Article

Serum Biomarker Concentrations on Admission in Acute Traumatic Brain Injury: Associations with, TBI Severity, *Toxoplasma gondii* Infection and Outcome in a Referral Hospital Setting, Cameroon

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Abstract: Despite the available literature on traumatic brain injury (TBI) biomarkers elsewhere, data are limited or non-existent in sub-Saharan Africa (SSA). The aim of the study was to analyse associations in acute TBI between admission serum biomarker concentrations and TBI severity, CT-scan findings and outcome as well as explore the influence of concurrent *Toxoplasma gondii* infection. Concentrations of serum biomarkers **(**GFAP, NFL Tau, UCH-L1, and S100B) were measured and *Toxoplasma gondii* detected in samples obtained <24 hours post-injury. The GOSE was used to evaluate 6 months outcome. All biomarkers levels increased with severity of TBI, but this increase was significant only for NFL (P=0.01). GFAP values significantly increased (P=0.026) in those with unfavorable outcome. Tau levels were higher in those who died (P=0.017). GFAP and NFL were sensitive to CT-scan pathology (p values respectively 0.004 and 0.002). S100B levels were higher (P<0.001) in TBI patients seropositive to *Toxoplasma gondii.* NFL was found to be sensitive to TBI severity while NFL and GFAP were predictive of CT intracranial abnormalities. Increased levels of GFAP and Tau were associated with poorer outcomes 6 months after TBI. S100B levels were significantly higher in *T. gondii* seropositive TBI patients.

Keywords: Serum biomarkers; Traumatic brain injury; CT-scan findings; outcome; *Toxoplasma gondii*

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1. Introduction

Traumatic brain injury (TBI) remains a major cause of mortality and disability globally [1,2], and more than 50 million people globally sustain a TBI each year [3]. The crude incidence of TBI at the regional level in Europe ranges between 83.3 to 849 per 100 000 populations per year [4]. In Low-middle-income countries (LMICs), the incidence of TBI ranges between 150 and 316 cases per 100 000 populations [5,6]. In sub-Saharan Africa (SSA), the incidence of TBI is expected to rise to 14 million yearly by 2050 [7-9]. TBI is extremely common and associated with complex biological and chemical changes in the brain resulting from the mechanical forces applied to the head [10].

Therefore, understanding the underlying pathomechanisms of TBI and accurate diagnosis is necessary for effective and patient-oriented treatment [11,12]. Presently, the primary clinical indicators for TBI are the Glasgow Coma Scale (GCS), pupil reactivity, and head computed tomography (CT). But these are however limited, as they do not show cerebral pathology at cellular or molecular level. Hence, several astroglial and neuronal proteins have been proposed as potential biomarkers [13,14].

The importance of biomarkers in TBI lies in their capacity to provide insight into injury-induced cellular, biochemical, and molecular changes and to demonstrate the presence of early micro-lesions that are difficult to detect using imaging techniques [15]. Protein biomarkers have been the most studied with a focus on S100 calcium-binding protein (S100B), neuron-specific enolase (NSE), glial fibrillary acidic protein (GFAP), ubiquitin C-terminal hydrolase-L1 (UCH-L1), neurofilament-light (NFL) [3,12,16,17]. In the USA, the Food and Drug Administration (FDA) has approved UCH-L1/GFAP-based blood test to aid in the diagnosis of mild TBI patients [18]. Generally, Magnetic Resonance Imaging (MRI) and blood biomarker measurement are reported to enhance the characterization of injury severity and type of TBI [19]. Therefore, the prospect of using peripheral blood-based markers synergistically with current clinical diagnostic and prognostic assessments in TBI is attractive for its clinical acceptability and compared to other invasive procedures, it is cost-effective and it may quickly and accurately provide specific information about the underlying pathophysiology of TBI, which clinicians need in the formulation of treatment strategies and prognosis [18].

Despite the available literature on TBI biomarkers elsewhere [3,12,20-24], data are limited or non-existent in SSA. In contrast to most developed countries, *T. gondii* is prevalent in SSA, and often involves the central nervous system. Neuroinflammation is considered an important component of TBI [25-27], and we hypothesized that concomitant *T. gondii* infections might lead to higher biomarker levels after TBI. TBI biomarker research may be particularly promising in resource-limited countries as the use of biomarkers may rule out the need for costly exams like the CT scan, which is particularly relevant to a setting where a major part of the population lives under poverty. This study aimed to analyze associations in acute TBI between admission serum biomarker concentrations and TBI severity, CT-scan findings, and outcome, and to explore possible influence with *Toxoplasma gondii* infection.

