Biolabs, Inc.) was used. Assay of MDA by the

FIG. 1. Distribution of patients with TBI by age groups.

TBARS method is used to monitor lipid peroxidation. The procedure was performed per the manufacturer's specifications.

SOD Assay

A SOD assay kit manufactured by Cayman Chemical USA was used. The SOD assay kit uses tetrazolium salt for the detection of superoxide radicals generated by xanthine oxidase and hypoxanthine. One unit of SOD is the amount of enzyme needed to exhibit 50% of dismutation of the superoxide radical. This assay measures all 3 types of SOD (Cu/Zn, Mn, and Fe SOD).

TAC Assay by the Ferric-Reducing Antioxidant Power Method

This method of evaluating the total antioxidant power assesses the ferric-reducing potential of the sample. During the procedure, inactivation of oxidants was done in a redox reaction by using reductants as the electron-donating antioxidants. Reduction of ferric tripyridyltriazone to a ferrous state occurs at a low pH to produce an intense blue color, which was measured by the change in absorbance at 593 nm. The change in absorbance was directly related to the total reduction of the electron-donating antioxidants in the reaction.

IL Assay

IL-1 β , IL-6, and IL-10 assay was performed using BioLegend's ELISA MAX deluxe sets (ISO 9001:2008 and ISO 13485:2003; BioLegend, Inc.). The assay was performed per the manufacturer's specifications.

Ethical Considerations

Prior to initiation of the study, ethical and institutional approval was obtained from the Walter Sisulu University and the department of health according to the ethical guidelines of the Helsinki Declaration of 1975.

Data and Statistical Analysis

Data analysis was performed using SPSS version 23.0 for Windows (IBM Corp.). Univariate and multivariate logistic regression models including receiver operating characteristic (ROC) curves, Cox regression, and Kaplan-Meier estimates were used to identify the variables that were independently associated with survival and recovery.

Results

Identification of Potential and Independent Predictors of Recovery of Cerebral Functional Status in Patients With Moderate to Severe TBI

During the study, 64 patients with moderate to severe TBI were treated. The age of our patients ranged from 10 years to 62 years. The patient age group with the highest frequency of TBI was the age group 20–39 years ($n = 35$), the intermediate frequency was in the age group 0–19 years ($n = 21$), and the lowest frequency was in the age group 40–62 years ($n = 8$) (Fig. 1). Of the 64 patients, 65.6% ($n = 42$) sustained injuries from assaults, 21.9% ($n = 14$) from motor vehicle accidents, 9.4% ($n = 6$) from falls from heights, and 3.1% ($n = 2$) for other unclear reasons ("other") (Table 2).

Frequencies of pathology among the intracranial findings in 64 patients were as follows: subdural hematoma in 13 (20.3%), extradural hematoma in 2 (3.1%), brain edema

TABLE 2. Mechanism of injury and number of patients

Mechanism of Injury	No. of Patients	%
Assault	42	65.6
Motor vehicle accident	14	21.9
Fall from height	6	9.4
Other	2	3.1
Total	64	100.0

FIG. 2. Pie chart of intracranial pathology in patients with TBI. EDH = extradural hematoma; ICH = intracerebral hemorrhage.

in 7 (10.9%), intracerebral hemorrhage in 7 (10.9%), combinations of 3 or more pathologies in 15 (23.4%), combined subdural and extradural hematoma in 1 (1.6%), combined subdural hematoma and brain edema in 3 (4.7%), combined subdural hematoma and intracerebral hemorrhage in 3 (4.7%), combined extradural hematoma and brain edema in 4 (6.3%), combined extradural hematoma and intracerebral hemorrhage in 3 (4.7%), and combined brain edema and intracerebral hemorrhage in 6 (9.4%) patients (Fig. 2).

The endpoint for assessment was the 3-month GOS score²² with its 5 categories: 1, dead; 2, persistent vegetative state; 3, severe disability; 4, moderate disability; and 5, good recovery. Good cerebral function or recovery was defined as GOS $\geq$ 4. Of the 64 patients, 0% ($n = 0$) and 65.6% ($n = 42$) presented with good functional status at admission and at final evaluation, respectively.

