NEUROTRAUMA: FROM EMERGENCY ROOM TO BACK TO DAY-BY-DAY LIFE

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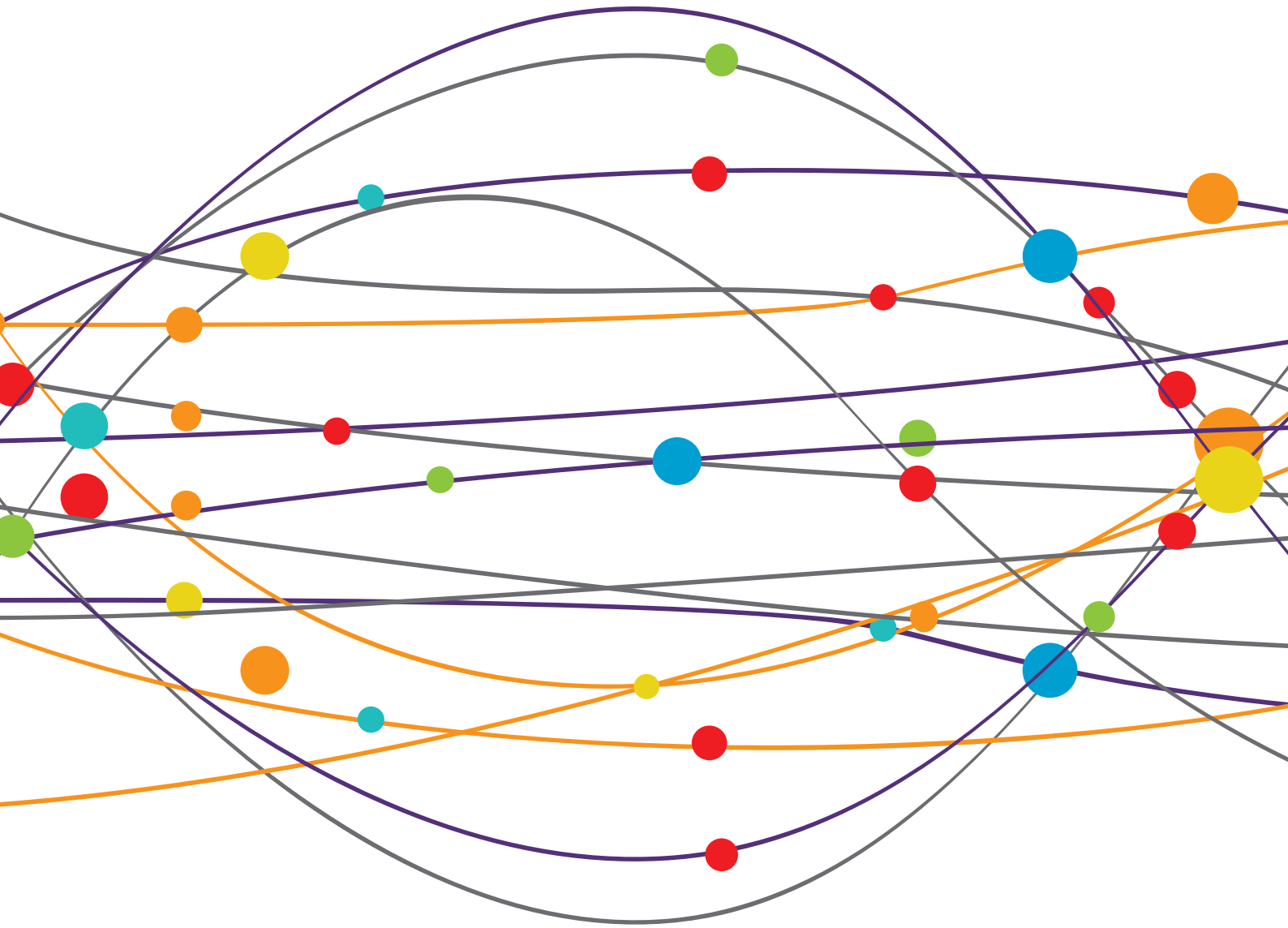

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NEUROTRAUMA: FROM EMERGENCY ROOM TO BACK TO DAY-BY-DAY LIFE

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EDITORIAL
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Editorial: NEUROTRAUMA: From Emergency Room to Back to Day-by-Day Life

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Keywords: traumatic brain injury, concussion, diffuse axonal injury, cognitive impairment, treatment

Editorial on the Research Topic

NEUROTRAUMA: From Emergency Room to Back to Day-by-Day Life

Traumatic brain injury (TBI) is a nondegenerative and non-congenital insult to the brain from an external mechanical force that can lead to permanent or temporary impairment of cognitive, physical, and psychosocial functions (1). TBI is considered a "silent epidemic" not only due to its magnitude, but also because it affects mostly young and productive individuals (2).

Most patients with TBI return home after the critical phase of hospital management. Although some patients manage to regain some degree of independence in their self-care, many are still incapable of applying critical thinking to decision-making processes, providing for their family needs, or continuing work, school or social activities. Moreover, many also manifest mood alterations and depression. As such, patient rehabilitation after hospital discharge is a critical step in returning to their day-by-day lives (3).

The objective of this *Frontiers in Neurology* Research Topic is to present the latest findings and views regarding the pathophysiology and treatment of TBI. It is comprised of 10 papers, each offering a unique view and understanding of how TBI can be detected and managed from the emergency room to back to day-by-day life.

Hayashi et al. evaluated the cortical excitability during the chronic phase of TBI in victims diagnosed with diffuse axonal injury (DAI). Amorim et al. in turn, studied the effects of transcranial direct current stimulation (tDCS) in patients with persistent post-concussion syndrome who demonstrated cognitive deficits in long-term episodic memory, working memory, and executive function following mild TBI. Hashim et al. used diffusion tensor imaging to investigate the apparently normal white matter (assessed by routine magnetic resonance imaging) in the brains of subjects with sub-acute and chronic TBI. Oliveira et al. studied the usefulness of transcranial color-coded duplex sonography for evaluating TBI patients. Vieira et al. described the outcome for patients with a primary diagnosis of DAI 6 months after trauma and identified sociodemographic and clinical factors associated with mortality and dependence at this time point. Dambinova et al. hypothesized that neurotoxicity AMPA, NMDA, and kainite receptor biomarkers might be utilized as part of a comprehensive approach to concussion evaluations. Popovic et al. in turn, described a case of a man with non-traumatic spinal cord injury that was submitted to functional electrical stimulation therapy to restore voluntary reaching and/or grasping function of his hand. Khong et al. conducted a systematic review regarding the evidence for the use of diffusion tensor imaging parameters in the human brain as a diagnostic tool for and predictor of post-concussion syndrome after a mild traumatic brain injury. Kirmani et al. reviewed the literature to understand the role

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Lastly, of anticonvulsants in the treatment of posttraumatic epilepsy. Rodrigues et al. conducted a review of existing literature regarding the effects of soccer heading on brain structure and function.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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ORIGINAL RESEARCH
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Altered Intracortical Inhibition in Chronic Traumatic Diffuse Axonal Injury

Cintya Yukie Hayashi[1], Iuri Santana Neville[1], Priscila Aparecida Rodrigues[1], Ricardo Galhardoni[1,2], André Russowsky Brunoni[3], Ana Luiza Zaninotto[1,4], Vinicius Monteiro de Paula Guirado[1], Ana Sofia Cueva[1], Daniel Ciampi de Andrade[1], Manoel Jacobsen Teixeira[1] and Wellingson Silva Paiva[1]*

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Background: Overactivation of NMDA-mediated excitatory processes and excess of GABA-mediated inhibition are attributed to the acute and subacute phases, respectively, after a traumatic brain injury (TBI). However, there are few studies regarding the circuitry during the chronic phase of brain injury.

Objective: To evaluate the cortical excitability (CE) during the chronic phase of TBI in victims diagnosed with diffuse axonal injury (DAI).

Methods: The 22 adult subjects were evaluated after a minimum of 1 year from the onset of moderate or severe TBI. Each of the subjects first had a comprehensive neuropsychological assessment to evaluate executive functions—attention, memory, verbal fluency, and information processing speed. Then, CE assessment was performed with a circular coil applying single-pulse and paired-pulse transcranial magnetic stimulation over the cortical representation of the abductor pollicis brevis muscle on M1 of both hemispheres. The CE parameters measured were resting motor threshold (RMT), motor-evoked potentials (MEPs), short-interval intracortical inhibition (SIICI), and intracortical facilitation (ICF). All data were compared with that of a control group that consisted of the healthy age-matched individuals.

Results: No significant differences between the left and right hemispheres were detected in the DAI subjects. Therefore, parameters were analyzed as pooled data. Values of RMT, MEPs, and ICF from DAI patients were within normal limits. However, SIICI values were higher in the DAI group—DAI SIICI = 1.28 (1.01; 1.87) versus the control value = 0.56 (0.33; 0.69)—suggesting that they had a disarranged inhibitory system ($p < 0.001$). By contrast, the neuropsychological findings had weak correlation with the CE data.

Conclusion: As inhibition processes involve GABA-mediated circuitry, it is likely that the DAI pathophysiology itself (disruption of axons) may deplete GABA and contribute to ongoing disinhibition of these neural circuits of the cerebrum during the chronic phase of DAI.

Keywords: brain injuries, craniocerebral trauma, diffuse axonal injury, neurophysiology, transcranial magnetic stimulation

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INTRODUCTION

Traumatic brain injury (TBI) is one of the major health consequences of trauma from motor vehicle accidents having its highest incidence among young male adults (1, 2). Considering that this age group is at its prime economic productivity, should they suffer a TBI which can cause long-term motor and cognitive disabilities, the negative impact of TBI stretches beyond just the individual victim (3, 4).

Traumatic brain injury is classified by clinical severity (mild, moderate, or severe), pathoanatomic type (focal or diffuse), and mechanism of injury (blunt or penetrating) (1). Focal lesions tend to have simpler management compared to diffuse injuries, whereas widespread damage has a complex mechanism that contributes to morbidity and limits clinical study and management.

Diffuse axonal injury (DAI) is a clinical condition often related to closed head traumas. It is the predominant finding in approximately half of TBI patients, and it has been found in all levels of TBI severity. Clinically, trauma victims with prolonged unconsciousness (6 h or more) unaccompanied by ischemic damage or mass lesions are usually diagnosed with DAI. Still, most of the features of DAI are microscopic and cannot be identified on conventional methods of neuroimaging, such as computed tomography (CT) scans or conventional magnetic resonance imaging (MRI) (1, 5, 6).

The diagnosis of DAI can only be confirmed by postmortem histopathological analysis and, for this reason, the development of new and more refined techniques—for instance, diffusion-weighted imaging and diffusion tensor imaging in MRI—enables further studies of DAI *in vivo*. In particular, transcranial magnetic stimulation (TMS) seems to be an interesting tool for neurophysiological testing as it allows a noninvasive real-time study of the brain, providing indirect information about intracortical interneuronal circuits through cortical excitability (CE) assessments (6–10).

Some studies using TMS show changes in CE after strokes, in psychiatric disorders, in pain syndromes, and even during acute phases of TBI (11–14). Mechanisms of intracortical facilitation (ICF) and intracortical inhibition are related to glutamatergic and GABAergic pathways, respectively, and the imbalance of these neurotransmitters is somehow involved in maladaptive plasticity (15, 16). Knowledge about how these imbalances lead to the pathophysiology that develops after brain injury would enable the development of new therapeutic strategies and rehabilitation options.

Unfortunately, there are few studies of CE after TBI, and most of those studies were carried out in patients with TBI of mild severity or only during the acute phase (14–16). Thus, in the present study, we sought to evaluate CE in patients during the chronic phase of TBI, diagnosed with DAI, using the diagnostic mode of TMS. In addition, we tried to correlate the neuropsychological profile of these patients with the CE data and clinical characteristics.

MATERIALS AND METHODS

Setting and Subjects

A convenience sample of 73 adults, 18–60 years old, of both genders, who had been clinically diagnosed with DAI during an acute hospitalization following trauma were initially evaluated at the neurotrauma outpatient center of a tertiary referral hospital in Sao Paulo, Brazil.

After this initial screening, 51 patients were excluded. Exclusion criteria included (1) associated focal lesions or the presence of any abnormality other than DAI (e.g., epidural/subdural hematoma); (2) having suffered more than one TBI; (3) major psychiatric disorders (e.g., major depression, bipolar disorder, any disorder requiring admission to a psychiatric ward); (4) history of surgical procedures to the brain/skull; (5) the presence of metallic devices/pieces in the brain/skull (clips, plates, electrodes, etc.); (6) pregnancy; (7) epilepsy/seizures; (8) severe language impairment (writing/reading/speaking); or (9) illicit drug and/or alcohol abuse.

To achieve the most homogeneous sample possible, DAI diagnosis was established for this study as (1) a clinical condition of prolonged unconsciousness (6 h or more) following TBI; (2) a head CT scan image taken during acute hospitalization, demonstrating a wide spectrum of findings such as a relatively normal examination, small hemorrhagic (hyperdense) or non-hemorrhagic (hypodense) lesions no more than 25 cm^3 in size (typically located at the gray–white matter junction, in the corpus callosum, and in more severe cases in the brainstem); small intraventricular hemorrhage, subarachnoid hemorrhage, and signs of brain swelling, such as compressed or even absent basal cisterns; (3) MRI obtained during the chronic phase (i.e., taken at least 6 months after TBI), demonstrating small regions of susceptibility artifact at the gray–white matter junction, in the corpus callosum, or the brain stem. Some lesions might be entirely non-hemorrhagic (even using susceptibility-weighted imaging sequences at high-field strengths). These would, however, be visible as regions of high fluid-attenuated inversion recovery signals on an MRI of the cranium.

Twenty-two subjects selected to participate in this exploratory study were assessed after a 1-year interval, at least, from the moment of trauma. The recruitment period was from May 2014 to 2015. For general comparisons, we used the normative data of CE as reference (17), and for supplementary analysis, we selected a control group consisting of 22 healthy subjects, with no history of brain injury or trauma, from a normative CE database (17), matching the DAI subjects for age and gender.

The protocol was approved by the Ethics Committee for Research of the respective institutions (Protocol #707.642), in compliance with the Declaration of Helsinki, and written informed consent was obtained from all the subjects participating in the study.

Neuropsychological Assessment

All of the selected patients underwent a broad neuropsychological assessment to evaluate attention, memory, information processing speed, dexterity, and executive functions (inhibitory control, verbal fluency, and working memory). All the neuropsychological tests were conducted at one session in a quiet room, with only the subject and the examiner, at 2–7 days before the CE assessment and included the following:

- STAI (State-Trait Anxiety Inventory) (18, 19),
- BDI (Beck Depression Inventory) (18, 20),

- HVLT (Hopkins Verbal Learning Test)—immediate recall/delayed recall/recognition (21),
- BVMT (Brief Visual Memory Test)—immediate recall/delayed recall/recognition (21),
- TMT A and B (Trail-Making Test parts A and B) focused visual attention and task-switching attention (22),
- Stroop test, Victoria version—selective attention and inhibition (22),
- Digit Span Test—working memory (23),
- COWAT (Controlled Oral Word Association Test)—phonologic and semantic verbal fluency (24),
- Symbol digit test—information processing speed (24),
- Five-point test—visual fluency (24),
- Grooved pegboard test—dexterity (25).

CE Assessment: TMS

The CE assessment was performed using MagPro X100 (MagVenture Tonika Elektronik, Farum, Denmark) with a C-100 circular coil connected to an electromyography amplifier of a one-channel, three-surface electrode output.

The stimulation target—*hotspot*—was determined using 70% intensity to identify the point of highest response of the hand muscles, which would correspond to the cortical representation of the abductor pollicis brevis muscle on M1 of both hemispheres. Each subject sat comfortably on a reclining armchair and wore a polyester swim cap on which the *hotspot* was marked.

The parameters measured were resting motor threshold (RMT), motor-evoked potentials (MEPs), short-interval intracortical inhibition (SIICI), and ICF. Peak-to-peak MEP amplitudes were considered in microvolts (μV). RMT was established as the lowest intensity at which MEP of at least 50-μV amplitude could be elicited in 5 of 10 consecutive stimuli (13, 17, 26–30).

We used single-pulse TMS for RMT and MEP measurements. The average value of four MEP curves taken at 120% of RMT was used for analysis. The same procedure was adopted for MEP curves at 140% of RMT. Paired-pulse TMS (pp-TMS) was used for SIICI and ICF measurements, with the conditioning stimulus set at 80% of RMT and the test stimulus at 120% of RMT (13). For SIICI analysis, response curves were taken using pp-TMS with 2 and 4 millisecond (ms) intervals between pulses [interstimulus intervals (ISI)], denominated ICI 2 ms and ICI 4 ms. As for ICF, ISI were 10 and 15 ms, denominated ICI 10 ms and ICI 15 ms. The average value of four MEP curves at each interval was used for analysis. CE measurements were performed using the same technique as in previous studies to facilitate comparison (17, 26–30).

Statistical Analysis

All neuropsychological and CE data were analyzed using the SPSS version 22.0 Statistical package (SPSS, IBM Inc., Chicago, IL, USA) with two-tailed tests and a 5% level of significance. Shapiro–Wilk tests were used to verify continuous variables for normal distribution, and Wilcoxon tests were used to compare the right and left hemispheres in the DAI group.

For inferential analysis, all subjects from DAI group were matched by age and gender to healthy subjects from a normative database of CE (17), and a Mann–Whitney *U*-test was performed. The Spearman test was performed to analyze correlation between neuropsychological and CE data results.