2.Methods

2.1. Study area, design and period

This prospective-cohort study was conducted at the Laquintinie Hospital of Douala (LHD) on acute traumatic brain injury patients. Douala is the economic capital of Cameroon and a highly cosmopolitan city. It is situated on the south eastern shore of the Wouri river estuary, on the Atlantic Ocean coast about 230 km west of Yaoundé. It has a wet and a dry season and has temperatures ranging from 74°F to 91°F with an estimated population of about 3.8 million inhabitants [28,29]. The LHD is a second-category referral hospital located in the heart of AKWA-Douala. It is implanted in a 09 hectares' surface area. Its mission is to ensure medical care and quantitative and qualitative medico-sanitary responses in major events like sporting activities, traffic accidents, catastrophes or epidemics. The LHD offers many services organized within departments amongst which the Department of Surgery (paediatric surgery, neurosurgery, ORL, Ophthalmology, Urology, and

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Orthopaedics / Traumatology, general surgery) and the Department of Emergency, Anaesthesiology and Reanimation. The neurosurgical department is well established and the hospital has 3 neurosurgeons, a Scanner and 0.5 tesla MRI. The hospital was chosen for the study because it receives the highest number of trauma cases in the Littoral Region of Cameroon, and probably Cameroon at large. The study was conducted over a period of 13 months from January 2021 to January 2022.

2.2. Study population and participants

The population of the study consisted of all those who sustained a traumatic brain injury, mild to severe, received at the emergency service of the Laquinitinie Hospital Douala within 24 hours of injury during the study period. Demographic details and outcome of this cohort have been reported previously (Buh et al., 2022, submitted). Fifteen healthy individuals were also included in the study as controls. **Inclusion criteria:** all individuals who were brought to the emergency services of the Laquintinie Hospital Douala who sustained a head trauma and who or their family members signed a written consent, were enrolled in the study. **Exclusion criteria:** all individuals who were not confirmed of a TBI (mild, moderate or severe) by the Physician, individuals with neuro-psychiatric problems, thieves and also those patients we could not get their blood samples for various reasons.

2.3. Sampling Method and Unit

Once a definitive diagnosis of TBI (mild, moderate or severe) was confirmed by the Physician for patients who sustained TBI within 24 hours; the families of patients were approached or the patients themselves depending on the severity of the TBI and their level of consciousness to obtain their consent to participate in the study. Those who gave their consent were enrolled in the study. Information on the sociodemographic, clinical and injury details, was registered. Blood samples were collected within 24 hours after injury for the determination of the serum concentrations of five biomarkers of TBI: glial fibrillary acidic protein (GFAP), ubiquitin C-terminal hydrolase-L1 (UCHL1), total tau protein, neurofilament light (NFL) and calcium-binding protein (S100B). The association of these biomarkers to the severity of TBI (mild, moderate, severe), T. *gondii* infection, CT scan findings and 6-month Glasgow outcome Scale-Extended (GOSE) outcome (favorable vs unfavorable, survival vs death) were explored. The GOS-E, was used to determine the 6 months' outcome after TBI. The evaluation was done through face-to-face visits or telephone calls. Some(n=8), however, were missed out due to contact difficulties after hospital discharge.

2.4. Laboratory methods

Blood samples from the study participants were collected into the dry-tubes (no anti-coagulant added). After which the tubes were coded for each patient. The samples were kept for 30 minutes to an hour for coagulation to take place. After this, the samples were centrifuged at 3500 rpm for 15 minutes to obtain blood serum. Each serum was aliquoted using micro-pipettes and emptied into two labelled cryo-tubes; two for each sample. Care was taken to ensure the cryotubes had the same code like the corresponding dry tubes. Serum samples were preserved at **-80 degrees** at the central laboratory of the LHD until shipment. Samples were shipped to the USA (University of Florida) for analysis under adequate conditions (World courier) and the University of Buea.