After 3 months of neurosurgical management, the outcome was characterized by 12.5% of deaths (GOS 1), 11.5% of persistent vegetative state (GOS 2), 10% of moderate disability (GOS 4), and 66% of good neurological functional state (GOS 5). Thus, at day 90, less than 15% of the patients who were still alive presented with severe disability or persistent vegetative state.

Univariate Analysis and Optimal Cutoff Points to Discriminate Recovery of Neurological Function and Unchanged GCS Score

The univariate risk of recovery of neurological functional status was computed using ROC curve methods to define optimal cutoff points of significant independent variables (Table 3, Fig. 3). There was no significant univariate association ($p > 0.05$) between sex, serum IL-6, serum IL-10, and recovery of good function (results not shown). However, significant univariate correlates of recovery of good function (GOS $\geq$ 4) were identified using optimal cutoffs to discriminate unchanged neurological status from recovery of good functional status by using ROC methods. Indeed, serum IL-1 β , serum MDA, serum SOD, serum TAC, CSF SOD, and CSF TAC were confirmed as either significant or important univariate correlates of recovery based on their prognostic performance (Table 3); the correlations for serum IL-1 β , serum SOD, and CSF TAC were not statistically significant ($p = 0.08$). To discriminate between recovery and nonrecovery using the ROC methods, the area under the curve (AUC) and 95% confidence interval (CI) were used.

According to the univariate analysis, the main fac-

TABLE 3. Optimal cutoff as defined by ROC curve analysis

Marker	Cutoff	AUC	95% CI	SE	% Sensitivity	% Specificity	p Value
Serum IL-1 β	<35 pg/ml	0.619	0.533–0.704	0.043	66	57	0.08
Serum MDA	<1.4 μ mol/L	0.653	0.567–0.738	0.044	70	52	<0.001
Serum SOD	$\geq$ 0.3 IU/ml	0.573	0.475–0.671	0.050	60	60	0.08
Serum TAC	$\geq$ 450 μ mol/L	0.662	0.566–0.757	0.049	72.2	52	<0.0001
CSF SOD	$\geq$ 0.3 IU/ml	0.635	0.547–0.734	0.045	63	52	0.002
CSF TAC	$\geq$ 300 μ mol/L	0.577	0.488–0.666	0.045	70	50	0.083

Diagonal segments are produced by ties.

FIG. 3. ROC curves for significant correlates of recovery of good functional status.

tors that strongly predicted recovery and improvement of functional status in patients with moderate to severe TBI included serum IL-1 β , serum MDA, serum SOD, serum TAC, CSF SOD, and CSF TAC (Tables 3 and 4), as was amplified by the calculated relative risk (Table 4).

Multivariate analysis was then performed to identify the most significant independent predictors of recovery, by using Cox regression analysis (Table 5) and stratified Kaplan-Meier curves (Figs. 4 and 5). In Cox regression analysis, after adjusting for serum SOD, serum TAC, serum IL-1 β , serum IL-10, and serum MDA, only the CSF SOD and CSF TAC remained as the most significant independent predictors of recovery of good functional status (Table 5). Indeed, the likelihood of recovery of good functional status—defined as GOS $\geq$ 4—was multiplied in excess of 90% by both CSF SOD $\geq$ 0.3 IU/ml and CSF TAC $\geq$ 300 μ mol/L (Table 4).

The comparisons of the mean time to recovery of good functional status according to the log-rank test by stratification of CSF SOD (Fig. 4) and CSF TAC (Fig. 5) were obtained by the Kaplan-Meier curves.

Kaplan-Meier Analysis of CSF SOD as an Indicator of Recovery

Based on Kaplan-Meier analysis of the CSF SOD activity, the cumulative proportion of patients with CSF SOD activity $\geq$ 0.3 IU/ml who recovered good functional status was 95.7%, with a mean time to recovery of 34 ± 1.9 days. This was statistically significant ($p < 0.0001$ by the log-rank Mantel-Cox test) when compared with the proportion of patients with CSF SOD activity $<$ 0.3 IU/ml who

TABLE 4. Summary of calculated significant association between correlates and recovery of good functional status