RESULTS

Demographic and Clinical Characteristics

Most participants had severe TBI. They were mostly young adult males (86%), as trauma in general is common in this group. For the outcome measure (functionality), participants were classified according to the Glasgow Outcome Scale-Extended (31). Most of them were independent, both inside and outside their homes but could not resume all their pre-injury social activities—*upper moderate disability*—mostly due to irritability, concentration problems, and memory failures (**Table 1**).

Three subjects had used medications in the past that could interfere with the neurophysiological tests, but by the time they were included in the study, they were no longer taking any medications and were not outliers on CE data. Therefore, they were not excluded from analysis.

CE Results

The CE data distribution was skewed, and there were no significant differences between hemispheres ($p = 0.125$). A pooled analysis was performed in which data from both hemispheres (left and right) were combined (22 subjects, 44 hemispheres). One subject had traumatic amputation of the right arm, and CE data of the left hemisphere were not collected. Another subject had a brachial plexus injury of the left arm, and CE data of right hemisphere were not collected. In summary, 42 hemispheres were analyzed for the DAI patients.

DAI Group Data Classified According to Normative Data

On a group analysis, CE values for the DAI group were classified according to normative data (**Table 2**). The confidence interval

TABLE 1 | Demographic and clinical characteristics of 22 subjects with DAI.

	Mean (±SD) or number (%)
Demographic	
Age, years	30.1 (±10.3)
Gender, male	19 (86.36)
Education, years	10.3 (±2.3)
Clinical characteristics	
Handedness, right	20 (90.9)
Time since TBI, months	18.7 (±2.5)
Glasgow Outcome Scale-Extended	
5—Lower Moderate Disability	2 (9.09)
6—Upper Moderate Disability	10 (45.45)
7—Lower Good Recovery	7 (31.82)
8—Upper Good Recovery	3 (13.64)
Glasgow Coma Scale, score <8 at admission	16 (72.73)
Mechanisms of injury	
Automobile accident	9 (40.91)
Motorcycle accident	8 (36.36)
Running-over	3 (13.64)
Interpersonal aggression	2 (9.09)
Medication	
None	19 (86.36)
Benzodiazepine (prior use)	2 (9.09)
Antidepressant (prior use)	1 (4.55)

TABLE 2 | CE data comparison between DAI patients and healthy controls, analyzed according to normative data.

CE parameters	Median (95% CI)		*p*-Value	95% CI normative data (17)[a]	Classification of DAI patients *n*(%)		
	Control ($n = 44$)	DAI ($n = 42$)			High	Normal	Low
RMT	48.5 (44; 52)	47.5 (43; 51)	0.604	46.3–49.8	19 (45.24)	3 (7.14)	20 (47.62)
MEP-120%	310.5 (260.03; 436.52)	435.85 (253.38; 581.28)	0.388	423.3–689.6	11 (26.19)	11 (26.19)	20 (47.62)
MEP-140%	756.5 (592.31; 1,300)	1,000 (742.02; 1,385.64)	0.346	987.0–1,385.7	15 (35.71)	9 (21.43)	18 (42.86)
Ratio 140/120	2.02 (1.80; 3.16)	2.46 (1.79; 3.12)	0.853	2.4–3.3	11 (26.19)	11 (26.19)	20 (47.62)
ICI 2 ms	0.26 (0.20; 0.47)	1.28 (0.79; 1.70)	**<0.001**	0.2–0.4	**36 (85.71)**	5 (11.90)	1 (2.38)
ICI 4 ms	0.36 (0.28; 0.62)	1.17 (1.0; 1.84)	**<0.001**	0.4–0.6	**36 (85.71)**	5 (11.90)	1 (2.38)
ICF 10 ms	1.31 (0.91; 1.77)	1.20 (0.94; 1.57)	0.638	1.5–2.1	7 (16.67)	10 (23.81)	25 (59.52)
ICF 15 ms	1.15 (0.87; 1.37)	1.47 (1.18; 1.88)	0.131	1.4–2.1	12 (28.57)	11 (26.19)	19 (45.24)
SIICI	0.56 (0.33; 0.69)	1.28 (1.01; 1.87)	**<0.001**	0.4–0.6	**36 (85.71)**	4 (9.52)	2 (4.76)
ICF	1.13 (0.96; 1.48)	1.40 (1.10; 1.68)	0.432	1.5–2.0	10 (23.81)	10 (23.81)	22 (52.38)

[a]*Normative data 95% CI obtained by Cueva (17).*

RMT, Resting motor threshold; MEP-120%, Motor-evoked potential at 120% of RMT; MEP-140%, Motor-evoked potential at 140% of RMT; Ratio 140/120, Motor-evoked potential amplitude ratio for stimulus intensity at 140 and 120% of RMT; ICI 2 ms, Motor-evoked potential amplitude ratio for 2 ms ISI and 120% of RMT; ICI 4 ms, Motor-evoked potential amplitude ratio for 4 ms ISI and 120% of RMT; ICF 10 ms, Motor-evoked potential amplitude ratio for 10 ms ISI and 120% of RMT; ICF 15 ms, Motor-evoked potential ratio for 15 ms ISI and 120% of RMT; SIICI, Short-interval intracortical inhibition; ICF, intracortical facilitation; CI, confidence interval.

range of normal values obtained by Cueva and collaborators (17) was utilized to classify CE results for each parameter of each patient. Values above the highest confidence limit were classified as "high," those within the confidence interval were classified as "normal," and those under the lowest limit were classified as "low."

For each CE parameter, there were patients with higher/lower values than normative data ones. For RMT values, there were 39 hemispheres (92.86%) out of common healthy values (normative data); for MEP-120% values, there were 31 hemispheres (73.81%); for MEP-140%, there were 33 hemispheres (78.57%); for Ratio 140/120, there were 31 hemispheres (73.81%); for ICI 2 ms, there were 37 hemispheres (88.09%); for ICI 4 ms, there were also 37 hemispheres (88.09%); for ICF 10 ms, there were 32 (76.19%); for ICF 15 ms, there were 31 (73.81%); for SIICI, there were 38 (90.47%), and for ICF, there were 32 (76.19%).

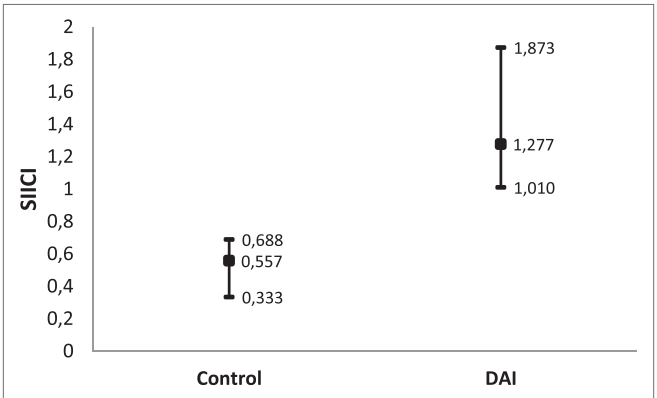

FIGURE 1 | Short-interval intracortical inhibition (SIICI) median values and 95% CI of diffuse axonal injury (DAI) patients compared to healthy subjects.

DAI Patients Compared to Healthy Controls

When data from the DAI patients on an individual level were analyzed, the difference on SIICI and its components was indeed statistically significant compared to those of healthy subjects (**Table 2**). Normal SIICI values usually range from 0.0 to 1.0, and our results showed SIICI median values of 1.28 (1.01; 1.87) (**Figure 1**).

Neuropsychological Results

The majority of neuropsychological tests of patients with DAI showed mean raw scores below the average expected for healthy individuals in our country. Assessments of HVLT, BVMT (immediate and delayed recall), TMT, Stroop, COWAT, Symbol Digit, Five Points, and Grooved Pegboard indicated that DAI patients were cognitively impaired considering age and/or years of schooling. On the other hand, full Digit Span test and only the recognition part of BVMT showed results within the normative average. **Table 3** presents mean scores of neuropsychological assessment. Each test has a standard score limit that defines normal and/or impaired function.

The hypothesis from the SIICI information and neuropsychological findings was that both of these could somehow be related *via* effects on inhibitory processes. For that reason, a correlation analysis was attempted using tests that assessed selective attention and inhibition (**Table 4**), but only weak correlations were found, though, and few of them were statistically significant (**Figures 2–4**).

DISCUSSION

From a pathophysiologic perspective on DAI, damage to axons occurs at the moment of trauma (primary axotomy) when abrupt acceleration–deceleration of the cranium results in shear forces and tensile strains on the white matter, generating small loci of hemorrhage. The resulting axonal degeneration can last for many hours after the trauma episode (caused by secondary axotomy and biochemical cascades) (1, 5, 6).

To minimize the influence of inflammation on these neurophysiological changes, and focus on the direct neuronal damage, we selected subjects during the chronic phase of DAI (at least 1

TABLE 3 | Results of the neuropsychological tests and inventories.

Tests/inventory		*n*	Mean Raw score (SD)	Mean *Z*-score (SD)
STAI	–	21	54.14 (8.79)	–
BDI	–	21	14.19 (12.03)	–
HVLT	Immediate recall	22	17.68 (4.19)	−1.79 (1.06)
	Delayed recall	22	5.23 (2.79)	−1.42 (1.21)
	Recognition	22	9.86 (1.78)	0.75 (1.30)
BVMT	Immediate recall	21	16.38 (8.65)	−1.10 (1.17)
	Delayed recall	21	6.76 (4.23)	−1.13 (1.47)
	Recognition	21	5.24 (1.09)	−0.44 (1.18)
TMT	Part A	22	49.72 (27.12)[a]	−0.80 (1.39)
	Part B	20	135.55 (91.36)[a]	−1.43 (1.22)
Stroop	Card 1	22	23.90 (14.88)[a]	−1.80 (1.25)
	Card 2	22	24.86 (10.22)[a]	−1.35 (1.20)
	Card 3	22	34.09 (11.88)[a]	−1.17 (1.41)
Digit Span	Original order	22	5.00 (1.07)	−0.01 (0.77)
	Reversed order	22	3.45 (0.80)	−0.42 (0.50)
COWAT	Phonologic	22	24.14 (9.77)	−1.48 (0.84)
	Semantic	21	14.38 (4.65)	−1.39 (0.97)
Symbol Digit	–	22	38.45 (14.29)	−1.86 (1.00)
Five Point	–	22	19.64 (7.21)	−1.47 (0.87)
Grooved	Dominant hand	19	87.42 (22.80)[a]	−1.43 (1.27)
Pegboard	Non-dominant hand	21	108.10 (35.89)[a]	−1.70 (1.15)

[a]*Task execution score measured by time in seconds.*

BDI, Beck Depression Inventory; BVMT, Brief Visual Memory Test; COWAT, Controlled Oral Word Association Test; HVLT, Hopkins Verbal Learning Test; STAI, State-Trait Anxiety Inventory; TMT, Trail-Making Test.

TABLE 4 | Correlation between SIICI values and inhibitory control main neuropsychological test results (Spearman's correlation coefficient).

Neuropsychological tests (inhibitory control assessment)		SIICI	
		rho	*p*-Value
Stroop Card 3	Time	0.080	0.611
	Z-score	−0.019	0.904
COWAT phonologic	Raw score	−0.407	**0.007**
	Z-score	−0.487	**0.001**
COWAT semantic	Raw score	−0.314	**0.047**
	Z-score	−0.300	**0.059**
Symbol digit	Raw score	−0.274	0.078
	Z-score	−0.385	**0.011**
Five point	Raw score	−0.129	0.414
	Z-score	−0.129	0.414
Grooved Pegboard dominant hand	Time	0.212	0.207
	Z-score	−0.151	0.370
Grooved Pegboard Non-dominant hand	Time	0.031	0.845
	Z-score	−0.121	0.449

SIICI, Short-interval intracortical inhibition; COWAT, Controlled Oral Word Association Test.

year after TBI). Although there are many mechanisms involved in the development and evolution of DAI, such as diffuse vascular injury and blood–brain barrier disruption that could also interfere with the function of the neural circuitry, the paucity of studies on neurophysiological changes after moderate and severe DAI prevents comparison.

From the CE assessment perspective, pp-TMS using short ISI applied to the motor cortex can indirectly evaluate inhibitory processes mediated by GABAergic circuits (26). The altered SIICI results we found drove us to consider a possible association of

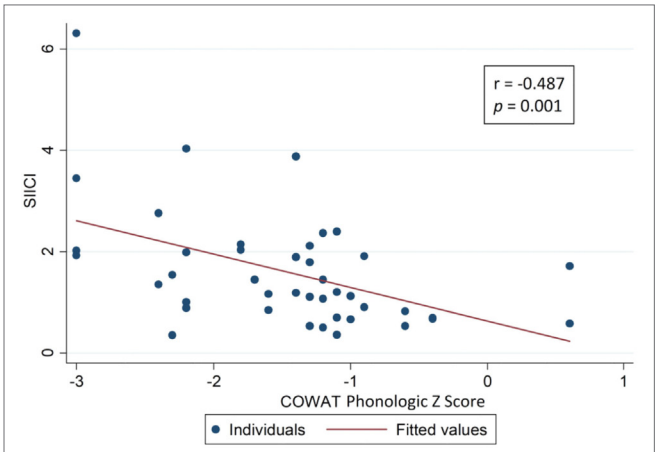

FIGURE 2 | Correlation between short-interval intracortical inhibition (SIICI) values and Controlled Oral Word Association Test (COWAT) phonologic test results.

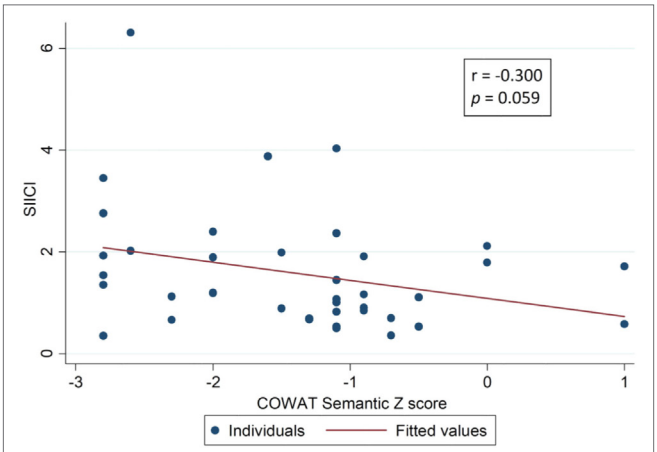

FIGURE 3 | Correlation between short-interval intracortical inhibition (SIICI) values and Controlled Oral Word Association Test (COWAT) semantic test results.

it with dexterity alterations (Grooved Pegboard Test scores) as specific motor task controlling involves intracortical inhibition process by activating a few selective cells and barring other motor neurons (32). The statistical analysis showed that it was not significant even though patients with TBI often present associated motor and cognitive injuries due to the various mechanisms of trauma (4, 33, 34).

Only few neuropsychological tests showed results within the average expected. Nevertheless, the recognition on visual memory (BVMT) and the original order of Digit Span are rather simple tests which might have the results overestimated, considering that the city we held this study in has a high schooling rate. All other aspects, such as information processing speed, selective and task-switching attention, episodic and working memory, verbal fluency, and inhibition process, were all impaired on DAI group, even though it was not possible to establish a relation between the also altered SIICI values.

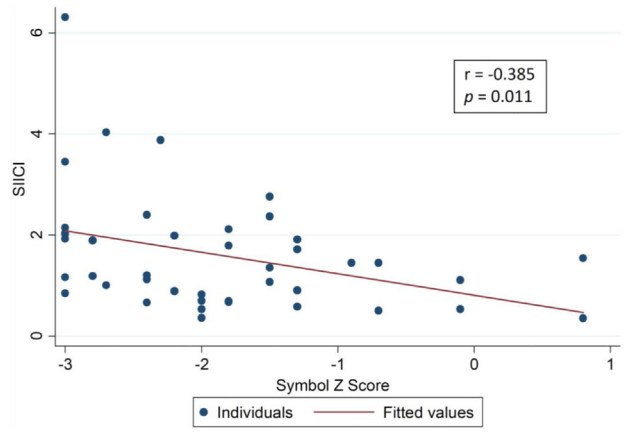

FIGURE 4 | Correlation between short-interval intracortical inhibition (SIICI) values and Symbol Digit test results.

It seems that the attention and memory impairment in DAI are not only affected by intracortical inhibition (GABAergic) and facilitation (glutamatergic) processes but also by many other circuits with complex influences that could not be fully identified in this study. Also, CE assessments can only be performed on a single area (the motor cortex), while cognitive tasks activate different areas of the brain at the same time in a complex pattern.

Another limitation of our study for correlating CE and neuropsychological data is the limited sample size ($n = 22$), so further studies with larger sample sizes are needed to elucidate this problem. Despite there being only a few statistically significant correlation of these aspects (**Table 4**), post-TBI cognition recovery still needs investigation, and unfortunately, the post-trauma setting restrained knowledge on how these individuals were before the incident and on any discrepancies from the neuropsychological perspective.

When considering the control group CE data alone, some values can be interpreted as already abnormal. Still, they are not pathological and just out of what would be considered "common"/"norm." It is worth mentioning that even in healthy individuals, CE values are subject to a large variability or be influenced by many environmental issues (lack of sleep, caffeine consumption, etc.) (17, 35). Minutely, as expected in normative data, there will be 5% of healthy sample that will not be within confidence interval (95% CI), by definition (36, 37). For this study, the control group was considered "healthy" from TBI perspective and free from any other CNS disease, so that the comparison we wanted to make was DAI patients and healthy (non-TBI) subjects.