2.5. Measurement of blood fluid biomarkers (GFAP, Tau, UCHL-1, and NFL)

The blood samples were collected within 24 hours of injury. GFAP, UCH-L1, t-tau, and NFL were analyzed at the University of Florida using Single Molecule Arrays (SiMoA) based 4-plex for research use only assay (N4PB, Item number 103345) on the SR-X benchtop assay platform (Quanterix Corp., Lexington, MA). The Simoa Human Neurology 4-Plex B assay (N4PB) measures four important neurology biomarkers in both cerebrospinal fluid (CSF) and blood. The four targets are neurofilament light (NF-L), total tau, glial fibrillary acidic protein (GFAP), and ubiquitin carboxyl-terminal hydrolase L1 (UCH-L1). The lower limit of detection (LLoD) was as follows: GFAP 1.32 pg/ml, NFL 0.0971 pg/ml, t-tau 0.0236 pg/ml and UCH-L1 0.67 pg/ml. The assay range was as follows:

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GFAP 0-40,000 pg/mL, NFL 0-2000 pg/mL, UCH-L1 0-40,000 pg/mL and Tau 0-400 pg/mL. All samples including the controls: GFAP, NFL and t-tau and UCH-L1 readout above the LLoD [22, 30].

The serum S100B concentration was measured using the Commercial Assay ELISA Kits following the manufacturer's instructions (MyBioSource INC, USA, MBS762374, sales@mybiosource.com). One hundred (100) µl of diluted Standards, Quality Controls, Dilution Buffer (=Blank) and samples, were pipetted into the appropriate wells. The plates were incubated at room temperature (37°C) for 90 minutes. Wells were washed 2-times with Wash buffer and the plates were inverted and tapped strongly against paper towel. After which, 100 µl of Biotin Labelled Antibody solution was added into each well. Incubated the plate at room temperature (37°C) for 60 minutes. Wells were washed 3-times. One hundred (100) µl of Streptavidin-HRP Conjugate was added into each well and incubated at 37°C for 30 minutes. Wells were washed 2-times and 90 µl of TBM Substrate Solution was added into each well. The plates were covered and incubated for 10 minutes. Fifty (50) µl of stop solution was added to each well and the colour immediately turned yellow as stated by the manufacturer. The optical density (OD) absorbance was read at 450nm in a microplate reader immediately after adding the stop solution. The normal serum concentration for S100B in this study was **<1pg/mL.**

2.6. Measurement of Toxoplasma gondii IgM antibodies (ELISA)

The seropositivity to *T. gondii* was measured using the Commercial Assay ELISA Kits following the manufacturer's instructions (MyBioSource INC, USA, MBS494548, sales@mybiosource.com). One hundred microliter (100μl) of diluted sera, calibrator and controls were dispensed into the appropriate wells. For the reagent blank, 100μl sample diluent was emptied into the wells. The holder was tapped to remove air bubbles from the liquid and mixed well and the plate was incubated for 20 minutes at room temperature. After which the liquid from all wells was removed and the wells were washed three times with 300μl of 1X wash buffer. The plate was then blotted on absorbance paper. One hundred microliters of enzyme conjugate were added to each well and incubated for 20 minutes at room temperature. The enzyme conjugate was removed from all wells and the wells were washed three times with 300μl of 1X wash buffer, 100 μl of TMB substrate was added and plates were incubated for 10 minutes at room temperature, after which 100μL of stop solution was dispensed into each well. The optical density (OD) was read at 450 nm using ELISA reader within 15 min. The **Antibody Index Interpretation** was as follows: Less than (<)0.9: No detectable antibody to Toxoplasma IgM by ELISA, 0.9-1.1: Borderline positive. >1.1: Detectable antibody to Toxoplasma IgM by ELISA.

2.7. Data management and analysis

Data collected were cross-checked for any errors. All questionnaires were given unique codes and the information was entered into the CSPro 7.6 data mask designed by the statistician. Continuous variables were reported as medians with 25th and 75th percentiles, and as means and standard deviations. Categorical variables were described as frequencies and percentages. The Wilcoxon and Kruskal-Wallis rank sum tests were used for comparisons between biomarker concentrations and TBI severity and outcomes. P-values <0.05 were considered statistically significant.