Variable of Interest	% w/ Recovery of Good Functional Status (no.)	% w/ Disability (no.)	Relative Risk	95% CI	p Value
Serum IL-1 β					
<35 pg/ml	94.7 (36)	5.3 (2)	1.4	1.2–1.5	<0.0001
$\geq$ 35 pg/ml	69.2 (18)	30.8 (8)			
Serum MDA					
<1.4 μ mol/L	94.4 (34)	5.6 (2)	1.4	1.2–1.5	<0.0001
$\geq$ 1.4 μ mol/L	71.4 (20)	28.6 (8)			
Serum SOD					
$\geq$ 0.3 IU/ml	93.3 (42)	6.7 (3)	1.5	1.3–1.8	<0.0001
<0.3 IU/ml	57.9 (11)	42.1 (8)			
Serum TAC					
$\geq$ 450 μ mol/L	92.5 (37)	7.5 (3)	1.4	1.2–1.6	<0.0001
<450 μ mol/L	66.7 (16)	33.3 (8)			
CSF SOD					
$\geq$ 0.3 IU/ml	95.7 (44)	4.3 (2)	1.7	1.4–2	<0.0001
<0.3 IU/ml	55.6 (10)	44.4 (8)			
CSF TAC					
$\geq$ 300 μ mol/L	95.5 (42)	4.5 (2)	1.5	1.3–1.8	<0.0001
<300 μ mol/L	60 (12)	40 (8)

FIG. 4. Kaplan-Meier curves for CSF SOD in TBI.

recovered to GOS ≥ 4 (55.6%, with mean time to recovery of 43 ± 4.4 days). Values for continuous variables are expressed as the mean $\pm$ SD throughout.

Kaplan-Meier Analysis of CSF TAC as an Indicator of Recovery

Kaplan-Meier analysis was used to estimate the survival of patients based on the biomarkers. The cumulative proportion of patients with CSF TAC ≥ 300 $\mu\text{mol/L}$ surviving and recovering to GOS ≥ 4 was 95.5%, with a mean time to recovery of 34.6 ± 1.97 days. This is significant when compared (log-rank Mantel-Cox test, $p < 0.001$) with the cumulative proportion of patients with CSF TAC < 300 $\mu\text{mol/L}$ who would recover (60%, mean time to recovery 41.4 ± 4.03 days).

Discussion

As in previous South African studies, assaults were a predominant mechanism of injury in our study population. This is unlike the findings in North American studies, in which motor vehicle accidents and falls are the most common causes of TBI.^{10,44,50}

In this study, we describe the impact of TBI and neurosurgical management on clinical and biomarker profiles to predict the likelihood of recovery as defined by the GOS score at 3 months. We attempt to provide an objective assessment of the likely outcome by discriminating recovery (GOS ≥ 4) from nonrecovery (poor outcomes) (GOS ≤ 3) after moderate to severe TBI.

The most significant prognostic information was observed from CSF TAC and SOD activity. None of these factors have been considered in prognostic models such as the International Mission for Prognosis and Clinical Trials in Traumatic Brain Injury (IMPACT) and Corti-

costeroid Randomisation After Significant Head Injury (CRASH-1).^{29,47} Indeed, these two characteristics have not been previously studied to determine any relationship with clinical outcomes in TBI.

Following neurosurgical intervention and management, some patients did not recover and were observed to have had an unchanged ("maintained") neurological functional status (GOS ≤ 3) by day 90. Univariate correlates of recovery by ROC curve analysis defined thresholds for serum IL-1 β , serum MDA (by TBARS assay), and serum and CSF SOD and TAC.

From this research it is evident that high levels of IL-1 β are associated with lack of recovery and poor outcomes after TBI among these patients. This is most likely to be related to the potent proinflammatory effects of IL-1 β , which lead to an increase in volume of hemorrhagic contusions and brain edema. These pathophysiological changes in TBI result in poor clinical outcomes as manifested by the low GOS score.^{18,43} Various studies have been done to determine the prognostic significance of some biomarkers, including the calcium-binding protein (S100B) and neuron-specific enolase as biomarkers in TBI. No universal consensus has been reached to define the role of these biomarkers in predicting outcomes, due to their low specificity and the small sample sizes used.^{40,53}

Analysis using ROC methods revealed that IL-1 β levels ≥ 35 pg/ml were associated with poor clinical outcomes (GOS ≤ 3). The proinflammatory effects of IL-1 β aggravate the pathological changes in TBI and affect the patient's capacity for survival and recovery. The association of elevated serum IL-1 β , a proinflammatory cytokine with poor clinical outcomes, has also been demonstrated in other studies.^{6,13,46,56} Indeed, the impact of IL-1 β on survival and recovery revealed in this study has also been demonstrated in various other studies.¹²

FIG. 5. Kaplan-Meier curves for CSF TAC in TBI.