Revising potential outliers on DAI group, they seemed mathematically outliers, however, not actual clinically outliers. This would be explained by the fact that maximum values for SIICI in healthy subjects over 50 years can be up to 6.7 and up to 3.5 for those who are under 50 years (17).

Regarding the mechanisms of TBI, Almeida-Suhett and collaborators (38) suggested that the loss of GABAergic interneurons after mild TBI reflects a reduction of neuronal inhibition. Miller and collaborators (39) also suggested an influence of mild TBI on intracortical inhibition, measured by silent period (SP) parameters. Bernabeu and collaborators (40) showed the abnormal corticospinal excitability in patients with DAI where motor recovery was related to the severity of TBI (the lower the severity, the better the motor recovery) using input–output curves and SP. These studies address mild TBI with a similar change in inhibition parameter as we found in moderate and severe TBI.

As the inhibition processes involve GABA-mediated circuitry, it is reasonable to infer that DAI pathophysiology itself (disruption of axons) may deplete GABA, contributing to a defective inhibition of neural system on the chronic phase of DAI. For better evidence of GABA depletion, we would recommend further studies measuring GABA noninvasively, if possible, using MR spectroscopy.

We acknowledge that measuring MEPs using cancelation techniques, such as the triple stimulation method, would provide better reliability and more accurate measurements of central conduction times and MEP amplitudes. However, our main aim was to have a concise and brief assessment of CE parameters that would provide a more general view of the excitability status on DAI patients. It is interesting that the present study could find altered intracortical inhibition on these patients but the imbalance of inhibition and facilitation processes is not limited only to GABA-mediated or glutamate-mediated pathways, suggesting that different mechanisms may influence on TBI recovery.

ETHICS STATEMENT

CAPPesq—Comissao de Etica para Analise de Projetos de Pesquisa do Hospital das Clinicas da Faculdade de Medicina da Universidade de Sao Paulo. Protocol #707.642. All eligible patients were interviewed and invited to voluntarily participate in our study. All aspects of our study were explained and clarified. Those who accepted gave written informed consent upon recruitment in compliance with Declaration of Helsinki. Not applicable.

AUTHOR CONTRIBUTIONS

All of the authors contributed to patient recruitment, data analysis, and preparation of the manuscript.

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CLINICAL STUDY PROTOCOL
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Transcranial Direct Current Stimulation for Post-Concussion Syndrome: Study Protocol for a Randomized Crossover Trial

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Background: Mild traumatic brain injury (MTBI) represents 70–80% of all treated brain injuries. A considerable proportion of MTBI patients experience post-concussion symptoms for a prolonged period after MTBI, and these symptoms are diagnosed as persistent post-concussion syndrome (PPCS). PPCS is defined as a range of physical, cognitive, and emotional symptoms. However, memory and executive dysfunction seems to be one of the most debilitating symptoms. Recently, non-invasive brain stimulation has been studied as a potential treatment method for traumatic brain injury (TBI) patients. Therefore, our primary goal is to verify the effects of transcranial direct current stimulation (tDCS) in patients with PPCS who demonstrate cognitive deficits in long-term episodic memory, working memory, and executive function following MTBI.

Methods/design: This is a randomized crossover trial of patients with a history of MTBI with cognitive deficits in memory and executive function. Thirty adult patients will be randomized in a crossover manner to receive three weekly sessions of anodal tDCS (2 mA) at left dorsolateral prefrontal cortex, left temporal cortex, and sham stimulation that will be performed at 7-day intervals (washout period). The clinical diagnosis of PPCS will be determined using the Rivermead Post-Concussion Symptoms Questionnaire. Patients who meet the inclusion criteria will be assessed with a neuropsychological evaluation. A new battery of computerized neuropsychological tests will be performed before and immediately after each stimulation. Statistical analysis will be performed to determine trends of cognitive improvement.

Discussion: There is paucity of studies regarding the use of tDCS in TBI patients, and although recent results showed controversial data regarding the effects of tDCS in such patients, we will address specifically patients with PPCS and MTBI and no brain abnormalities on CT scan other than subarachnoid hemorrhage. Moreover, due

to the missing information on literature regarding the best brain region to be studied, we will evaluate two different regions to find immediate effects of tDCS on memory and executive dysfunction.

Clinical Trial Registration: www.ClinicalTrials.gov, identifier NCT02292589 (https://register.clinicaltrials.gov).

Keywords: brain injuries, post-concussion syndrome, transcranial direct current stimulation, non-invasive brain stimulation, crossover studies

INTRODUCTION

Traumatic brain injury (TBI) is the leading cause of death and disability among children and young adults. Approximately 90% of more than two million annual traumatic brain injuries in the United States are classified as mild traumatic brain injury (MTBI) (1). The criteria for clinical identification of MTBI consists of one or more of the following: a Glasgow Coma Scale (GCS) score of 13–15, confusion and disorientation, loss of consciousness for 30 min or less, posttraumatic amnesia for less than 24 h, and/or other transient neurologic abnormalities (2).

Mild traumatic brain injury has been referred to as a "silent epidemic" because the problems experienced by patients after injury are often unnoticed but can have profound consequences, such as long-term physical, mental, social, or occupational sequelae (3–5). For the majority of patients, MTBI follows a natural course in which the symptoms rapidly resolve within 3 months. However, a considerable proportion of patients with MTBI experience post-concussion symptoms (PCS) for a prolonged period after injury (6). The range of these symptoms can include headache, dizziness, fatigue, irritability, sleep disturbance, difficulties with concentration, memory loss, stress intolerance, light and sound sensitivity, balance problems, anxiety, and a depressed mood. Such prolonged post-injury effects are referred to as persistent post-concussion syndrome (PPCS).

The consequences of PPCS are overwhelming and include a broad spectrum of cognitive, behavioral, and sensorimotor disabilities that dramatically reduce the quality of life; therefore, PPCS is a worldwide public health problem that requires long-term care (7). Given the magnitude of the problem and the lack of specificity of PPCS symptoms, there is an obvious need for studies to examine whether early intervention might reduce the duration of PPCS symptoms.

Neurological and Neuropsychological Findings concerning PPCS

Along with changes in emotional regulation, impairments in attention, memory, and executive function dominate the clinical profile of PPCS (8). However, a variety of symptoms can exist following concussion. The most common symptoms are a disruption of consciousness and a brief period of posttraumatic amnesia. The individual may also report feeling as though he or she is "in a fog." Somatic symptoms, such as headache, fatigue, and balance problems, are also very common. During the acute stages following concussion, a patient may demonstrate disturbances in memory and concentration and feel "slowed down" (9).

It has been hypothesized that PPCS is caused by microstructural damage to the brain due to shearing injury, which is not detectable with conventional imaging techniques and may be responsible for functional deficits (10, 11). The brain regions affected by a concussion seem to especially involve the mesial regions and deeper regions including the hippocampus and corpus callosum. This "preference" would justify the deficits found in post-concussion patients who have memory complaints. Another area that is frequently involved is the prefrontal cortex, which would explain the executive function deficits that can persist even 3 months after the trauma (12, 13).

Cognitive dysfunction is characterized by impairments in attention, concentration, memory, and/or executive function. Patients may have difficulties following instructions and performing tasks or jobs that would have been routine before the trauma (14).

The rapid resolution of symptoms after MTBI raises questions of whether patients can directly benefit from neuropsychological interventions. However, PCS can undoubtedly persist in some cases. Addressing such cases through research in neuropsychology and neuroscience would help to improve our understanding of the progression and etiology of PCS, as well as produce new interventions to help patients who do not improve as expected (15).

Neuropsychological assessment provides diagnostic information about the nature and extent of cognitive dysfunction in neurological conditions, including MTBI. The National Institute of Mental Health and Neurosciences suggests that some neuropsychological batteries have adequate sensitivity and ecological validity to assess the cognitive deficits associated with MTBI (16).

Cognitive Rehabilitation—The Role of Non-Invasive Neuromodulation

Recent reports have documented the therapeutic potential of non-invasive neuromodulation techniques for cognitive enhancement (17–23). The main techniques used for this purpose are repetitive transcranial magnetic stimulation (rTMS) and transcranial direct current stimulation (tDCS).

Transcranial direct current stimulation is a non-invasive neuromodulatory technique that is inexpensive, is easy to use, and applicable to the modification of cerebral excitability. This technique delivers weak polarizing direct current to the cortex *via* two electrodes placed on the scalp. One electrode is an active electrode that is placed over the targeted cortical region, whereas the second electrode is a reference electrode that is typically placed over the contralateral supraorbital area or a non-cephalic region (24). Several studies have demonstrated that a single session of rTMS or tDCS can improve performance on computerized

neuropsychological tests that measure cognitive functions, such as working memory, verbal fluency, reaction time, cognitive interference, and sustained attention in patients with TBI (23, 25–28). These stimulation techniques appear to modulate not only "cold" (non-emotional) cognitive functions but also cognitive processes that involve decision-making, attention, and working memory, as shown in studies of patients with depression (29) and eating disorders (30, 31).

Most studies show that anodal instead of cathodal tDCS is better to enhance cognitive function (32–44). Repetitive anodal tDCS (A-tDCS) applied to the dorsolateral prefrontal cortex (DLPFC) was reported to enhance cognition (37–39), reduce depression (40), and suppress food craving (41). Some studies showed short-term facilitation effects on visual recognition memory and memory peformance after prefrontal and temporal A-tDCS applied 30 min at 2 mA, in patients with Alzheimer disease (42–44). Moreover, A-tDCS over the DLPFC may also improve working memory in patients with Alzheimer disease (42). Another recent study showed that applying tDCS to the left temporal lobe effectively improved auditory memory of patients with poststroke cognitive impairment (45). To date, no study evaluated the effects of A-tDCS over left temporal lobe on cognitive function in patients with TBI.

Considering the possible cognitive effects of tDCS and the clinical importance of TBI, the purpose of this study is to investigate the early effects of tDCS in patients diagnosed with PPCS exhibiting cognitive deficits in long-term episodic memory, working memory, and executive function. tDCS was chosen instead of rTMS for several reasons, including that tDCS is more suitable for conducting neuropsychological tests (rTMS causes noise and slight discomfort at the stimulation site, which could interfere with patient performance on the tests), that blinding to tDCS is more reliable considering the study design, and that rTMS is expensive. Furthermore, there is a lower risk of seizures related to tDCS than to rTMS, which is contraindicated for patients with an elevated risk of seizures (46).

Study Purpose and Objectives

The purpose of this study is to determine the early effects of a single session tDCS in patients with MTBI and PPCS with cognitive deficits in long-term episodic memory and executive function (inhibitory control).

Primary Outcome Measures

The primary hypothesis is that there will be evidence of improvement of patient's episodic memory and executive function measured by neuropsychological test after the stimulation over the left DLPFC (L-DLPFC) in comparison to the other two types of stimulation [sham stimulation and stimulation over the left temporal cortex (L-TC)].

METHODS AND ANALYSIS

Trial Design

This is a randomized, sham-controlled, crossover trial. All patients will be selected from the outpatient services at the Neurotrauma Clinic of the Hospital das Clínicas of the University of São Paulo Medical School (HCFMUSP). The recruitment period will be from February 2016 to April 2018.

This trial will follow the main Consolidated Standards of Reporting Trials guidelines.

Participants

Thirty patients with a history of MTBI who are least 18 years of age will be recruited through our outpatient services at the Neurotrauma Clinic. In our institution, all patients sustaining MTBI at the emergency department are advised to come to our outpatient clinic if they have persistent symptoms. Participants must be diagnosed with clinically defined PPCS based upon established criteria for the presence and frequency of three or more current PCS-like symptoms. Those symptoms will be assessed using the Rivermead Post-Concussion Symptoms Questionnaire (RPQ) (47). The inclusion criteria are as follows: (1) a history of MTBI on hospital admission, (2) age between 18 and 60 years, (3) current subjective complaints related to memory and executive function, (4) able to sign an informed consent form, and (5) consent to participate in the study. The exclusion criteria are as follows: (1) outside the age limits, (2) no specific complaints related to memory or executive function, (3) severe symptoms of major depression (Beck Inventory >35), (4) drug addiction, (5) uncontrolled epilepsy, (6) presence of a metallic prosthesis implant, (7) presence of a cochlear implant, (8) intracranial hemorrhage other than subarachnoid hemorrhage on admission CT scan, or (9) unable to sign an informed consent form.

All patients will be informed about tDCS and the experimental protocol, which has been approved by the Ethics Committee of our hospital.

Recruitment

First the patients will be evaluated by a senior licensed neurosurgeon in the outpatient clinic. Those who present with PCS for a minimum of 3 months will be diagnosed with PPCS. The diagnosis of PPCS will be established using the RPQ (42). Patients will then be referred for a neuropsychological evaluation. Individuals with cognitive impairment related to episodic memory, working memory, and/or executive function will be eligible for the study.

Procedure

The study will consist of three phases: (1) a baseline neuropsychological assessment before starting the stimulation sessions; (2) a single tDCS session (L-DLPFC, L-TC, or Sham stimulation) once a week for three consecutive weeks. **Figure 1** show the fluxogram of the study.

All the subjects will receive all types of stimulation (L-DLPFC, L-TC, or Sham). The interval between each session will be 7 days to avoid carry-over effects. Because this is a crossover study, all participants will receive all proposed stimulation sessions. A computerized neuropsychological test will be performed before and immediately after each stimulation session.

(A) Frontal stimulation: patients will receive A-tDCS over the L-DLPFC at an intensity of 1.5 mA for 20 min.

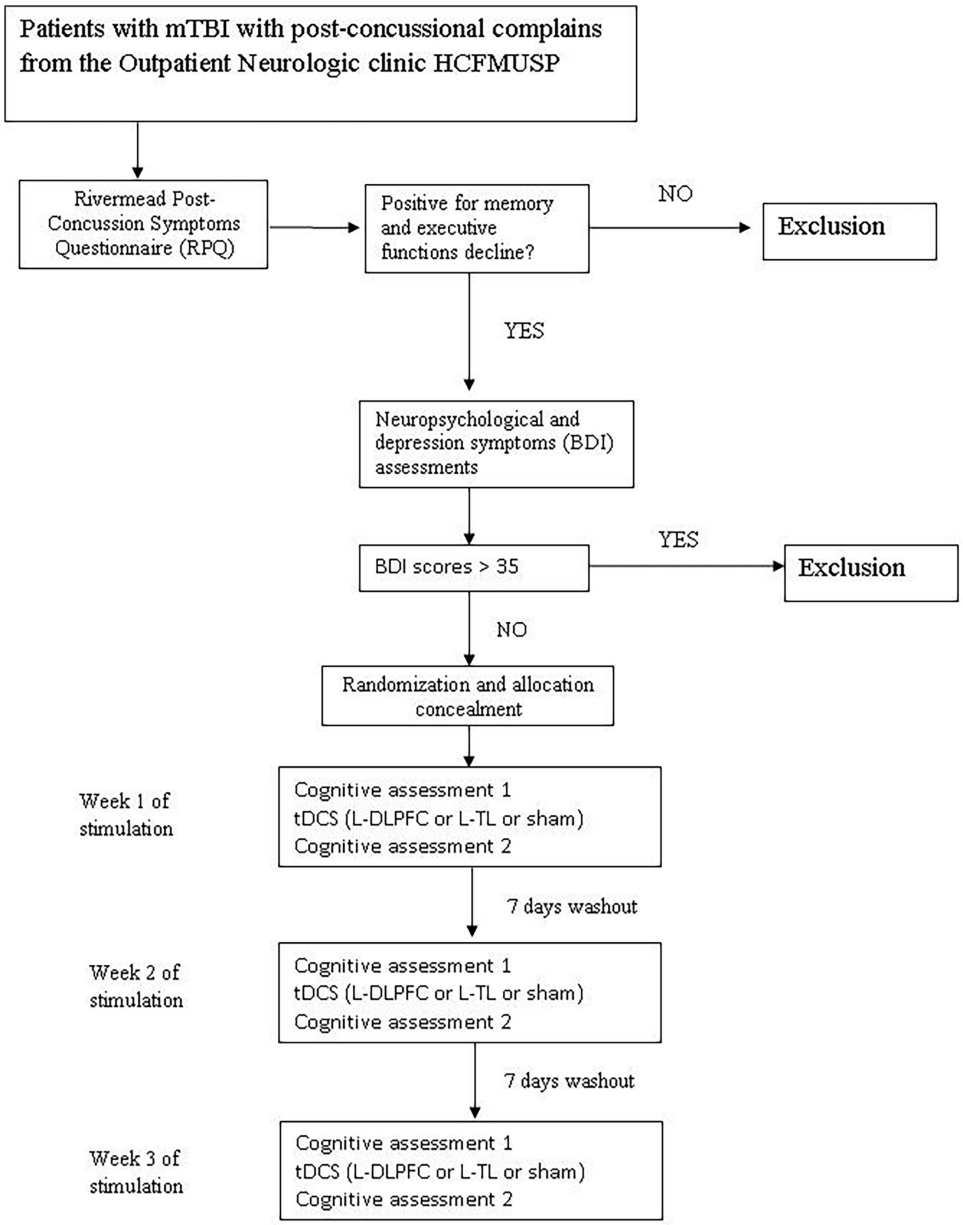

FIGURE 1 | **Fluxogram of the study**.

(B) Temporal stimulation: patients will receive A-tDCS over the L-TC at an intensity of 1.5 mA for 20 min.
(C) Sham stimulation: patients will receive sham stimulation over the occipital area for only 30 s, after which the current will be turned off automatically without the patient's knowledge. To guarantee blinding to the stimulation parameters, the A-tDCS electrode will be placed over the occipital region to simulate a study protocol using three different stimulation positions.