2.8. Ethical clearance and administrative authorizations

Ethical clearance for the study was obtained from the Institutional Review Board of the Faculty of Health Sciences (IRB-FHS), University of Buea; **Reference N^{o} 1238-08**. Administrative authorization was obtained from the Laquantinie Hospital of Douala.

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3. Results

3.1. Characteristics of Participants

A total of One hundred and sixty (160) patients with TBI of median age 32 (IQR26, 39) years were enrolled in the study. between January 2021 and February *2022.* Detailed clinical and demographic characteristics of the study cohort has previously been described (Buh et al., 2022, submitted). In brief, the median age of patients was 32 (IQR26, 39). Most patients were adolescents and young adults aged 15-45 years (78%; 125), 90% (144/160) of patients were males (144/160). Most participants (76%) had not finished secondary education. The median GCS was 12.0 (8.0 -14.0). Driving after alcohol consumption was suspected in 33 (21%) (33) of cases. Most of the patients (59%, 95) were referred directly (59%, 95) and 65 (41% () 65) had secondary referrals. Mild TBI cases were the most common (41%; 66/160) presentation form of presentation (41%; 66/160) followed by moderate (34%; 55/160) and severe (24%; 39/160) TBI. CT scan was performed in 78% (125) cases and showed traumatic intracranial abnormalities in 64% (; 77/125) of cases. The two most common types of TBI were cerebral contusion (54%; 65/160) and extradural haemorrhage (49%; 59/160); neurosurgical intervention was carried out in 22% (17/77) of cases (Table 1).

Table 1. Sociodemographic and clinical characteristics.

Characteristic	**N (%)**
N	**160**
Age, Median (IQR) in years	32 (IQR26, 39)
Gender	
Female	16 (10%)
Male	144 (90%)
Education	
Graduate	19 (12%)
No formal education	9 (5.6%)
Matriculated	7 (4.4%)
Not Known	2 (1.2%)
Post-graduate	1 (0.6%)
Primary	40 (25%)
Secondary	82 (51%)
Profession	
Employee in service	30 (19%)
Manual workers	24 (15%)
Bike riders	43 (27%)
Student	16 (10%)
Unemployed	21 (13%)
Others	26 (16%)
Marital status	
Married	67 (42%)
Not applicable	6 (3.8%)
Single	86 (54%)
Widowed	1 (0.6%)
Medico-social history	
Diabetes	2 (1.2%)
Hypertension	14 (8.8%)
Smoking	22 (14%)
Alcohol	97 (61%)
Influence of alcohol	
None	108 (68%)

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Suspected	33 (21%)
Unknown	19 (12%)
Symptoms of TBI	
Loss of consciousness	152 (95%)
Vomiting	55 (34%)
Nausea	21 (13%)
Ear bleed	20 (12%)
Nasal bleed	43 (27%)
Headache	103 (64%)
Seizure	7 (4.4%)
Agitation	43 (27%)
Classification of TBI	
Mild	66 (41%)
Moderate	55 (34%)
Severe	39 (24%)
Blood pressure	**N=160**
Elevated	15 (9.4%)
Hypertension	62 (39%)
Hypotension	19 (12%)
Normal	64 (40%)
Median Glasgow Coma Scale	12.0 (8.0, 14.0)
Referrals	
Direct	95 (59%)
Indirect	65 (41%)
Complementary exams done to characterize injury	**N=160**
CT Scan	125 (78%)
If scan or MRI, traumatic abnormalities present	77 (64%)
Type of TBI	**N=77**
Cerebral contusion	25 (32%)
Extradural hematoma	22 (29%)
Acute subdural haemorrhage	18 (23%)
Intracerebral haemorrhage	12 (16%)
Cerebral oedema	10 (13%)
Meningeal haemorrhage	8 (10%)
Mass effect pressure	3 (3.9%)
Other type of TBI	4 (5.2%)
Neurosurgery	**N=77**
Yes	17 (22.1%)
No	60 (78%)

TBI: Traumatic Brain Injury.

3.2. Serum concentrations of biomarkers and TBI severity and outcome

Levels of all biomarkers were significantly higher in acute traumatic brain injury patients compared to controls **(Table 2).**

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Table 2. Comparison of Serum biomarkers levels in TBI cases and controls.