This study identifies high levels of MDA ($\geq 1.4 \mu\text{mol/L}$; $p = 0.041$) as associated with decreased survival and poor outcomes ($\text{GOS} \leq 3$) in patients with TBI. ROC curve analysis identified MDA as a significant predictor of recovery ($\text{AUC } 0.653$; $p < 0.001$; sensitivity 70%, specificity 52%) after TBI. Based on these findings, levels of MDA, a by-product of lipid peroxidation, can be used to prognosticate outcomes in TBI. Early after TBI oxidative stress changes occur, leading to increased lipid peroxidation and decreased enzymatic antioxidant activities, which can influence clinical outcomes in these patients.^{16,31} These findings are further supported by the Kaplan-Meier analysis, which revealed that elevated MDA levels ($\geq 1.4 \mu\text{mol/L}$) are associated with decreased survival and increased fatality.

Unlike previous studies on the role of biomarkers in TBI, this research shows the chronobiological changes in biomarkers over several days. In this study, although the levels of MDA were high immediately following trauma and admission, prompt neurosurgical intervention and ICU management led to a progressive decline in the levels over several days and to a reduction in the oxidative stress imbalance among our patients. The impact of the reduction in MDA levels below the threshold value on the recovery of our patients highlighted the predictive significance of this biomarker. As has been documented in other studies, MDA is a significant biomarker of lipid peroxidation and oxidative stress.³⁶ The increased lipid peroxidation and elevated MDA levels were associated with a decrease in enzymatic antioxidant activity, which may have led to poor clinical outcomes in our patients. This association of increased lipid peroxidation with poor outcomes has also been shown in other studies.^{16,31}

The enzymatic antioxidant activity of SOD is necessary to counter the prooxidant response and to correct the oxidative stress imbalance in TBI, and by so doing, SOD aids in neurological recovery.^{19,60} ROC curves identified

CSF SOD activity $\geq 0.3 \text{ IU/ml}$ as being correlated with recovery, with sufficient power to discriminate recovery from nonrecovery ($\text{AUC } 0.635$; $p = 0.002$; sensitivity 63%, specificity 52%). In Kaplan-Meier analysis, when CSF SOD activity was $\geq 0.3 \text{ IU/ml}$, more patients (95.7%) had a greater probability of recovery ($p < 0.0001$). Additionally, the recovery period was shorter (34 ± 1.9 days) than when the CSF SOD activity was $< 0.3 \text{ IU/ml}$ (55.6% over 43 ± 4.4 days). The chance for survival and recovery of good functional status was shown to be 4 times greater when the CSF SOD activity was $\geq 0.3 \text{ IU/ml}$.

Free radicals generated during oxidative stress response cause ischemic cerebral damage and may result in reduced patient survival and poor outcomes. Increased antioxidant biomarkers including SOD in TBI scavenge the reactive species and correct the oxidative stress imbalance and inflammatory response to get better clinical outcomes.^{23,35} Our study demonstrates these observations and shows that the resolution (elevation) of antioxidant biomarkers including SOD and total antioxidant activity in CSF significantly impacts clinical outcomes.