Tolerability and Safety

After each session, patients will be questioned about adverse events. If a major adverse event occurs, the patient will receive medical assistance and further examination and investigation will be provided as needed.

Instruments

To obtain the necessary data to perform this clinical trial and to analyze the results, the following instruments will be used.

Demographic Questionnaire

Information such as age, gender, initial score on the GCS, trauma mechanism, medications in use, and imaging findings will be collected.

Rivermead Post-Concussion Symptoms Questionnaire (47)

The RPQ is used to determine the presence and severity of PPCS according to a set of 16 different symptoms commonly found after MTBI. These symptoms are reported by their severity on a scale from 0 (not experienced) to 4 (severe problem). This instrument will be the screening test to identify patients with PPCS.

Beck Depression Inventory (BDI) (48)

Brazilian version (49) of the BDI will be used. The BDI is a 21-question multiple-choice self-report inventory designed for individuals aged 13 and over to assess depressive symptoms, such as hopelessness, irritability, guilt, and feeling of being punished, as well as physical symptoms, such as fatigue, weight loss, and lack of interest in sex (31). The BDI ranges from 0 to 63 points. The BDI will be assessed only at baseline.

The above neuropsychological tests will be assessed before and after each tDCS session.

Hopkins Verbal Learning Test (50)—Computer Version

This test consists of a list of 12 words. The computer program verbally reproduces the list at a 2-s interstimulus interval. Afterward, the patient is asked to recall as many items as possible in any order. Two additional learning trials are performed, and the delayed recall trial is conducted after a 25-min interval (51).

Forward and Backward Digit Span

The computer version of the Wechsler Intelligence Scale for adults (WAIS III) (52) will be used. The digits forward test assesses attention and short-term memory, whereas the digits backward test measures working memory. A random number sequence is presented to the patient at a rate of approximately one number per second. At the end of each sequence, the patient must repeat the digits in the exact sequence (for the forward sequence) in which they were presented or in the opposite order (for the backward sequence). The test is stopped when the patient has consecutive failure on a sequence with the same digit span.

Stroop Color–Word Test (53)

The Stroop test measures selective attention, cognitive flexibility, and processing speed. It consists of three cards presented by the examiner. The first card (word card) has 24 rectangles painted in brown, pink, blue, or green; the second card (color card) has 24 words (EACH, NEVER, TODAY, ALL) painted in brown, pink, blue, or green; the third card (color–word card) has 24 words (BROWN, PINK, GREEN, and BLUE) painted with mismatched colors. For each card, the subject is asked to say the name of the color as fast as he/she can. The score is calculated based on the time required to respond for each card.

Corsi Block Test (Computerized Version) (53)

This test assesses visual–spatial short-term working memory. This test requires the subject to observe the sequence of blocks "tapped" (illuminated in the computer version) and then repeat the sequence in the same order. The task starts with a short sequence of blocks that gradually increases in number for up to nine blocks. The test measures both the number of correct sequences and the longest sequence remembered.

Inhibitory Control Test (ICT)—Computerized Version (54)

This test assesses attention and inhibitory control of action. In this computerized test, the patient is shown a series of letters and is asked to press the backspace of the keyboard when the letter X is followed by the letter Y or if Y is followed by X. X and Y are the target letters; however, during the presentation of the series, other letters are included and serve as distracters. Patients are instructed not to respond to X following X or Y following Y. The ICT is administered as a practice test followed by a series of six similar 2-min trials separated by breaks to allow the subjects to rest. Performance is evaluated as the number of times the patient misses by clicking following an incorrect letter sequence (55).

Randomization and Blinding

Randomization will be done *via* a computer-produced randomized controlled table. All the 20 patients will be randomized into the three types of stimulation: frontal stimulation, temporal stimulation, and sham stimulation. The neuropsychologists and the patients will be blinded for the type of stimulation performed in each session.

Electronic Data Collection and Management

Data will be stored in a database developed with the Research Electronic Data Capture system (56), which is hosted on the server of the University of São Paulo. This software developed at Vanderbilt University (TN, USA) is fully web-based and enables electronic data collection, management, and also study process management, while meeting the criteria set by the international policies on data privacy and security in the health sector (57).

Sample Size Calculation

Most studies which aimed to assess the effects of non-invasive neurostimulation on cognitive function in TBI were case reports or small open labels studies. Moreover, there have been no previous studies comparing the cognitive effects of tDCS stimulation in patients with PPCS. Considering that there were no prior data on the effects of A-tDCS on patients with PPCS using our primary outcome measure, a formal sample size calculation was not possible; thus, we estimated that enrolling 30 patients would be a reasonable approach for an exploratory trial.

Statistical Analysis

All analysis will be performed using Statistical Package for Social Sciences software version 23.0 for Windows (Prentice Hall, Chicago, IL, USA). A significance level of $p < 0.05$ will be considered for all tests. The quantitative variables will be described using the mean and SDs for normally distributed data or median with inter-quartile range for non-parametric data. The qualitative variables will be presented as absolute and relative frequencies. The five neuropsychological assessments will be summed and averaged to create a composite score. Cohen's *d* will

be calculated to compare the changes in the neuropsychological scores between the groups. An analysis of variance will be used to test whether there is an overall effect of any type of active stimulation on each outcome measure. When appropriate, we will perform *post hoc* paired comparisons using Bonferroni correction for multiple comparisons.

Ethical Issues

Considering the study's context and design, there will be minimal risk to patients. Non-invasive neuromodulation techniques follow the ethical criteria for studies involving human participants by respecting the principles of autonomy, beneficence, non-maleficence, and proportionality to ensure that the subject will not be harmed if he/she participates in the study.

Transcranial direct current stimulation can be considered a safe intervention for several reasons: (A) the electric current applied is very low (1–2 mA over an area of 25–35 cm^2), (B) there is no direct contact between the electrodes and the brain, and (C) the electrodes are embedded in a saline solution, minimizing tissue resistance and avoiding overheating (58–60).

The most common adverse effects observed in safety studies were tingling sensations, itching, mild transient redness of the skin and discomfort on the site of stimulation, moderate fatigue, difficulty concentrating, nausea, and headache. However, these effects were short-lived and were presented at the same frequency between the experimental and placebo groups (60). Patients will be queried after each tDCS session as to whether they experienced adverse effects and how these effects were related to the tDCS treatment. Stimulation sessions have been established by Dr. André Russowski Brunoni, Assistant Professor in the Division of Psychiatry of HC-FMUSP, who will provide any assistance if necessary.

Several advantages of tDCS have been highlighted in clinical practice. These advantages include few side effects that are usually benign, high tolerability, and good potential for efficacy. Notably, it has been emphasized that this technique "has been used in several clinical trials in the last decade and to date, no serious adverse effect has been reported" (58).

DISCUSSION AND DISSEMINATION

This study protocol aims to investigate the neuromodulatory effects of tDCS in patients with a history of MTBI who developed PPCS with a current subjective complaint involving long-term episodic memory, working memory, and executive function. Additionally, the study aims to verify the hypothesis that tDCS exerts pro-cognitive effects in the described population.

Interesting findings have emerged from both clinical trials and neuropsychological studies using tDCS. Knowledge about the cognitive and behavioral functions of brain lesions together with sophisticated neuroimaging techniques have provided major contributions to the fields of neuropsychology and cognitive neuroscience.

The interest in this topic arises from the understanding that neuromodulation techniques can provide causal data that answer questions about the effect of stimulation on cortical structures and specific cognitive functions. The modulatory effect of neuromodulatory stimulation on executive function is of particular interest for understanding the mechanisms underlying the integration of cognition with behavior.

The data that will be obtained in this study may help to provide a step forward for neuropsychology and cognitive neuroscience, as the results will help to reveal brain functioning and the effects derived from interventions. This study also may produce new information regarding the possible pro-cognitive effects of tDCS. Therefore, therapeutic interventions in subsequent studies may be investigated, since the present study will use only a single session of tDCS. We decided to initially study the immediate effects of tDCS in such patients because of the following reasons: (1) we will study a specific population of TBI patients, then, as the first study to evaluate the referred outcomes, we believe that we need to have preliminary data to move forward. (2) We do not know what is the best region to be stimulated, and this trial probably will be able to solve this issue.

This study is expected to initiate a discussion about PPCS thus contributing to the creation of public health policies to treat this underdiagnosed disease. On one hand, PPCS affects a patient's life in social, work, and cognitive contexts. On the other hand, these patients are poorly supported, and their condition is rarely established based on the findings of imaging exams; this lack of evidence could lead to difficulty in diagnosing and treating PPCS patients.

We believe that tDCS holds great promise. It has been shown in previous studies that tDCS is successful, capable, inexpensive, and safe for use in the treatment of a wide range of neurological conditions. Thus, the application of tDCS might improve the efficiency of different neurorehabilitation techniques and provide further relief to patients suffering from long-term disabilities.

ETHICS STATEMENT

Ethics approval has been obtained from the Ethics Committee for Analysis of Research Projects, Hospital das Clínicas, University of São Paulo (CAPPesq 612.643/14). Written informed consent will be obtained from all participants.

AUTHOR CONTRIBUTIONS

RA participated in conception and design of the study, manuscript writing, and its final approval. AB designed the study and helped with data analysis. MO recruited patients, designed the neuropsychological assessment battery, and performed the neuropsychological assessments. AZ recruited patients and designed the neuropsychological assessment battery. MN performed tDCS applications, collected data, reviewed the literature, recruited patients, and contributed to patient follow-up. VG, IN, GB, ML, WP, and AA participated in the conception and design of the study. MT conceived the study and revised the manuscript.

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Investigating Microstructural Abnormalities and Neurocognition in Sub-Acute and Chronic Traumatic Brain Injury Patients with Normal-Appearing White Matter: A Preliminary Diffusion Tensor Imaging Study

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For a significant percentage of subjects, with chronic traumatic brain injury (TBI), who report persisting cognitive impairment and functional loss, the diagnosis is often impeded by the fact that routine neuroimaging often does not reveal any abnormalities. In this paper, we used diffusion tensor imaging (DTI) to investigate the apparently normal white matter (as assessed by routine magnetic resonance imaging) in the brains of 19 subjects with sub-acute (9) and chronic (10) TBI. We also assessed memory, executive function, and visual-motor coordination in these subjects. Using a voxel-wise approach, we investigated if parameters of diffusion were significantly different between TBI subjects and 17 healthy controls (HC), who were demographically matched to the TBI group. We also investigated if changes in DTI parameters were associated with neuropsychological performance in either group. Our results indicate significantly increased mean and axial diffusivity (MD and AD, respectively) values in widespread brain locations in TBI subjects, while controlling for age, sex, and time since injury. HC performed significantly better than the TBI subjects on tests of memory and executive function, indicating the persisting functional loss in chronic TBI. We found no correlation between diffusion parameters and performance on test of executive function in either group. We found negative correlation between FA and composite memory scores, and positive correlation between RD and visuomotor coordination test scores, in various tracts in both groups. Our study suggests that changes in MD and AD can indicate persisting micro-structure abnormalities

in normal-appearing white matter in the brains of subjects with chronic TBI. Our results also suggest that FA in major white matter tracts is correlated with memory in health and in disease, alike; larger and longitudinal studies are needed to discern potential differences in these correlations in the two groups.

Keywords: diffusion tensor imaging, chronic traumatic brain injury, white matter microstructure, human, voxel-based analysis, sub-acute traumatic brain injury, normal-appearing white matter

INTRODUCTION

Traumatic brain injury (TBI) often results in cognitive and functional deficits, impairs daily life functioning, and degrades the quality of life of the injured person significantly in the acute stage (1). Furthermore, the cognitive and functional deficits may persist and yet remain untreated in the chronic stage, particularly when routine diagnostic imaging [e.g., structural magnetic resonance imaging (MRI), and computed tomography (CT)] does not reveal any gross structural abnormalities, indicative of the cognitive or functional loss, in the brain of the injured patients. A large literature indicates that these persisting deficits are linked to microstructural damage in the brain (2). Diffusion tensor imaging (DTI) (3), which uses the diffusion of water molecules to assess microstructure in both pathological and normal brain tissue, has frequently been used in the past decade to investigate microstructural damage in patients with TBI (4–6) [also see the review by Hulkower et al. (7)]. The DTI studies of TBI often investigate the change in one or more of the classic diffusion parameters—axial diffusivity (AD), radial diffusivity (RD), mean diffusivity (MD), and fractional anisotropy (FA)—using either region of interest (ROI) (8, 9) or whole brain voxel-wise (10, 11) approaches. Most of these studies have focused on FA alone (10) or FA and MD (12, 13), the two parameters that describe the overall or average diffusion trends. These studies suggest a general trend of decrease in directionality of diffusion as measured by FA (7, 10, 14, 15) and an increase in the overall diffusion as measured by MD (7, 16, 17) in subjects with chronic TBI and associate these changes with diffuse axonal injury (DAI).

The DAI, an example of microstructural disruption resulting from TBI, is thought to be one of the major causes of neurocognitive deficits after TBI (18, 19). However, given the complexity of the various cognitive and executive function networks in the brain, it is important to investigate the role of DAI in neurocognitive deficits after TBI in situations where no other confounding structural anomalies (e.g., focal lesions indicative of the cognitive or functional loss) are present and by adopting a whole-brain analysis approach. The DTI is an excellent technique to investigate the DAI in TBI, however, with notable exceptions (20, 21), most of the DTI studies investigating the correlation between neurocognitive function and DTI parameters did not exclude subjects with white matter abnormalities as assessed with routine diagnostic imaging (22–25). Furthermore, many of these DTI studies investigating neurocognitive function after TBI are restricted only to FA and MD and only to TBI patients, excluding healthy controls (HC) from the correlation analysis (9, 22, 23, 26–28). A few studies investigated this correlation in all subjects including HC using an ROI approach (22, 23, 29, 30) and only a fraction of these extended the correlation analysis to whole brain white matter tracts using the voxel-wise approach (31–33). While the aforementioned studies present similar conditions, these studies either did not investigate the presence of gross or focal white matter abnormalities (31, 33) or did not exclude the subjects with such abnormalities from the extended investigation of neurocognitive deficits. The present study builds on these latter studies of the whole brain white matter tracts using a voxel-wise analysis approach to elucidate the relationship between neuro-cognition and white matter microstructure in sub-acute and chronic TBI patients with normal-appearing white matter (NAWM).

In this pilot study, we investigate if the classic DTI parameters (FA, MD, RD and AD) exhibit any changes in patients with sub-acute and chronic TBI of all severities but with no gross abnormalities on routine MRI. We also aim to explore if the relationship between the classic DTI parameters and neurocognitive function is altered when compared with HC. This knowledge is essential as part of considering whether classic DTI parameters are useful biomarkers of the degree of neurocognitive deficit after TBI.

MATERIALS AND METHODS

Participants

This study was performed at St. Michael's Hospital in Toronto, ON, Canada with approval from the hospital's Research Ethics Board. Patients with a positive history of TBI were recruited from the Trauma/Neurosurgery Ward, Neurosurgery and Head Injury Clinics, and Trauma Registry Centre at the hospital. Inclusion criteria for TBI patients required that they were within 16–70 years of age and had sustained an isolated, closed head TBI with Glasgow Coma Score [GCS; (34)] in the range 5–15 as assessed within 12 h of injury. The HC were recruited through the word-of-mouth and through online advertisement. For HC, the inclusion criterion was to be within 16–70 years of age, while the exclusion criterion included having a history of TBI, motor vehicle collisions, or falls. The exclusion criteria, as applicable to both groups, included the presence of non-hemorrhagic or micro-hemorrhagic brain lesions as assessed by fluid-attenuated inversion recovery (FLAIR) and gradient recalled echo (GRE) MRI. The presence of small superficial contusions and superficial hemosiderin staining was not considered to be an exclusion criterion, as these lesions are not expected to affect the central white matter tracts that this study aimed to analyze. The exclusion criterion for both groups also included incomplete assessment of neuro-cognition as tested with standardized neuropsychological

(NP) tests (see below for more details). Subjects with existing substance abuse were excluded from the study, as this dependency can potentially alter cognition (35), and is known to be associated with microstructural abnormalities with altered FA values (36). Both groups also were subject to standard MRI exclusion criteria (e.g., claustrophobia, ferromagnetic implants) and were required to possessed adequate verbal English language skills. All participants provided informed consent to participate in the study, and received monetary compensation for the time spent on the study.

NP Testing

Cognitive function was assessed in all subjects in the three categories of memory, executive function, and visual-motor coordination. These assessments were performed using the following standardized NP tests. Memory was assessed with the Digit Span subtest (37) of the Wechsler Adult Intelligence Scale (WAIS-III) (38) and the Hopkins Verbal Learning Test (HVLT) (39). Executive function was assessed using the Token Test (40), the Phonemic Fluency subtest, which is a part of the Neurosensory Center Comprehensive Examination for Aphasia (41), and the Digit Symbol subtest of the Wechsler Adult Intelligence Scale (WAIS-III) (38). The visuomotor coordination skill was assessed using the Grooved Pegboard test (42). The National Adult Reading Test (NART) (43) was used as a measure of verbal IQ. Except for the Grooved Pegboard, all tests were administered using pen and paper.

In the Digit Span test, the subjects were asked to recall numbers in forward and backward order and the number of correct responses was recorded in each case. The HVLT consisted of three trials; the total correct responses, incorrect responses, and intrusions were recorded for each trial and the number of correct delayed recall responses at the end of the three trials was also recorded. For the Token test, the total correct responses in each of the two trials performed were recorded. The Phonemic Fluency test required subjects to list words starting with each of the letters F, A, and S; total correct responses, incorrect/repeated responses, and intrusions were recorded in each case. For the Digit Symbol test, the total number of correctly completed, incorrectly completed, skipped, and incomplete boxes were recorded. For the Grooved Pegboard test, the time (in seconds) taken to put all the pegs in the board and the number of pegs dropped during the procedure were noted. Subjects completed the task first with their dominant hand, then the non-dominant hand. The test thus resulted in four scores: time to complete with the dominant hand (dominant time), the number of drops with the dominant hand (dominant drop), time to complete with the non-dominant hand (non-dominant time), and the number of drops with the non-dominant hand (non-dominant drop).