Biomarkers as Median (IQR)	TBI cases N = 160	Controls N = 15	P-value
S100B (pg/mL)	28 (26, 33)	0 (0, 0)	**<0.001*** **
GFAP (pg/mL)	1,244 (277, 4,042)	13 (11, 21)	**<0.001*** **
NFL (pg/mL)	7 (2, 16)	1 (1, 1)	**<0.001*** **
Tau (pg/mL)	1.15 (0.44, 2.87)	0.32 (0.20, 0.49)	**<0.001*** **
UCH-L1 (pg/mL)	31 (12, 86)	7 (4, 9)	**<0.001*** **

P- value is based on non-parametric comparison of **median(IQR)** (Wilcoxon rank sum test). UCHL-1: ubiquitin C-terminal hydrolase, NFL: neurofilament light, GFAP: glial fibrillary acidic protein, S100B: calcium binding protein.

Serum concentrations all TBI biomarkers generally increased with injury severity (GFAP: 1020pg/mL in mild, 1,876 pg/mL in severe; Tau: 0.76pg/ml in mild, 1.65 in severe; UCH-L1: 20 pg/mL in mild and 50 pg/mL in severe), but was statistically significant only for NFL (P=0.010). However, when the concentrations in the controls were compared to either of the severities; mild, moderate, or severe, there were significant differences (p<0.001) as shown in **Table 3.**

Table 3. Concentration of neurobiomarkers according to TBI severity.

Biomarkers as Median (IQR)	Controls	Traumatic brain injury severity			P-value	[1]P-value
		Mild	Moderate	Severe		
N	**15**	**66**	**55**	**39**		
S100B (pg/mL)	0.0 (0, 0)	28.1 (27.1, 32.6)	27.6 (25.6, 33.0)	28.3 (27.2, 31.9)	0.6	**<0.001**
GFAP (pg/ml)	13.0 (11, 21)	1,020 (147, 2,831)	1,723 (503, 7.403)	1,876 (532, 5,903)	0.2	**<0.001**
NFL (pg/ml)	1.0 (1, 1)	3.0 (1, 11)	10.0 (4, 19)	10.0 (3, 22)	**0.010**	**<0.001**
Tau (pg/ml)	0.32 (0.20, 0.49)	0.76 (0.39, 2.18)	1.17 (0.40, 3.35)	1.65 (0.88, 2.85)	0.11	**<0.001**
UCH-L1 (pg/ml)	7.0 (4, 9)	20.0 (10, 75)	28.0 (12, 87)	50.0 (22, 86)	0.10	**<0.001**

P value obtained by comparison of TBI severity. [1]**P** value obtained by comparison of controls and mild TBI. UCHL-1: Ubiquitin C-terminal hydrolase, NFL: Neurofilament light, GFAP: Glial fibrillary acidic protein, S100B: Calcium binding protein, pg/mL: picogram/millilitre.

Patients with unfavourable outcome had higher levels of GFAP (p=0.026) compared to those with favourable outcome. No significant difference between patients with favourable and unfavourable outcomes were found for the other biomarkers considered, although a clear trend towards higher values of NFL in patients with unfavourable outcome was demonstrated. Total tau levels were significantly higher in patients who died from the injury compared to survivors (p=0.017). Except for S100B, levels of the other biomarkers (GFAP, NFL, and UCH-L1) were higher in non-survivors, but differences did not reach statistical significance **(Table 4).**

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Table 4. Neurobiomarkers according to the GOSE.

Biomarkers as Median (IQR)	Survival N= 130	Death, N = 22	Favorable N = 122	Unfavorable N = 38	p-value (favourable vs unfavourable GOSE	p-value (Death vs. survival)
S100B (pg/ml)	28.1 (26.3, 32.6)	28.2 (27.2, 33.7)	28.1 (26.3, 32.6)	28.0 (26.4, 32.1)	>0.9	0.4
GFAP (pg/ml)	1,161 (219, 3,349)	2,811 (569, 8,611)	1,100 (132, 3,310)	2,545 (582, 7,356)	**0.026**	0.087
NFL (pg/ml)	7.0 (2, 15)	12.0 (2, 27)	5.0 (2, 15)	10.0 (5, 26)	0.11	0.3
Tau (pg/ml)	0.98 (0.40, 2.31)	2.84 (1.03, 3.88)	1.15 (0.40, 2.62)	1.55 (0.63, 3.44)	0.3	**0.017**
UCH-L1 (pg/ml)	24.0 (12, 83)	53.0 (20, 78)	30.0 (12, 88)	40.0 (13, 83)	0.6	0.2

[1]Median (IQR); [2]Wilcoxon rank sum test. All TBI cases are included. UCHL-1: Ubiquitin C-terminal hydrolase, NFL: Neurofilament light, GFAP: Glial fibrillary acidic protein, S100B: Calcium binding protein, pg/mL: picogram/millilitre.