TABLE 5. Independent predictors of recovery by Cox regression analysis

Variable	B Statistic	SE	Wald Test	HR (95% CI)	p Value
CSF TAC					
$\geq 300 \mu\text{mol/L}$	0.363	0.180	4.085	1.4 (1.01–2.1)	0.043
$< 300 \mu\text{mol/L}$			Referent	1	
CSF SOD					
$\geq 0.3 \text{ IU/ml}$	0.471	0.189	6.224	1.6 (1.1–2.3)	< 0.01
$< 0.3 \text{ IU/ml}$			Referent	1	

ROC curve analysis furthermore identified better clinical outcomes when serum TAC was ≥ 450 $\mu\text{mol/L}$ (AUC 0.662; $p < 0.0001$). However, analysis of CSF TAC showed that the optimal cutoff value for recovery was ≥ 300 $\mu\text{mol/L}$ (AUC 0.577), which defines the important role of antioxidants in averting the oxidative stress imbalance. Further analysis using Cox regression models defined CSF TAC as a major determinant of good outcomes in TBI (hazard ratio [HR] 1.4, 95% CI 1.01–2.1; $p = 0.043$) (Table 5).

Additionally, Kaplan-Meier analysis showed that when CSF TAC levels were ≥ 300 $\mu\text{mol/L}$, the cumulative proportion of patients surviving was 95.5%, with a shorter time to recovery (34.6 ± 1.97 days) when compared with levels < 300 $\mu\text{mol/L}$, where the proportion of patients recovering was smaller (60%) and the time to recovery was prolonged (41.4 ± 4 days). Indeed, various studies show that antioxidant defenses can prolong survival, enhance recovery, and reduce the destructive effects of free radicals and oxidant factors.^{34,35} Thus, both Kaplan-Meier and Cox regression analysis demonstrate that the resolution and improved antioxidant activity of CSF SOD and CSF TAC not only shorten the time to recovery but also result in an increased proportion of patients with better outcomes (GOS ≥ 4).

Thus, in multivariate analysis an elevation in CSF TAC and CSF SOD activity was independently associated with recovery of good functional status (GOS ≥ 4). The findings in this study reveal that antioxidant factors, as defined by the CSF TAC levels and SOD activity, are important in the recovery of patients with moderate to severe TBI. Multivariate Cox regression analysis revealed that recovery (GOS ≥ 4) in TBI was significantly dependent on factors such as increased antioxidant function, manifested by increased CSF SOD ≥ 0.3 IU/ml (HR 1.6, 95% CI 1.1–2.3; $p < 0.01$) and increased CSF TAC ≥ 300 $\mu\text{mol/L}$ (HR 1.4, 95% CI 1.01–2.1; $p = 0.043$).

This study reveals that the resolution in levels of antioxidants as evidenced by the CSF SOD and CSF TAC is more predictive of functional recovery. The study further justifies the important role of endogenous antioxidants in functional recovery. The impact of antioxidant and anti-inflammatory biomarkers in correcting the oxidative stress imbalance, BBB dysfunction, and cerebral autoregulation has been documented in various other studies.⁵

Studies on the role of calcium-binding protein (S100B) and neuron-specific enolase show that elevated levels of these biomarkers may be predictive of poor outcomes in patients with TBI.^{11,27} Our investigation is a comprehensive study that relates the trends of biomarker profiles over several days to the clinical recovery of patients with moderate to severe TBI. As illustrated in other studies, this research defines the role of antioxidants in reversing the destructive effects of free radicals.^{34,35}

Limitations of the Study and Future Perspectives

This sample size was relatively small and the study was conducted in a single institution; therefore, institutional bias may be a valid limitation. It would be preferable to conduct a larger, multicenter, institutional study that evaluates the impact of replenishment of antioxidants and relates it to clinical outcomes in patients with moderate to severe TBI.

Conclusions

Independent predictors of recovery in patients with moderate to severe TBI include CSF SOD and CSF TAC. An elevation in the antioxidant levels in the CSF was associated with better clinical outcomes. Although other studies have evaluated the role of biomarkers in TBI, none of them reviewed the trends in these biomarkers and their impact on survival, as has been demonstrated in this study.

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Disclosures

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

Author Contributions

Conception and design: Muballe. Acquisition of data: Muballe. Analysis and interpretation of data: Muballe. Drafting the article: Muballe. Critically revising the article: Muballe. Reviewed submitted version of manuscript: Muballe. Approved the final version of the manuscript on behalf of all authors: Muballe. Statistical analysis: Longo-Mbenza. Administrative/technical/material support: Iputo. Study supervision: Sewani-Rusike, Longo-Mbenza.

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