For each of the NP tests administered, a higher score indicates better performance. The one exception is the Grooved Pegboard test, in which a higher score indicates poorer visuomotor coordination.

MRI Protocol

Imaging was performed with a research-dedicated MRI system at 3.0 T (MR750, GE Healthcare, Waukesha, WI, USA) at Sunnybrook Health Sciences Centre in Toronto. The MRI protocol for each subject (TBI patients and controls) included a three-dimensional (3D) longitudinal relaxation time (T_1)-weighted sequence to rule out gross structural abnormalities and for anatomical reference and two-dimensional (2D) FLAIR and GRE sequences to detect microbleeds, along with a 2D DTI sequence. The parameters for these MRI sequences were as follows: 3D axial T_1-weighted inversion-recovery prepped fast gradient echo imaging—in-plane resolution = 0.9 mm × 0.9 mm; field of view (FOV) = 220 mm × 165 mm; slice thickness = 1.4 mm; flip angle = 15°; repetition time (TR) = 8.2 ms; echo time (TE) = 3.2 ms, axial FLAIR imaging—resolution = 0.9 mm × 1.1 mm × 3.0 mm; FOV = 220 mm × 200 mm; flip angle = 90°; TR = 9,950 ms; TE = 96 ms; axial GRE imaging—resolution = 0.8 mm × 1.0 mm × 3.0 mm; FOV = 200 mm × 200 mm; flip angle = 20°; TR = 784 ms; TE = 35 ms; and axial DTI imaging—FOV = 240 mm × 240 mm; resolution = 1.9 mm × 1.9 mm; slice thickness = 3 mm; non-collinear diffusion directions = 11 with b = 1,000 s/mm^2 and with two volumes applied without diffusion sensitizing gradients (b = 0 s/mm^2), averages = 4; TR = 9,400 ms; TE = 89.7 ms. The MRI protocol lasted approximately 40 min.

DTI Data Processing

Diffusion tensor imaging data were processed using the Oxford Centre for Functional MRI of the Brain (FMRIB) Software Library (FSL- version 5.0.5—http://www.fmrib.ox.ac.uk/fsl) (44). The diffusion data were corrected for distortions due to eddy currents and minor head motion using the $b = 0$ image as a reference. Brain extraction was then performed on these data using the FSL brain extraction tool (45). The diffusion tensor model was fitted to the images of the extracted brain tissue to produce maps of FA, MD, RD, and AD in individual subjects. Voxel-wise analyses of the DTI parameters were performed using the Tract-Based Spatial Statistics (TBSS) package (46). As part of TBSS processing, the FA image of each subject was transformed into the Montreal Neurological Institute (MNI)-152 standard space (47), using non-linear registration (44, 48) to the FMRIB58_FA_1mm image (average high-resolution FA image of 58 subjects in MNI 152 standard space). The mean FA maps of all subjects enrolled in the study were then created. A mean white matter skeleton was subsequently generated using a threshold of FA > 0.2 to include only the centers of white matter tracts common to the entire group. The FA data from each subject were then projected onto this mean FA skeleton to generate a 4D data matrix (x, y, z, FA) characterizing the group. The non-linear transformations used in this procedure were then applied to create analogous 4D data matrices of the skeletonized MD, RD, and AD values, respectively. These matrices were used for the inter-group statistical analyses.

Statistical Analyses

A Student's t-test was used to compare the mean NP test scores between the two groups, after assessing for normality of the data. Differences were considered statistically significant using a threshold p-value ≤ 0.05.

A General Linear Model (GLM) (49) was applied using a voxel-based approach to each of the 4D data matrices of FA, MD, AD, and RD within the FSL framework to investigate the relationship of DTI parameters with the group (TBI versus HC). The GLM was applied using non-parametric permutation-based statistics by employing the *randomize* tool in FSL with threshold-free cluster enhancement and 5,000 permutations (50). Age, sex, and time since injury were included as non-interacting covariates in the GLM. The contrasts were designed to investigate if the two groups had statistically significant differences in DTI parameters (TBI > HC and HC > TBI).

The investigation of whether DTI parameters were correlated with NP test performance was also performed with the *randomize* tool within the framework of GLM in FSL. For this analysis, a composite score for each category of NP function (memory, executive function, and visuomotor coordination) was calculated using the following procedure. First, the Pearson correlation coefficient (R) was calculated to assess the correlation between scores and sub-scores of various tests within the same category and only those subtests that had significant positive correlation with each other ($R > 0.5$, p-value ≤ 0.05) were included in the subsequent analyses. These selected tests/subtests scores for all subjects (TBI and HC) were then converted into ranks. Finally, a composite score for each category was calculated as the mean of ranks of all subtests and tests in that category. The correlation and rank calculations were performed using the Statistical Analysis System, 9.4 (SAS Institute Inc. 2013, Cary, NC, USA). These composites scores for NP tests were entered in the GLM design matrix for investigation of the correlation between DTI parameters and NP test performance. First, an investigation of whether the correlation between NP test performance and DTI parameters varied according to the group was performed. For this, an interaction term for each NP test category was added to the design matrix and contrasts were designed to determine positive and negative interactions between composite test scores in the three NP categories and group predictor variable. Afterward, correlations of the composite scores in the three NP categories were investigated with contrasts designed to calculate positive as well as negative correlations. For these analyses involving NP tests, IQ and education were also included in the GLM as non-interacting covariates.

A p-value ≤ 0.05, corrected for multiple comparisons within *randomize* by controlling for the family-wise error rate (51), was considered significant for all statistical comparisons performed within the framework of the GLM in FSL.

Identifying the Regions with Significant Inter-Group Differences/Correlations

The *randomize* outputs consisting of p-values corrected for multiple comparisons were thresholded to keep voxels with p-value ≤ 0.05. The FSL atlas query and cluster identification tools (merged as the *autoaq* algorithm) were used on the thresholded images to identify various white matter tracts using the Johns Hopkins University White Matter Labels atlas (52). To eliminate noise due to averaging and normalization, tracts with <50 significantly different voxels were not considered as tracts exhibiting structural differences between the two groups and were excluded from the reported results.

Post Hoc Analyses

The *post hoc* analyses, where mentioned, were performed using only those voxels that were found significant at $p \leq 0.05$ level. The *post hoc* analyses were also performed within the framework of the GLM in FSL and the results were corrected for multiple comparisons within *randomize* by controlling for the family-wise error rate (51).

RESULTS

Subjects

A total of 52 patients and 18 HC met the inclusion criteria and underwent MRI and standardized NP tests. FLAIR and GRE images of all the subjects were reviewed by two board-certified neuroradiologists to rule out non-hemorrhagic (53) and micro-hemorrhagic (54) lesions. Twelve patients were excluded from the study: five did not complete the MRI exam, four had significant head motion during MRI and three withdrew their consent after MRI. Twenty-one patients and one HC were excluded from the analyses due to gross abnormalities in the central cerebral white matter, visible on FLAIR or GRE images. Most of the TBI subjects had small superficial contusions and superficial hemosiderin staining but were not excluded, as these lesions are not expected to affect the central white matter tracts that this study aimed to analyze. Nineteen patients with a history of trauma from a motor vehicle collision (5 patients) or fall (14 patients) were thus included in the study. Based on the GCS scores at the time of emergency department arrival, 15 patients had mild TBI (reference GCS: 13–15), three patients had moderate TBI (reference GCS: 9–12) and one patient had severe TBI (reference GCS $\leq$8). Time elapsed between injury and MRI for the present study ranged from 0.2 to 5.1 years (**Table 1**). Nine subjects were in the sub-acute stage (2 weeks < time since injury < 1 year), whereas the rest of the subjects were in the chronic stage after TBI (time since injury > 1 year) (7).

The group of HC consisted of individuals recruited through online advertisement as well as friends and family members of patients who attended the injury, trauma, or neurosurgery clinics at the hospital. The group of HC was matched by age, sex, and education to the group of TBI patients. **Table 1** lists the demographic details of all subjects in the study.

TABLE 1 | Group demographic variables.

	TBI (N = 19; 12 males)		Healthy controls (N = 17; 10 males)	
	Median	**IQR**	**Median**	**IQR**
Age (years)	47	22.5	42	31
Education (years)	15	2.5	15	3
Glasgow Coma Score	14	2	Not applicable	
Time since injury (years)	1	1.8		

Median and interquartile range (IQR) for demographic variables of traumatic brain injury (TBI) patients and control subjects.

NP Performance

Healthy controls performed better than the TBI group on all NP tests, based on score results. However, only the scores for Digit Span, Phonemic Fluency, the third trial of the HVLT, and Digit Symbol tests were significantly higher in HC at p-value ≤ 0.05. A significant positive correlation was found between the Digit Span scores (forward and backward), the total correct responses in each of the three trials of HVLT, and the correct delayed recall score in the memory category. In the executive function category, a significant positive correlation was found between the total correct phonemic score, the total correct score in the Digit Symbol test, and the total score in each of the two Token test trials. In the visuomotor coordination category, the dominant and non-dominant times were found to have a significant positive correlation. No significant difference was found in the verbal IQ of the two groups as measured with the NART. The results of NP test performance are summarized in **Table 2**.

Inter-Group Differences in DTI Parameters

Significantly increased MD and AD values were found in the TBI group compared to HC in several brain regions (controlling for age, sex, and time since injury). **Table 3** lists MD and AD values in those tracts where significant inter-group differences in these variables were found. In particular, significant differences in MD and AD were found in the corpus callosum and bilateral internal capsule, external capsule, corona radiata, posterior thalamic radiations, and various other structures. Changes in AD were more widespread compared to those in MD (**Figures 1** and **2**). No significant differences between the two groups were found in FA and RD. Although trends of increased RD and FA in TBI were visible in several major white matter tracts, notable exceptions included a trend of decreased FA in TBI in the corpus callosum and the bilateral cingulum.

TABLE 2 | NP performance results.

NP test	TBI	Healthy controls	p-Value
	Median/ interquartile range (IQR)	Median/ IQR	
National Adult Reading Test (verbal IQ)	34/15	39.5/14	0.1
Memory			
Digit span (forward and backward)	9/4; 6/3.5	12/3; 7.5/4.8	0.003*; 0.03*
Hopkins Verbal Learning Test (3 trials and delayed recall)	6/3; 9/2.5; 8/4; 8/2.5	5.5/2.8; 8.5/3; 10/2; 8.5/3.8	0.8; 0.4; 0.04*; 0.2
Executive function			
Token test (2 trials)	21/3; 22/0	21/2; 22/1	0.2; 0.7
Digit symbol (total correct)	64/9	71.5/29.3	0.02*
Phonemic fluency (total correct)	36/12	42/13.5	0.02*
Visuomotor coordination			
Pegboard (dominant and non-dominant time- seconds)	70/11; 79/30.5	60/28.5; 70/20	0.16; 0.08

List of subtests and tests in each category of neuropsychological (NP) function that were found to have significant positive correlation with each other. Higher values imply better performance on the corresponding NP test except for the Pegboard where the reverse is true. The last column lists the p-value for Student's t-test.
**represents statistically significant difference between the mean scores of the two groups.*

Post Hoc Analyses of the Inter-Group Differences in DTI Parameters

The *post hoc* analyses, involving only the significant voxels at $p \leq 0.05$ level confirmed the inter-group differences in the DTI variables. The p values of the *post hoc* analyses are also listed in **Table 3**.

Interactions

No significant interactions between the composite test scores in the three NP function categories and group variables were found, indicating that both groups exhibited similar relationships between the NP explanatory variables on the DTI parameters. Therefore, the NP-group interaction terms were excluded, and contrasts were appropriately modified for the subsequent analyses to investigate the effect of group and NP explanatory variables on the DTI parameters.

Correlation of NP Test Scores with DTI Parameters

Continuing with the GLM design matrix with the composite test scores in the three NP function categories added as covariates along with age, sex, time since injury, education and the NART (verbal IQ), the correlation between NP test scores and DTI parameters was investigated with appropriate contrasts. In this analysis, a negative correlation was found between the composite memory score and FA in all subjects, in several tracts widespread in the entire brain. These tracts included bilateral internal and external capsules, corona radiata, cingulum, posterior thalamic radiations and superior longitudinal fasciculi. A positive correlation between composite visuomotor score and RD was also found in both groups, in a few tracts including the corpus callosum and only the right components of corona radiata, cingulum, and superior longitudinal fasciculus. **Table 4** lists the tracts where a significant correlation between the DTI parameters and the composite NP test scores was found. No significant correlations between the composite NP function scores and MD or AD were found.

DISCUSSION

This study was designed to investigate the microstructural abnormalities in NAWM using DTI and to explore their potential impact on cognitive function in post-acute TBI patients compared to HC using a voxel-wise approach. Most often, TBI patients report persisting cognitive deficits and hence are not able to return to their pre-morbid daily activities, sometimes also including the inability to resume work, resulting in financial stress and increased psychological stress. The clinical assessment of this persisting cognitive deficit is challenging, especially when no gross abnormalities indicative of the cognitive or functional loss are detected on routine diagnostic imaging (e.g., structural MRI and CT). The DTI method, however, has been shown to

TABLE 3 | Tracts with significantly increased MD and AD values in TBI.

Structure	MD			AD		
	Mean ± SD ($\times 10^{-4}$ – mm^2 s^{-1})		*p*-Value *(post hoc)*	Mean ± SD ($\times 10^{-3}$ – mm^2 s^{-1})		*p*-Value *(post hoc)*
	TBI	HC		TBI	HC	
Corpus callosum	8.1 ± 0.4	7.8 ± 0.3	0.03 (0.008)	1.5 ± 0.7	1.5 ± 0.5	0.03 (0.004)
Corticospinal tract-R	–	–	–	1.3 ± 0.7	1.2 ± 0.7	0.03 (0.005)
Corticospinal tract-L	–	–	–	1.2 ± 1	1.2 ± 0.5	0.04 (0.007)
Cerebral peduncle-R	6.8 ± 0.4	6.6 ± 0.4	0.04 (0.01)	1.5 ± 0.8	1.4 ± 0.6	0.03 (0.005)
Cerebral peduncle-L	–	–	–	1.4 ± 0.8	1.4 ± 0.8	0.03 (0.005)
Internal capsule-R	7.5 ± 0.3	7.2 ± 0.2	0.03 (0.006)	1.4 ± 0.6	1.3 ± 0.4	0.02 (0.003)
Internal capsule-L	7.4 ± 0.3	7.2 ± 0.2	0.04 (0.01)	1.3 ± 0.5	1.3 ± 0.3	0.02 (0.003)
External capsule-R	7.6 ± 0.3	7.3 ± 0.2	0.03 (0.009)	1.2 ± 0.6	1.1 ± 0.2	0.03 (0.003)
External capsule-L	7.4 ± 0.3	7.2 ± 0.2	0.04 (0.02)	1.2 ± 0.6	1.1 ± 0.3	0.02 (0.004)
Corona radiata-R	7.4 ± 0.3	7.2 ± 0.2	0.02 (0.003)	1.2 ± 0.5	1.2 ± 0.4	0.02 (0.002)
Corona radiata-L	7.4 ± 0.3	7.2 ± 0.2	0.03 (0.007)	1.2 ± 0.5	1.2 ± 0.4	0.02 (0.002)
Cingulum-R	7.8 ± 0.3	7.4 ± 0.2	0.03 (0.005)	1.2 ± 0.5	1.2 ± 0.3	0.03 (0.004)
Cingulum-L	7.7 ± 0.4	7.4 ± 0.2	0.03 (0.01)	1.2 ± 0.6	1.2 ± 0.4	0.03 (0.004)
Thalamic rad Post-R	8.3 ± 0.4	7.9 ± 0.4	0.03 (0.007)	1.5 ± 0.7	1.4 ± 0.5	0.03 (0.004)
Thalamic rad Post-L	8.0 ± 0.4	7.6 ± 0.4	0.04 (0.01)	1.4 ± 0.6	1.3 ± 0.5	0.04 (0.007)
Longitudinal fasc Sup-R	7.4 ± 0.3	7.1 ± 0.2	0.02 (0.006)	1.2 ± 0.6	1.1 ± 0.4	0.03 (0.004)
Longitudinal fasc Sup-L	7.5 ± 0.3	7.2 ± 0.2	0.03 (0.009)	1.2 ± 0.34	1.1 ± 0.3	0.03 (0.005)
Frontooccipital fasc Sup-R	7.1 ± 0.3	6.7 ± 0.2	0.02 (0.005)	1.1 ± 0.6	1.1 ± 0.5	0.02 (0.003)
Frontooccipital fasc Sup-L	6.8 ± 0.4	6.5 ± 0.3	0.03 (0.008)	1.1 ± 0.7	1.1 ± 0.5	0.02 (0.002)

White matter tracts where mean diffusivity (MD) and axial diffusivity (AD) were found to be significantly higher in the traumatic brain injury (TBI) group compared to healthy controls (HC), after controlling for age, sex, and time since injury at p-value ≤ 0.05, corrected for multiple comparisons. For each structure, the mean value of the diffusion parameters is first calculated in every subject for only those voxels where significant differences were identified, and then the mean across all subjects is calculated. The p-values quoted are the mean values calculated only for those voxels where significant differences were identified. The numbers in parenthesis in the p-value column represent p-value obtained in the post hoc analyses which was performed with only those voxels where a significant difference at p-value ≤0.05 was earlier found.
fasc, fasciculus, L, left, Post, posterior, rad, radiations, R, right, Sup, superior.

detect micro-abnormalities and hence can be helpful in explaining the functional or cognitive deficit in sub-acute and chronic TBI with NAWM. Voxel-wise analysis of the DTI parameters and of the correlation of cognitive function with DTI parameters performed over the entire brain has the advantage of identifying partial regions or tracts with significant inter-group differences and significant correlations. Such regions may not be successfully detected *via* analyses which involve ROI selection based on *a priori* hypotheses. Partial regions or tracts with significant inter-group differences may also be masked by spatial averaging in ROI analyses.