GFAP and total tau levels were significantly higher in CT positive compared to CT negative patients (GFAP: P=0.004; NFL: P=0.002). UCHL-1 was also found to be sensitive as values were relatively high in those who showed traumatic intracranial abnormalities with the CT-scan (21pg/ml for negative CT-scan vs 42 pg/ml for positive CT-scan), although this increase was not statistically significant. However, when restricting the analysis to patients with mild TBI (n=42), no significant difference between CT-positive and CT-negative patients was found. Significant differences for GFAP (P=0.038) and NFL (P=0.005) remained when mild and moderate TBI cases (n=86) were combined **(Table 5).**

Table 5. Biomarkers concentrations and CT-scan outcome.

Biomarkers as Median (IQR)	CT negative N = 44	CT positive N = 77	P-value (all severities; mild, moderate, severe)	P-value (only mild TBI)	P-value (mild and moderate)
S100B (pg/mL)	28.3 (26.3, 33.0)	28.0 (26.4, 32.5)	>0.9	0.6	0.8
GFAP (pg/mL)	928 (146, 1,955)	1,809 (475, 7,130)	**0.004****	>0.9	**0.038***
NFL (pg/mL)	3.0 (2, 10)	10.0 (4, 22)	**0.002****	0.2	**0.005****
Tau (pg/mL)	0.95 (0.39, 1.96)	1.35 (0.46, 3.14)	0.2	0.4	0.7
UCH-L1 (pg/mL)	21.0 (9, 48)	42.0 (15, 86)	0.085	0.9	0.076

[1]Median (IQR); [2]Wilcoxon rank sum test. All TBI cases are included. UCHL-1: Ubiquitin C-terminal hydrolase, NFL: Neurofilament light, GFAP: Glial fibrillary acidic protein, S100B: Calcium binding protein, pg/mL: picogram/millilitre.

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3.3. Toxoplasma gondii infection and biomarkers concentrations

Toxoplasma gondii infection was recorded in 33% (52/160) of TBI cases. The median age for *T. gondii* positive patients was 30 (IQR23, IQR39) years and there were no significant differences between *T. gondii* positive and negative TBI patients in terms of TBI severity (P=0.7) as shown in **Supplemental Table 1.** When the biomarker concentrations between *T. gondii* negative vs positive cases were compared, only S100B was found in significant higher levels in *T. gondii* positive TBI cases **(Table 6).**

Table 6. Influence of Toxoplasma status on biomarkers.

Biomarker as Median (IQR)	**Toxoplasma Negative, N = 108[1]**	**Toxoplasma Positive, N = 52[1]**	**p-value[2]**
S100B (pg/ml)	27.2 (24.9, 28.1)	36.2 (32.6, 37.9)	**<0.001****
GFAP (pg/ml)	1,651 (351, 5,394)	839 (244, 3,360)	0.4
NFL (pg/ml)	7.0 (2, 15)	7.0 (2, 21)	>0.9
Tau (pg/ml)	1.39 (0.53, 3.39)	0.82 (0.38, 2.12)	0.10
UCH-L1 (pg/ml)	33.0 (13, 94)	28.0 (10, 68)	0.2

[1]Median (IQR), [2]Wilcoxon rank sum test. IQR: Interquartile range.

4. Discussion

The development of clinically validated traumatic brain injury biomarker tests can improve treatment approaches and prognostic estimates in patients with TBI in acute care settings. In what is, to our knowledge the first TBI biomarker study in Cameroon and SSA, we report how 5 biomarker concentrations vary with TBI severity, CT scan positivity and outcome, and explore the influence of *Toxoplasma gondii* seropositivity on biomarker levels.