Our neuroradiologists confirmed the absence of gross abnormalities in the central white matter of the brain in only 19 of the 40 TBI patients after reviewing their FLAIR and GRE data. These numbers suggest that some gross abnormalities due to TBI are likely to persist for lengthy time periods and may account for the changes in DTI parameters in chronic TBI, as widely quoted in the literature. It is noteworthy, however, that most of the TBI patients with NAWM that were included in our analyses had mild TBI (15 out of 19 subjects). Mild TBI is more frequent than other forms of TBI world-wide (55), and is less likely to cause gross lesions in the brain.

Our finding of widespread increase in the overall diffusivity of water molecules (as measured with MD) in the post-acute TBI subjects, while controlling for age, sex, and time since injury, robustly reaffirms the findings of previous studies (12, 13, 21, 24, 32, 33, 56, 57) and indicates the persistence of DAI in sub-acute and chronic TBI with NAWM. We also found an accompanying increase in AD in the TBI group. Many DTI studies of TBI have not analyzed AD. However, those studies that explored inter-group differences in AD also found an increase in this parameter in post-acute TBI (21, 32). The changes in AD were more widespread compared to changes in MD, and potentially significantly contributed to increase in MD.

We did not find any significant differences between the two groups in FA. Several DTI studies of TBI reported a decrease of FA in post-acute TBI; however, these studies included more acute patients, up to 1 month from the injury (22, 23, 27, 30). Some other studies with a minimum post-injury time similar to ours (2–3 months) reported variable FA results: some reported a decrease (12, 24, 26, 31, 32); one reported an increase (58); and others reported no change (9, 25, 59). One potential reason for this variability may be the presence of gross abnormalities in the brain, as some of the studies that reported decreased FA either included subjects with gross abnormalities (24, 32) or did not screen subjects for gross abnormalities (26). Furthermore, one study that found reduced FA (12) focused on TBI subjects using prescribed medicines for major depression or post-traumatic stress disorder, which constitute a known confounding factor (60).

We also did not find any significant differences between the two groups in RD; however, trends of increased RD in TBI were evident. Very often, studies of DTI in post- acute TBI have not investigated the RD parameter. A few studies involving RD reported variable findings, with increase (32) or no difference (26) observed in chronic TBI compared to HC.

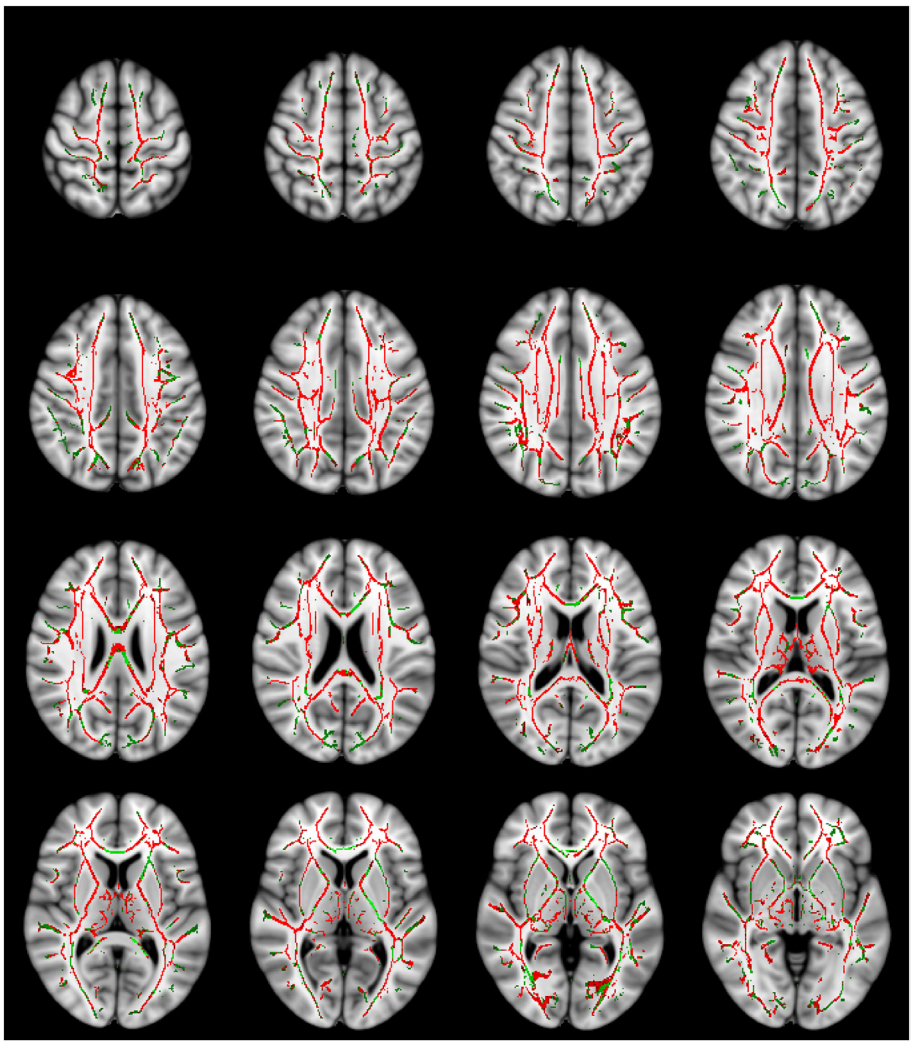

FIGURE 1 | **Mean diffusivity (MD) values are significantly higher in the traumatic brain injury (TBI) group with normal-appearing white matter**. The MD contrast is overlaid on a standard Montreal Neurological Institute 152 T_1 1 mm brain and the mean fractional anisotropy skeleton (green—display threshold 0.2–0.8). The voxels where MD was found to be significantly higher in the TBI group (p-value ≤ 0.05) are shown in red. Every fourth transverse slice only is shown here to approximately cover the entire brain along the superior–inferior axis. Anatomical right side of the brain is shown on the left in this figure.

A survey of the TBI literature suggests that DTI parameters change dynamically with time post-injury. One longitudinal study assessing FA values in TBI patients reported a decrease at the acute and sub-acute stage (range of time since injury = 3–55 days) when compared with controls, an increase at the chronic stage (minimum of 238 days since the first scan) in comparison with patients' first measurements and no difference at the chronic stage when compared with controls (25). The same study also reported an increase in MD in the TBI group in comparison with controls at both time-points, and an increase in MD with time in the TBI group. Another longitudinal study reported increases in AD, MD, and FA in the acute stage, and no differences in RD; and sustained increase in MD in the chronic stage accompanied by an increase in RD, normal AD levels, and a reduction in FA compared to HC (33). These observations suggest that the absence of statistically significant differences in RD and FA in our study might be due to inclusion of both sub-acute and chronic TBI patients.

Considering these findings collectively with our results, we conclude that DAI, as manifested in elevated diffusivity, potentially persist from the acute stage to well into the chronic stage after TBI, whereas changes in FA may be more sensitive to time since injury and may not remain detectable later on in the chronic stage. The different effects shown by these parameters are interesting, and may reflect their biophysical underpinnings. Whereas MD is a linear combination of diffusivities in the three orthogonal directions and will increase if all three diffusivities are increased, FA is a measure of difference among the three diffusivities and may show no change if the three diffusivities are all modulated proportionally in the same manner. An

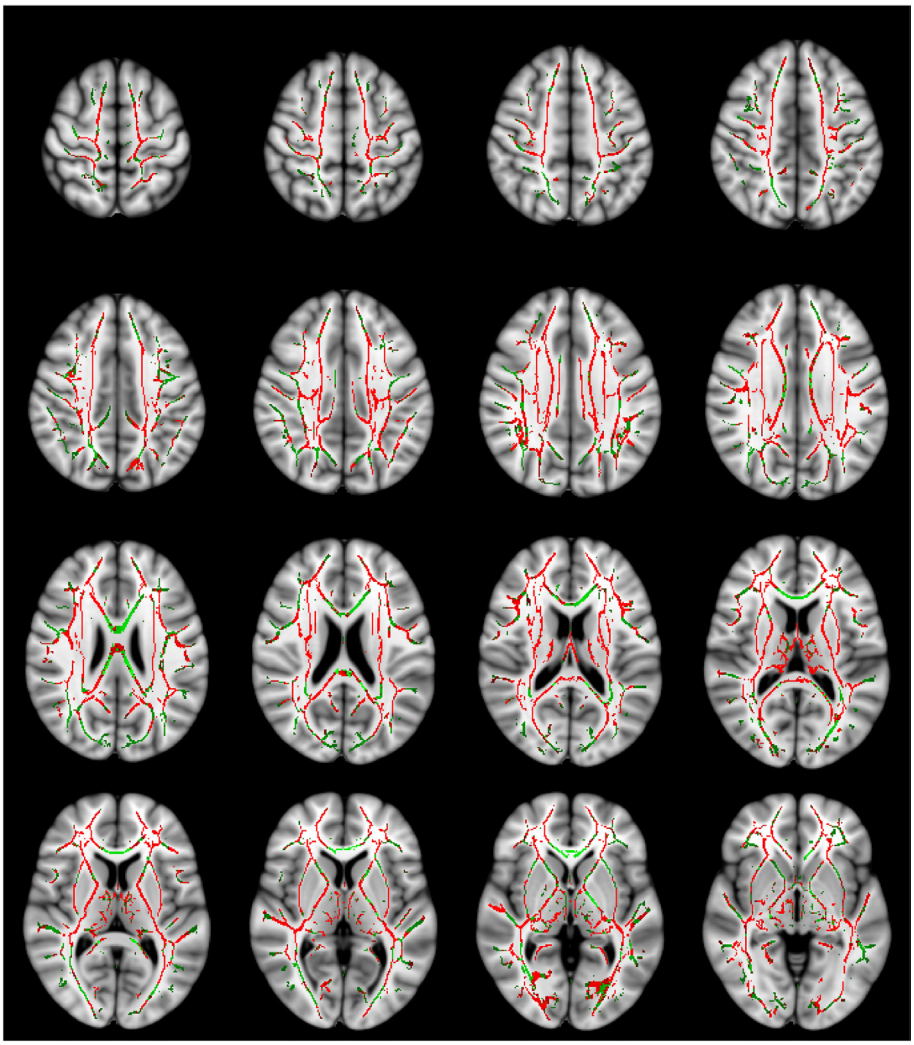

FIGURE 2 | Axial diffusivity (AD) values are significantly higher in the traumatic brain injury (TBI) group with normal-appearing white matter. The AD contrast is overlaid on a standard Montreal Neurological Institute 152 T_1 1 mm brain and the mean fractional anisotropy skeleton (green—display threshold 0.2–0.8). The voxels where mean diffusivity was found to be significantly higher in the TBI group (p-value ≤ 0.05) are shown in red. Every fourth transverse slice only is shown here to approximately cover the entire brain along the superior–inferior axis. Anatomical right side of the brain is shown on the left in this figure.

increase in AD and MD and no difference in FA in our study, therefore, suggest a trend of increased RD values in TBI subjects. Furthermore, AD is thought to be a measure of axonal integrity (61). By extension, our results suggest that the repair of the axonal cytoskeleton, thought to result in rising AD values during recovery (24), happens before the repair of axonal membranes or re-myelination of axons, both of which are thought to restrict perpendicular diffusion and to help normalize increased RD values (62).

Persisting elevated MD values in sub-acute and chronic TBI, encompassing association (e.g., external capsule), projection (e.g., corona radiata, thalamic radiation), and commissural (e.g., corpus callosum) fibers, despite overall NAWM, suggest that microstructural damage or pathological change remains a salient feature of chronic TBI. The significant changes in MD which represent a cumulative effect of AD and RD values are of primary importance, possibly suggesting that MD can be used for assessing the burden of microstructural damage in sub-acute and chronic TBI characterized with NAWM. Our *post hoc* analyses also confirmed these differences.

Our investigation of the relationship between NP function and DTI parameters primarily revealed effects involving FA and RD, and not AD and MD. The absence of interaction effects between NP test scores and DTI parameters implies that positive and negative correlations hold similarly in all subjects regardless of the group status. A significant negative correlation was found between the composite memory score and FA in several white matter tracts. Notably, the negative correlation was found in the structures of the corpus callosum, bilateral external capsule, bilateral posterior thalamic radiations, and bilateral superior longitudinal fasciculi, which constitute either the commissural, projection, or long-range

TABLE 4 | Tracts with significant correlations between diffusion tensor imaging parameters and composite neuropsychological scores.

Structure	*p*-Value
Corpus callosum[a]	0.02 (0.05)
Cerebral peduncle-R	0.03
Internal capsule-R	0.03
Internal capsule-L[a]	0.03 (0.05)
External capsule-R	0.03
External capsule-L	0.03
Corona radiata-R[a]	0.03 (0.05)
Corona radiata-L	0.03
Cingulum-R[a]	0.03 (0.05)
Cingulum-L	0.03
Thalamic radiations Post-R	0.02
Thalamic radiations Post-L	0.02
Longitudinal fasciculus Sup-R[a]	0.02 (0.05)
Longitudinal fasciculus Sup-L	0.02

White matter tracts where fractional anisotropy was found to be negatively correlated with the composite memory score, after correcting for multiple comparisons.
[a]The tracts where a significant positive correlation between radial diffusivity (RD) and the composite visuomotor score was also found. The p-values for significant positive correlations for RD are mentioned in parentheses in the second column.

association fibers. Working and short-term memory is known to be associated with various regions in the frontal, parietal, and anterior-cingulate cortices and basal ganglia (63). The white matter tracts involved in memory are the relatively short-length or U-association fibers connecting these cortical regions. The major white matter tracts that are known to be involved in memory include the fornix (64, 65), which projects to the cingulate cortex, and the internal capsule (66), which connects the cortex with basal ganglia. Furthermore, the DTI literature suggests a positive correlation of FA with function, indicating higher FA (and hence intact structure of axons, with no disruption in myelin or axonal membranes) in subjects with better performance on memory (67) as well as other cognitive tasks (68, 69). Interestingly, our results show a negative correlation of FA in the white tracts which are not directly involved in memory. Keeping in mind the evidence of widespread DAI in the brain, we explain this finding by suggesting that whereas FA increases within the network of interest are correlated with improved performance, the negative correlation of FA in another network or some sub-network might indicate hyperfunction resulting in less effective mental processing and thus a decrement in behavioral performance.

We also found a significant positive correlation between RD and visuomotor performance in the corpus callosum and right aspects of corona radiata, cingulum, and superior longitudinal fasciculus. This means that poor visuomotor coordination (higher scores on the Pegboard task) were associated with elevated RD values. Each of the structures where a positive correlation of RD with visuomotor task performance is found is known to be involved in the visual or motor tasks: the corona radiata are associated with the corticospinal tract, the superior longitudinal fasciculi are involved in integrating the motor and decision-making centers with ipsilateral visual centers (70), and the posterior aspect of cingulum is involved in visual skills. Although other studies have reported tracing of superior longitudinal fasciculi associated with investigation of language networks (71), our study is the first to report the involvement of this structure in visuomotor skills using DTI parameters.

Our study has some limitations, which include the collection of DTI data in only 12 diffusion-weighted directions with coarse resolution, the small sample size, and the inclusion of subjects with all TBI severities. Another limitation is that we did not use susceptibility-weighted imaging (SWI) to detect microstructural abnormalities in the NAWM because the protocol was not available on the MRI scanner at the time of data collection. As SWI is known to be superior to GRE in detecting micro-hemorrhages (72), it can be argued that the differences between the two groups might be influenced by undetected micro-hemorrhages in NAWM, which were not visible on GRE. However, the intergroup differences that were found are well supported by earlier studies (12, 24, 32, 73). The similarity of the effect of NP performance on the DTI parameters across both groups and the lack of interaction between NP performance and group variable also indirectly supports that no gross abnormalities were present in the white matter of subjects with TBI.

Conclusion and Future Directions

Overall, this pilot study shows DTI to be helpful in evaluating the microstructural abnormalities in sub-acute and chronic TBI patients even when there are no apparent abnormalities in the central white matter. Elevated diffusivity values appear to persist well into the chronic stage after TBI, and hence MD can be used to assess persisting microstructural damage in chronic TBI. No clear trend for FA values in post-acute TBI is visible. The study also indicates the potential use of DTI in assessing neurocognitive functions in both TBI patients and HC. The authors recommend larger and longitudinal studies for each of mild, moderate, and severe TBI, to establish the use of DTI parameters as biomarkers of DAI and neurocognitive function in patients with NAWM.

ETHICS STATEMENT

Research Ethics Board, St. Michael's Hospital, Toronto, ON, Canada. M5B 1W8. Telephone: 416-864-6060 extension: 2557. REB # 11-282 (Strategic Teams in Applied Injury Research Protocol). Informed consent was obtained from the participants on a paper form, after a research team member explained the objectives and methods of the study to them.

AUTHOR CONTRIBUTIONS

EH and EC performed the neuroimaging analyses with help from CL and OC. NP provided input for TBSS analyses. RJ helped with the statistical analyses. MC and AB were involved in study design. SZ assisted with patient recruitment and research coordination. EH, EC, NP, and MC wrote the manuscript. All authors participated in discussing the results and editing the manuscript.

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Comparison between Brain Computed Tomography Scan and Transcranial Sonography to Evaluate Third Ventricle Width, Peri-Mesencephalic Cistern, and Sylvian Fissure in Traumatic Brain-Injured Patients

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Introduction: Transcranial color-coded duplex sonography (TCCS) may help guide multimodal monitoring in the neurocritical setting. It may provide indirect information about intracranial hypertension, such as midline shift, third ventricle width, and peri-mesencephalic cistern obliteration. We aim to assess the agreement between brain computed tomography scan (CT scan) and TCCS in traumatic brain injury (TBI) patients.

Methods: In this retrospective cross-sectional observational study, TCCS was performed within 6 h before a brain CT scan. Only the first CT and TCCS after ICU admission were included. The agreement between the CT scan and TCCS was assessed by Bland–Altman plots and evaluating the intraclass correlation coefficient.

Results: Overall, 15 consecutive patients were included (80% male, 42 ± 23 years of age, Glasgow Coma Score 5 [4,6]). The mean difference between the brain CT scan and TCCS in measuring the midline shift was 0.30 ± 2.1 mm (intraclass correlation coefficient: 0.93; $p < 0.01$). An excellent correlation was also observed between the methods in assessing the third ventricle width (intraclass correlation coefficient: 0.88; $p < 0.01$). Bland–Altman plots did not show any systematic bias in either agreement analysis. TCCS showed good accuracy in predicting non-compressed peri-mesencephalic cisterns (AUC: 0.83, 95% CI 0.46–1.0) and the presence of the Sylvian fissure (AUC: 0.91, 95% CI 0.73–1.0) on CT scan.

Conclusion: TCCS is a promising tool and may be an alternative to CT scans for evaluating TBI patients.

Keywords: traumatic brain injury, transcranial color-coded sonography, midline shift, perimesencephalic cisterns, sylvian fissure

INTRODUCTION

Severe traumatic brain injury (TBI) remains an important cause of death and severe disability in adults. Thus, serial computed tomography (CT) brain scan imaging plays a crucial role in monitoring patients and helping guide intensive care management in the acute TBI phase (1). In addition to the identification of neurosurgical lesions, brain CT scanning provides important information about intracranial pressure features, such as midline structural shifts, peri-mesencephalic cisterns, and third ventricle widths (2).

In addition to brain CT scans, transcranial color-coded sonography (TCCS) has been commonly applied in neurocritical care scenarios as a valuable tool to monitor acute brain-injured patients because of its non-invasive feature and bedside application. TCCS has been widely used as a standard technique to evaluate the cerebral blood flow velocity of the intracranial arterial system, mainly in the acute stroke setting (3–6). However, TCCS also allows an accurate description of the cerebral anatomy, including hematomas or ventricular enlargements, in patients with intact skulls. Additionally, several authors demonstrated that midline structural displacement and hyperdense lesions could be accurately measured by TCCS compared to brain CT scanning in acute brain-injured patients, with either intact skulls or decompressive craniectomy (7).

However, it is unknown whether TCCS is able to assess features usually seen in brain CT scans to evaluate intracranial hypertension, such as compressed peri-mesencephalic cisterns, third ventricle obliteration, and Sylvian fissure effacement (8). Thus, considering the potential role of TCCS to evaluate intracranial hypertension in TBI, we hypothesized that this technique could be used as an adjuvant neuroimaging methodology in scenarios where the patient cannot be transported to the CT room or if CT is not available.

Thus, in this study, we aimed to evaluate the agreement between cerebral CT scanning and TCCS to visualize third ventricle widths, Sylvian fissure effacement, and peri-mesencephalic cistern compression in severe traumatic brain-injured patients.

MATERIALS AND METHODS

We performed a cross-sectional retrospective observational study at the 18-bed Surgical Emergencies and Trauma Intensive Care Unit of the General Hospital of the University of São Paulo Medical School. The data were collected between July 2015 and October 2015. All consecutive patients with severe TBI admitted to the ICU were evaluated during this period. The hypotheses were generated before data analysis and after data collection. The ethics committee approved the study (CAPPesq), and written informed consent was waived because of the study's rigorous observational design.

Only the first brain CT scan and TCCS of each patient was included. All exams were performed conforming to medical decisions and within 24 h after ICU admission. The Marshall classification was used to assess the severity of the injury (9). An experienced neuro-intensive care physician who was blinded to the TCCS results evaluated the CT scans. TCCS was performed within 6 h before the CT scan by an experienced single-operator using a Sonosite® device with a 2.5 MHz phased array transducer probe with the transcranial Doppler setting through the transtemporal acoustic bone window (axial plane), as described by Seidel (10). Initially, the mesencephalon was tracked with a classic "butterfly-wing" structure (mesencephalic plane). We supposed that well-defined mesencephalon margins on TCCS denoted the peri-mesencephalic cisterns clearly seen on CT scan, which would indicate a non-compressed state (**Figure 1**). Later, the probe was moved 10° upward to locate the third ventricle with its hyperechogenic margins and the surrounding hypoechogenic talami and posterior hyperechogenic pineal gland (diencephalic plane). We also observed the Sylvian fissure on this plane and characterized it as the hyperechogenic structure perpendicular to the probe on the contralateral side, nearly to the skull (**Figure 2**). The third ventricle width was measured in the axial plane between its hyperechogenic margins. We presumed that in cases of high cerebral complacency, we would observe the presence of the Sylvian fissure on TCCS, as is normally seen on brain CT scans in this scenario. Furthermore, the presence of the third ventricle on TCCS was interpreted as indicating a normal cerebral complacency status.

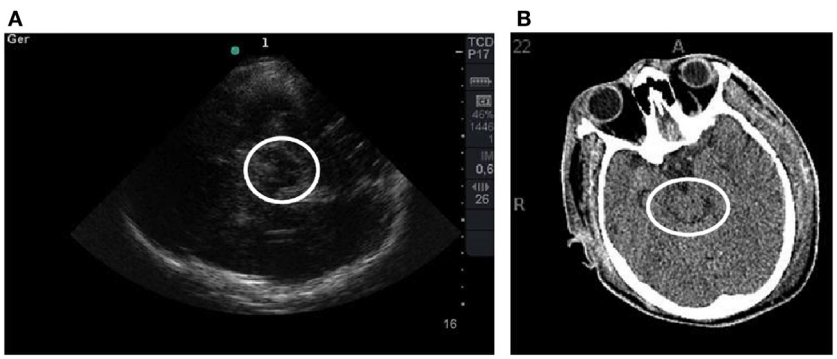

FIGURE 1 | **(A)** TCCS image shows the mesencephalic plane with a "butterfly-wing" structure surrounded by a white circle. Note the hyperechogenic margins. **(B)** Corresponding tomographic imaging is shown.

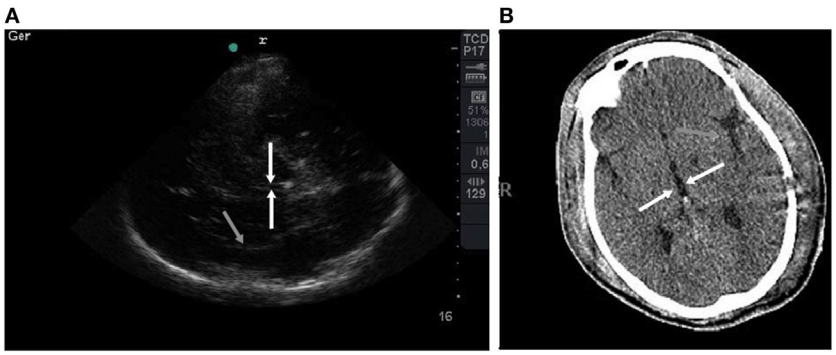

FIGURE 2 | **(A)** TCCS image shows the diencephalic plane with third ventricle with hyperechogenic margins emphasized by white arrows. The Sylvian fissure is shown highlighted by a gray arrow. **(B)** Corresponding tomographic imaging is shown.

The distance between the probe and the center of the third ventricle was measured along a line perpendicular to the walls of the third ventricle from both the ipsilateral and contralateral sides, called the *A* and *B* distances, respectively. The midline shift was calculated by $(A-B)/2$ (11).

Clinical data such as age, gender, Simplified Acute Physiology Score 3 (SAPS 3), Glasgow Coma Score, TCCS results, and hospital mortality were extracted from our electronic database to perform a baseline analysis.

Statistical Analysis

Parametric variables were expressed as the mean (SD), and non-parametric variables were expressed as the median [IQ]. Inferential analysis was performed to evaluate the agreement between the CT scan and TCCS in measuring the midline shift and third ventricle width. The agreement between the techniques was evaluated by analyzing the intraclass correlation coefficient (>0.75 was considered a good correlation) and Bland–Altman plots (12). The area under the curve, specificity, and sensitivity were analyzed to evaluate the performance of TCCS in predicting non-compressed peri-mesencephalic cisterns and the presence of the Sylvian fissure compared to the CT scan. The correlation between parametric variables was evaluated using Pearson's correlation. $p < 0.05$ was considered statistically significant. Statistical analyses were conducted using SPSS 19 (SPSS Inc., Chicago, IL, USA) and the R Project.

RESULTS

Fifteen consecutive severe traumatic brain-injured patients with intact skulls were evaluated. The mean age was 42 ± 23 years, and 80% were male. The SAPS 3 score was 56 ± 12. The Glasgow coma score at the trauma scene after initial stabilization was 5 [4,6]. The observed hospital mortality rate was 53%. The individual characteristics at baseline are presented in **Table 1**.

An excellent correlation was observed between CT scanning and TCCS concerning midline structural shifts (*b*: 0.978, $p < 0.01$) and third ventricle widths (*b*: 0.85, $p = 0.01$). The mean difference between the CT scan and TCCS was −0.308 [95% confidence interval (CI), −4.42–3.80, $p = 0.57$] and −0.08 (95% CI, −14.97–14.81, $p = 0.96$) for the measure of midline structure shift and third ventricle width, respectively. The agreement between the methods for both measures was excellent (**Table 2**), and no systematic bias was observed on the Bland–Altman plot (**Figure 3**).

TCCS showed a good performance in predicting non-compressed peri-mesencephalic cisterns (AUC: 0.83, 95% CI 0.46–1.0), with 100% sensitivity and 50% specificity (**Figure 4**). TCCS also presented the same performance in predicting the

TABLE 1 | Individual characteristics at baseline and outcome.

Patient	Age, years	Gender	Simplified Acute Physiology Score 3	GCS[a]	Marshall CT	Hospital mortality
1	27	F	63	7	III	Yes
2	22	M	48	4	III	Yes
3	15	M	53	5	V	Yes
4	32	F	51	5	V	Yes
5	79	M	67	5	V	Yes
6	23	M	82	3	III	No
7	90	F	65	5	V	No
8	42	M	54	8	II	No
9	52	M	41	3	V	No
10	20	M	56	4	V	No
11	51	M	68	6	VI	Yes
12	38	M	40	5	II	No
13	67	M	66	8	V	No
14	61	M	67	4	V	Yes
15	22	M	42	6	III	Yes

[a]*Glasgow coma score at hospital admission.*

TABLE 2 | Results of comparison between TCCS and brain computed tomography scan (CT scan).

Variable	TCCS[a]	Computed tomography	Intraclass correlation (95% CI)	*p*
Shift medium line, mm	3.6 ± 4.5	3.3 ± 4.17	0.93 (0.81, 0.98)	<0.01
Third ventricle width, mm	35.5 ± 12	33.1 ± 14	0.88 (0.63, 0.96)	<0.01

[a]*Transcranial color-coded sonography.*

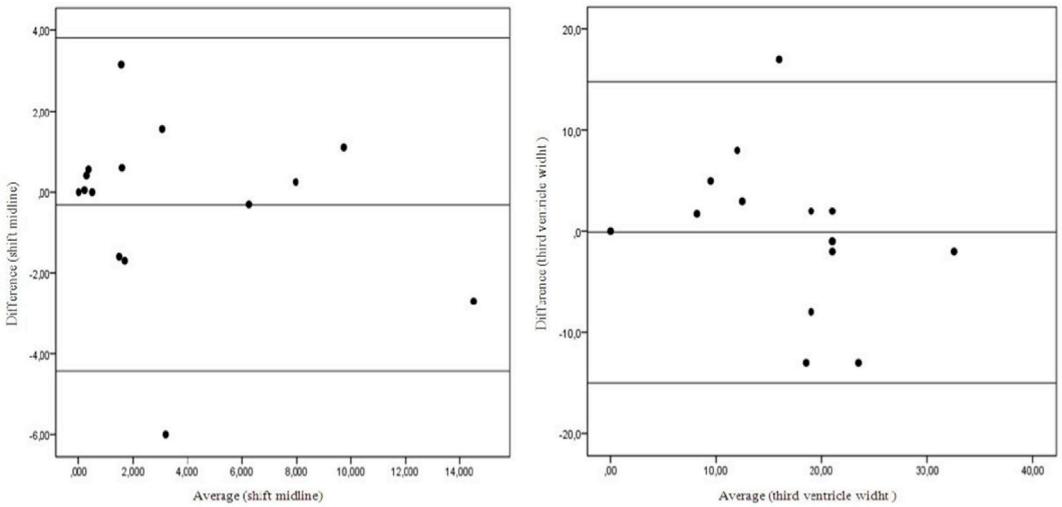

FIGURE 3 | Bland–Altman plot shows measurement agreement between TCCS and brain computed tomography scan (CT scan).

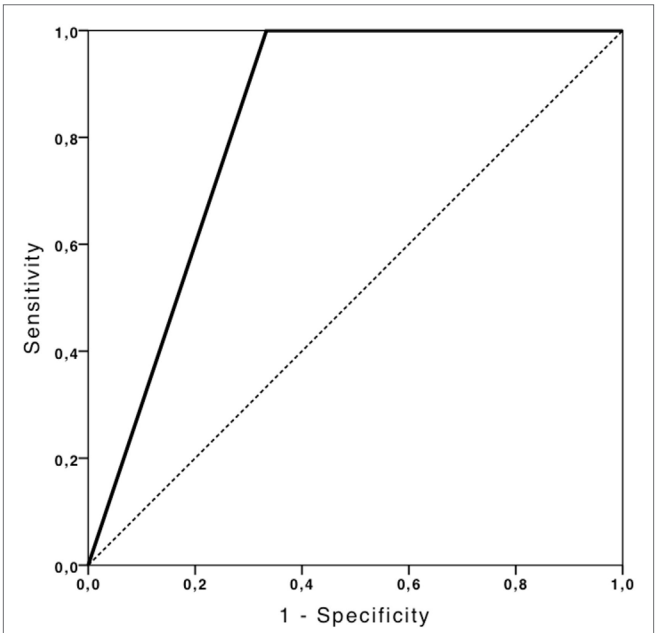

FIGURE 4 | ROC curve depicting the ability of TCCS to predict non-compressed peri-mesencephalic cisterns.

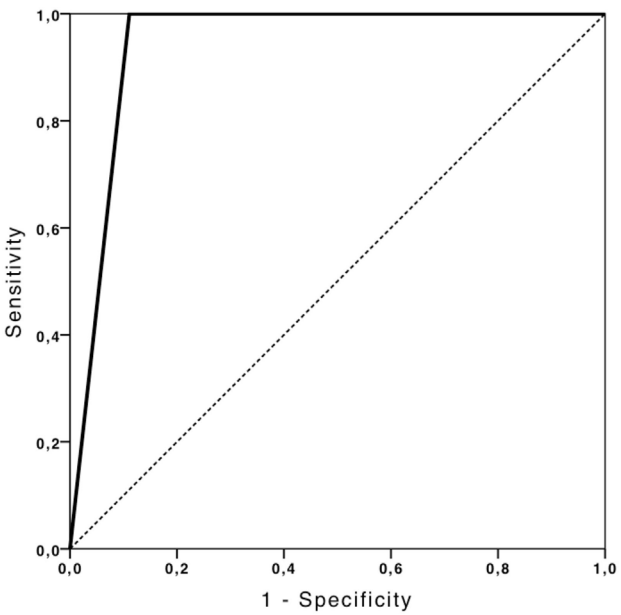

FIGURE 5 | ROC curve depicting the ability of TCCS to predict the presence of Sylvian fissure.

presence of the Sylvian fissure (AUC: 0.91, 95% CI 0.73–1.0), with 83% sensitivity and 100% specificity (**Figure 5**).

DISCUSSION

In this study, TCCS showed an excellent performance in predicting non-compressed peri-mesencephalic cisterns and the presence of the Sylvian fissure compared to brain CT scanning. We also observed a good correlation and agreement between TCCS and CT scanning regarding the shift of midline structures and the third ventricle width.

Recently, TCCS has been used increasingly frequently in the ICU environment because of its non-invasive characteristic, and it has become a reliable tool for assessing the brain parenchyma and cerebral vasculature in patients with an adequate acoustic window (13). Some authors have demonstrated that midline shifts could be reliably evaluated non-invasively at bedside in patients with intact skulls and with decompressive craniectomy (7). It has brought an opportunity to develop new forms of imaging for neuromonitoring without the risks of serial CT scans, especially in the transport of patients to the CT room.