4.1. Concentrations of serum biomarkers and the association with traumatic brain injury severity

The serum concentrations of the TBI biomarkers studied (S100B, NFL, UCH-L1, Tau, GFAP) were significantly increased compared to controls. These proteins are mainly found in the CNS and can be found in blood and CSF in traumatic brain injury or other disruption in the CNS.[14,16,21] The serum concentrations of the TBI biomarkers considered in this study generally increased with TBI severity (mild to severe), similar to reports in literature [3,12,22,31,32]. There was a significant increase in the serum levels of NFL while serum levels of S100B almost remained the same from mild to severe TBI. This is in line with reports by Shahim *et al*. [33], where NFL was found to reflect TBI severity after traumatic brain injury. However, our results in part do not corroborate those of the above cited studies as most of the biomarkers in the above studies showed significant variations from mild to moderate. Differences in sample sizes and study setting may be accountable. The high level of biomarker concentration in non-survivors may be explained by neuronal and glial damage.[34] Moreover, biomarker levels may reflect damage at the cellular level, which may not be seen with imaging techniques,[15] and motivate further biomarker research to support their role in clinical decision making.

4.2. Concentrations of the biomarkers and 6 months outcome

We found significantly higher levels of t-Tau in non-survivors compared to survivors, which is in line with the results obtained by Wang et al.,[35] where increased levels of tau were found to be associated to poorer outcomes after TBI. It was also consistent with findings by Korley et al. [24], where higher values of tau and UCH-L1 predicted mortality. However, in contrast to other studies, we did not find significant differences in the levels of other biomarkers considered (GFAP, NFL, S100B and UCH-L1) between non-survivors and survivors. Nevertheless, except for S100B, the levels of other biomarkers were higher in non-survivors. This corroborates studies reporting higher values of these biomarkers in non-survivors [23,24,34,36]. Regarding the comparison between favourable vs unfavourable outcome, GFAP was significantly higher in patients with unfavourable outcome 6

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months after TBI, in line with results reported by Korley et al. [24] and Helmrich et al. [23]. These studies, however, also reported significant associations with outcome for other biomarkers which were not demonstrated in our findings. This may be explained by our relatively small sample size (160) compared to the over 2000 participants considered in the studies mentioned above, as well as to differences in case-mix and time of sampling.

4.3. Concentrations of the biomarkers and CT positive and negative scans

Correlation of the biomarker concentrations between TBI positive CT-scan (77%) vs TBI negative CT-scan (23%) showed two of the five biomarkers; GFAP and NFL were significantly associated with CT positivity after traumatic brain injury. This result is partly in line with several studies [3,21.22,37], where the sensitivity of GFAP and UCH-L1 are demonstrated. Furthermore, McMahon *et al.* [38] demonstrated that GFAP biomarker testing in emergency services could eliminate unnecessary CT-scans in 12 to 30% of TBI patients. However, when the comparison was made considering mild TBI alone, no significant differences were obtained between CT-positive and CT-negative scans with all the tested biomarkers. This observation is not consistent with reports by Czeiter et al. [22], where they reported incremental diagnostic value for GFAP in mild TBI cases. The difference in results could be accounted for by our relatively low event rate in mild TBI (n=14), and differences in study settings.

Although our sample size was too small to draw definitive conclusions, the significantly higher levels of NFL in CT-positive cases may motivate further research into the role of this biomarker in predicting presence of CT abnormalities with a focus on timing of sampling.

The use of TBI biomarker testing for informing the need for CT scanning is particularly relevant in resource limited settings as the price of CT-scans and MRI are high. More than half of the population of Cameroon (60%) cannot access appropriate healthcare because of the high costs and 70% spend out-of-pocket for their healthcare as no universal healthcare services are yet offered [39]. Therefore, the development and implementation of biomarkers for ruling out CT-scans when not necessary is promising and could reduce health expenditure of the patients and proxies in mild TBI, and therefore improve healthcare provision and health.

4.4. Concentrations of the biomarkers according to Toxoplasma gondii infection

We hypothesized that a latent *T. gondii* infection might influence the neuropathology in traumatic brain injury. Recent literature attests that latent infections with *T. gondii* may cause neuro-inflammation which could worsen the neuro-inflammatory component of in TBI [40]. However, it has not yet been experimentally established how *T. gondii* affects the brain in TBI. We therefore aimed at investigating if TBI patients infected with *T. gondii* would show higher levels or not of serum TBI biomarkers.

Toxoplasma gondii positivity was observed in 33% of cases, with a median age of 30 (23, 39) years similar to reports in literature where one third of the world population is said to be infected with *T. gondii* [40, 41,42]. S100B levels were higher in *T. gondii* positive patients. To our knowledge this is the first study exploring effects of *T. gondii* infection on biomarkers in TBI. However, Ayyildiz et al [43]. studied S100B serum levels association to *T. gondii* positivity in Alzheimer disease and found no significant variation in the levels of serum S100B between *T. gondii* positive and negative Alzheimer patients. This variation in results may be due to the fact that their sample size was small (33) or simply to a difference in the underlying pathology, e.g chronic versus acute. The authors suggested the use of different genotypes of *T. gondii* in future studies may add to literature. As of today, it is not clear if TBI outcomes may differ in individuals infected with neurotrophic parasites like *T. gondii a*nd how seropositivity to *T. gondii* in traumatic brain injury influence biomarker concentrations. The possibility that elevated S100B levels may have had an extracerebral origin cannot be excluded. However, experimental studies have shown that *T. gondii* infection reduces cerebral microvascular perfusion and induces neuro-inflammation through activation of cerebral endothelial cells [40,41]. These results would support the concept that latent neuro-parasitic disease, like *T. gondii* infection, might aggravate the disease process in acute neurological disorders, which could be reflected in biomarker concentrations. Currently, work is ongoing at the Monash University in Australia to

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determine the effects *of T. gondii* infection on acquired brain injury (TBI and stroke) and its outcome [44]. Further studies considering larger sample sizes are needed to elucidate effects of latent neuroparasitic disease on the pathophysiology and outcome of TBI. This would contribute to the knowledge of the pathomechanisms after TBI and thus the diagnosis and care of TBI patients, particularly in low-resource settings.

5. Strengths and limitations

This is the first study designed to study TBI biomarkers in Cameroon and their association with TBI severity, CT scan positivity / negativity, 6 months outcome with GOSE as well as influence of *Toxoplasma gondii* seropositivity. It is the first time *Toxoplasma gondii* seropositivity is measured in acute traumatic brain injury and predetermining possible interaction between *T. gondii* infection and serum TBI biomarkers concentrations. This could serve as baseline information for future research on TBI biomarkers in Cameroon and other parts of Africa and also on *T. gondii* neuropathology in acute TBI. This study also reported the potential of NFL as a sensitive marker of CT abnormalities which will subsequently need to be studied further for more accurate findings. As of now, this characteristic is noted with two of the TBI biomarkers: GFAP and UCH-L1. We wish to carry out similar studies in future with larger sample sizes and considering more trauma centres in Cameroon. A limitation of our study was a relatively low sample size, and that CT scans could not be obtained in all patients. Another limitation was loss to follow-up of a few cases (8) in the 6 month's evaluation due to inaccurate contact information. Despite these limitations, our results are pertinent, offering insights to the TBI biomarker testing in a LMIC as well as determining possible influence of *T. gondii* seropositivity on TBI biomarker concentrations.

6. Conclusion and implications

The serum concentrations of the five TBI biomarkers considered in this study generally increased from mild to severe TBI, although this increase was statistically significant only for NFL. However, when the concentrations in the controls were compared to either of the severities; mild, moderate, or severe, there were significant differences. We report that GFAP was associated with unfavourable outcome 6 months after TBI. The concentrations of tested biomarkers were generally increased in non-survivors, although the increase was significant for only one of these biomarkers (Tau). Two (GFAP and NFL) of the five biomarkers were found to predict CT abnormalities. When the biomarker concentrations between *T. gondii* negative vs positive cases were compared, S100B was significantly higher in *T. gondii* positive TBI cases. Future studies should be conducted with larger sample sizes and recruiting more trauma centres around Cameroon and SSA in order to draw more definite conclusions on the use of biomarkers as TBI diagnostic and prognostic tools in resource limited settings as well as on *Toxoplasma gondii* neuropathology in traumatic brain injury. NFL could be studied further to explore it sensitivity towards CT-positive or negative scans. Finally, the governments of SSA countries should promote and encourage research in this area, which is promising in the prognosis and care of TBI in SSA.

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