In this study, we evaluated the TCCS ability to measure and predict some important findings that are usually seen only on brain CT scans. We observed an excellent correlation between the methods in measuring midline structure shifts, as demonstrated previously (11), and an excellent correlation in measuring the third ventricle width. This is an important finding and suggests some evidence about the cerebral complacency status because its absence could be interpreted as a high elastance cerebral system.

Another relevant and outstanding finding is the good performance in predicting non-compressed peri-mesencephalic cisterns, with high sensitivity (100%) and median specificity (50%). Usually, the peri-mesencephalic cistern's obliteration on brain CT scan is interpreted as a strong predictor of intracranial hypertension. Thus, TCCS could lead to obtaining valuable knowledge at the bedside regarding the intracranial pressure status. Analogously, the excellent performance of TCCS in predicting the presence of the Sylvian fissure promotes the same information regarding good cerebral complacency, with 100% specificity and 83% sensitivity. To our knowledge, this is the first study to show the ability of TCCS in predicting peri-mesencephalic cisterns and Sylvian fissure status in neurocritical care patients.

This study has several limitations. First, the small number of patients could compromise the interpretation of the results. Second, although we did not identify any inadequate acoustic windows in our sample, it has been reported in 5–18% of patients (14, 15), which could be a limitation of our findings' reproducibility. Finally, cerebral blood flow velocity and pulsatility index, which could be used as potential intracranial hypertension surrogates, were not evaluated because they were missing on our electronic database.

Thus, our findings showed that TCCS affords valuable information at the bedside in the neurocritical care setting. Its good accuracy in evaluating parameters that are surrogates for intracranial hypertension provides the much-needed possibility to manage severe traumatic brain-injured patients without the risks associated with patient transport and radiation dosage with a serial CT scan approach. Although we believe that more data are necessary to validate our results, the harmless and non-invasive nature of TCCS provides the feasibility to add it to the imaging armamentarium for the management of severe TBI.

ETHICS STATEMENT

The study was approved by the *Comissão de Ética para Análise de Projetos de Pesquisa – CAPPesq*.

AUTHOR CONTRIBUTIONS

All authors listed, have made substantial, direct and intellectual contribution to the work, and approved it for publication.

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ORIGINAL RESEARCH
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Diffuse Axonal Injury: Epidemiology, Outcome and Associated Risk Factors

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Diffuse axonal injury (DAI), a type of traumatic injury, is known for its severe consequences. However, there are few studies describing the outcomes of DAI and the risk factors associated with it. This study aimed to describe the outcome for patients with a primary diagnosis of DAI 6 months after trauma and to identify sociodemographic and clinical factors associated with mortality and dependence at this time point. Seventy-eight patients with DAI were recruited from July 2013 to February 2014 in a prospective cohort study. Patient outcome was analyzed using the Extended Glasgow Outcome Scale (GOS-E) within 6 months of the traumatic injury. The mean Injury Severity Score was 35.0 (SD = 11.9), and the mean New Injury Severity Score (NISS) was 46.2 (SD = 15.9). Mild DAI was observed in 44.9% of the patients and severe DAI in 35.9%. Six months after trauma, 30.8% of the patients had died, and 45.1% had shown full recovery according to the GOS-E. In the logistic regression model, the severity variables – DAI with hypoxia, as measured by peripheral oxygen saturation, and hypotension with NISS value – had a statistically significant association with patient mortality; on the other hand, severity of DAI and length of hospital stay were the only significant predictors for dependence. Therefore, severity of DAI emerged as a risk factor for both mortality and dependence.

Keywords: head trauma, diffuse axonal injury, Glasgow Outcome Scale, recovery, severe traumatic brain injury, cohort study

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INTRODUCTION

Diffuse axonal injury (DAI), the microscopic damage to the axons in the brain neural tracts, corpus callosum, and brainstem, is associated with significant mortality and morbidity. The occurrence of DAI depends on the mechanism of injury; it is more common in higher energy trauma, especially traffic accidents (1–3).

Diffuse axonal injury is clinically defined by coma lasting 6 h or more after traumatic brain injury (TBI), excluding cases of swelling or ischemic brain lesions (2). DAI is considered the most important factor in determining morbidity and mortality in victims of TBI and is the most common cause of posttraumatic coma, disability, and a persistent neurovegetative state (1, 2).

Diffuse axonal injury causes cognitive, physical, and behavioral changes that compromise social reintegration, return to productivity, and quality of life of patients and their families (1–10). These changes persist beyond the acute phase of treatment and continue for a long period after the traumatic

event. Because the brain tissue is functionally impaired but not destroyed, the brain may gradually regain normal function as the clinical condition stabilizes and neural connections are remodeled due to plasticity (9–12).

According to some authors (7, 9, 10, 13), understanding the variables associated with recovery after TBI is needed for the development of individualized therapy, the evaluation of care provided, and the development of systematic care focused on patient rehabilitation. It is also important for the evaluation of the efficacy of new techniques and treatments, as these should result in better outcome and survival.

Diffuse axonal injury, and more generally TBI, often results in physical, cognitive, and behavioral impairments that can be temporary or permanent (1–10). Research on outcome after TBI, using scales of function and performance in activities of daily living (ADLs), depicts the individual and social consequences of these changes suffered by patients after TBI.

The outcome of patients after DAI has been linked to the number of lesions identified through imaging. A longitudinal study that analyzed the evolution of traumatic axonal injury using magnetic resonance imaging (MRI) of 58 patients with moderate or severe TBI showed that the greater the number of lesions observed early after trauma, the greater the impairment of functionality after 12 months (14). A study of 26 DAI patients (15) indicated that the volume and number of lesions identified by MRI performed within 48 h of hospital admission strongly correlated with the level of disability observed at the time of hospital discharge.

Few studies on DAI patients have focused on the clinical and sociodemographic factors associated with outcome and mortality. Thus, the aim of this study was to describe the outcome of patients with primary diagnosis of DAI and to identify clinical and sociodemographic factors associated with mortality and functional capacity 6 months after the injury.

MATERIALS AND METHODS

Patients

This was a prospective cohort study, with data collected at the time of hospital admission and 6 months after DAI in the Neurosurgical Outpatient Clinic and Trauma at the Central Institute of the Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (IC/HCFMUSP) in São Paulo, Brazil.

Seventy-eight patients with DAI admitted to the IC/HCFMUSP from July 2013 to February 2014 were enrolled in the study. Patients eligible for the study had Glasgow Coma Scale (GCS) scores of ≤8 at admission, were between 18 and 60 years old, and had a computed tomography (CT) scan showing either normal outcome or signs of DAI. DAI was confirmed by signs of injury identified in CT or MRI scans by neurosurgeons experienced with this type of injury. Cases with GCS ≤8, and without MRI and normal CT were also diagnosed as DAI (16–21). The study criteria excluded patients who were admitted to these hospitals more than 6 h after trauma, who were transferred from other hospitals with previous diagnosis of TBI, or who had psychiatric disorders or other injuries to the head or spinal cord with a severity score ≥3, according to the Abbreviated Injury Scale (21).

For each patient, the following data were recorded:

- Sociodemographic characteristics: age, sex, marital status, race, and occupational status.
- Characteristics related to trauma and pre-hospital care (PHC): traffic accident, type of PHC, orotracheal intubation (OTI) at the scene, and alcohol intake.
- Characteristics related to admission: sedation, respiratory rate (RR), hypotension, bradycardia, tachycardia, hypoxia by peripheral oxygen saturation (SpO_2), hypoxia by partial pressure of oxygen (PO_2), hypoglycemia, hyperglycemia, and pupillary abnormalities.
- Characteristics related to hospital stay: intensive care unit (ICU) stay, use of continuous sedation, treatments with drugs that act on the central nervous system (CNS), surgery, second surgery, infection, other complications, early DAI signs in CT, intracranial pressure (ICP) monitoring, intracranial hypertension (ICH), hypotension, hypertension, hypothermia, hyperthermia, hypoglycemia, and hyperglycemia.

The secondary systemic injuries recorded for each patient during admission and hospital stay are as follows:

- RR: normal 10–29 breaths per minute and RR changes <10 or >29 breaths per minute.
- Hypotension: systolic blood pressure <90 mmHg;
- Hypertension: systolic blood pressure ≥160 mmHg;
- Bradycardia: heart rate variability <50 beats per minute;
- Tachycardia: heart rate variability >100 beats per minute;
- Hypoxia by SpO_2: SpO_2 <90%;
- Hypoxia by PO_2: PO_2 <60%;
- Hypoglycemia: glycemia <70 mg/dL;
- Hyperglycemia: glycemia >160 mg/dL;
- Hypothermia: axillary temperature ≤35°C;
- Hyperthermia: axillary temperature ≥38°C;
- Early DAI signs in CT: based on cranial CT scan in the first 72 h of hospital admission, this included individuals with intraventricular hemorrhage, subarachnoid hemorrhage, gliding contusion, or diffuse swelling with deletions of the basilar cisterns or grooves (indirect signs of injury) (16–21).

Procedure

Sociodemographic data, clinical variables related to trauma, PHC, admission and hospitalization details, and variables related to the severity and consequences of DAI were collected for all of the patients. During the 6-month follow-up interview, sociodemographic data and data related to the traumatic event were confirmed, and information on the functional outcome of victims was recorded using the Extended Glasgow Outcome Scale (GOS-E).

Trauma severity was estimated with the Injury Severity Score (ISS) (22) and the New Injury Severity Score (NISS) (23). The Maximum Abbreviated Injury Scale (MAIS), referring to the head region (MAIS-Head) (21), was used to characterize the severity of TBI.

For DAI severity, Gennarelli's clinical classification, rating diffuse lesions as mild, moderate, or severe, was applied (1, 2). In mild DAI, coma lasts 6–24 h. In moderate DAI, coma lasts longer than 24 h but without abnormal posturing. In severe cases of DAI, coma duration is longer than 24 h, and signs of brainstem impairment can also be observed (1, 2). Patients were considered to have awakened from the coma when they scored 6 on the best motor response (BMR) in the GCS.

To evaluate the functional outcome, GOS-E was applied, encompassing seven categories: upper good recovery, lower good recovery, upper moderate disability, lower moderate disability, upper severe disability, lower severe disability, and persistent vegetative state. For the patients alive at 6 months after trauma, level of dependence was determined according to the criteria of this scale; patients included in the categories of upper good recovery, lower good recovery, upper moderate disability, and lower moderate disability were grouped as independent, whereas those with upper severe disability, lower severe disability, or in a persistent vegetative state were classified as dependent (24, 25).

The study was approved by the Research Ethics Committee of the Escola de Enfermagem da Universidade de São Paulo and of the Escola de Medicina da Universidade de São Paulo (certificate of submission to ethics review number 14115513.1.3001.0068). All participants freely consented to participation and signed the informed consent form. Written and informed consent was given by all participants who were clinically able to do so; otherwise, the forms were signed by their legal representatives.

Statistical Analysis

The information related to this investigation was stored in a computerized database in the Statistical Package for Social Sciences software version 17.0 (SPSS®, IBM).

To identify associations between the variables of interest and the outcomes of mortality and dependence 6 months after trauma, comparisons were made between groups of individuals who died or survived and between those who were dependent or independent, as determined by the GOS-E. In these comparisons, Pearson's chi-square and Fisher's exact tests were applied for categorical and numerical variables, respectively. Both discrete and continuous numerical variables were compared using Student's *t*-test.

Multiple logistic regression analysis with stepwise forward method was performed on the variables associated with risk factors for mortality and dependence. Separate models were created with ISS and NISS for these variables, as ISS and NISS estimate the overall severity of the trauma and thus display multicollinearity problems. During modeling, the final model used only those variables that showed statistical significance in the logistic regression model ($p \leq 0.05$).

RESULTS

Between July 2013 and February 2014, 78 patients with DAI admitted to IC/HCFMUSP met the inclusion criteria of the study and participated in the survey at the hospital admission stage. Of these patients, 24 (30.8%) died during the following 6 months, 51 (65.4%) were evaluated 6 months after DAI, and 3 (3.8%) withdrew from the study after hospital discharge.

Table 1 shows that the vast majority of the study participants were male (89.7%) and employed at the time of the injury (89.7%). The sample consisted mostly of young people between 18 and 28 years of age (43.6%), and the mean age of patients was 32 years (SD = 11.2). Participants who did not complete primary education only constituted 48.7% of the sample, and the average length of education was 9.1 years (SD = 9.1). Most of the trauma victims were white (65.4%) and single (51.3%); 73.0% had monthly per capita family income between one and five times the minimum wage, with an average income of R$1,290.98 (SD = R$2,282.64).

As shown in **Table 2**, the main cause of DAI in this study was traffic accidents, with motorcyclists being the largest group of

TABLE 1 | Comparisons between patient conditions (dead or alive, independent or dependent) at 6 months after diffuse axonal injury (DAI) in relation to sex, race, marital status, and occupational status at the time of trauma.

	Survival			GOS-E		
Sociodemographic characteristics	**No** ***n*** **(%)**	**Yes** ***n*** **(%)**	***p*-Value**	**Independent** ***n*** **(%)**	**Dependent** ***n*** **(%)**	***p*-Value**
Sex						
Male	22 (91.7)	48 (88.9)	>0.999	40 (88.9)	5 (83.3)	0.548
Female	2 (8.3)	6 (11.1)		5 (11.1)	1 (16.7)	
Marital status						
Single	12 (50.0)	28 (51.9)	0.971	23 (51.1)	4 (66.7)	0.659
Married	10 (41.7)	21 (38.9)		18 (40.0)	2 (33.3)	
Separated	2 (8.3)	5 (9.2)		4 (8.9)	–	
Race						
White	16 (66.7)	35 (64.8)	0.874	29 (64.4)	4 (66.7)	>0.999
Black	8 (33.3)	19 (35.2)		16 (35.6)	2 (33.3)	
Occupational status						
Employed	20 (83.3)	50 (92.6)	0.242	42 (93.3)	6 (100)	>0.999
Unemployed	4 (16.7)	4 (7.4)		3 (6.7)	–	

HCFMUSP, 2013–2014.
GOS-E, Extended Glasgow Outcome Scale.

TABLE 2 | Comparisons between patient conditions (dead or alive, independent or dependent) at 6 months after DAI in relation to characteristics related to trauma and pre-hospital care (PHC).

	Survival			GOS-E		
Characteristics related to trauma and PHC	**No** ***n*** **(%)**	**Yes** ***n*** **(%)**	***p*-Value**	**Independent[a]** ***n*** **(%)**	**Dependent[a]** ***n*** **(%)**	***p*-Value**
Traffic accident						
Yes	20 (83.3)	45 (83.3)	>0.999	36 (80.0)	6 (100.0)	0.575
No	4 (16.7)	9 (16.7)		9 (20.0)	–	
Type of PHC						
Air	11 (45.8)	26 (48.1)	0.850	22 (48.9)	2 (33.3)	0.671
Land	13 (54.2)	28 (51.9)		23 (51.1)	4 (66.7)	
OTI at the scene						
Yes	18 (75.0)	44 (83.0)	0.535	38 (86.4)	4 (66.7)	0.242
No	6 (25.0)	9 (17.0)		6 (13.6)	2 (33.3)	
Alcohol intake						
Yes	8 (33.3)	25 (46.3)	0.285	20 (44.4)	2 (33.3)	0.688
No	16 (66.7)	29 (53.7)		25 (55.6)	4 (66.7)	

HCFMUSP, 2013–2014.

[a]Excludes 1 case without information.

OTI, orotrachial intubation.

trauma victims (43.6%) in those events, followed by car occupants (25.6%). A large proportion of the patients (42.3%) referred to alcohol intake in the period immediately preceding the trauma event. All participants were transported to the hospital by pre-hospital emergency care services, with a significant proportion of air transport use (47.4%) for the victims. Individuals in a coma (GCS $\leq$ 8) constituted 75.7% of trauma victims, with 79.5% of survey participants intubated at the scene of the incident.

Regarding the severity of the trauma, ISS ranged from 17 to 75, with a mean of 35 (SD = 11.9) and median of 33. As for the distribution of the victims in the three categories of severity, there were no patients with mild trauma (ISS < 16), 19.2% with moderate trauma ($\geq$16 and <25), and the majority had severe trauma (ISS $\geq$ 25). According to NISS, almost all of the victims (91.0%) had severe trauma (NISS $\geq$ 25). The average for this index was 46.2 (SD = 15.9), and the median was 43, ranging from 18 to 75.

All DAI patients had MAIS score $\geq$4 in the head region, with an average of 4.6 (SD = 0.5). Critical injuries, i.e., MAIS score = 5, were found in 55.1% of the sample. Mild DAI was observed in 44.9% of the victims, moderate DAI in 19.2%, and severe DAI in 35.9%.

The clinical conditions of the victims on arrival at IC/HCFMUSP are shown in **Table 3**. RR was altered in 10.3% of the sample, with tachypnea as the most frequent change (6.4%). Hypoxia was detected by SpO_2 measurement in 15.3% (SpO_2 < 90%) and by PO_2 in 14.1% (PO_2 < 60%) of the trauma victims. Of the study participants, 19.2% had an SBP <90 mmHg, 2.5% experienced cardiac arrest, and most (52.5%) had tachycardia at this stage of treatment. Glycemic alterations were found in 32.0% of the sample, with hyperglycemia being the most frequent change (28.2%). All patients had a GCS score $\leq$8, as this was an inclusion criterion, and 60.3% had pupillary changes.

As shown in **Table 4**, almost all of the victims were hospitalized in the ICU (92.3%), with an average length of stay in this unit of 11.7 days (SD = 15.4) and a median of 7 days (range: less than 1–109 days). Of all patients, 69 (88.5%) were continuously sedated for an average of 4.1 days (SD = 4.3) and median of 3 days (ranging from less than 1–18 days). Fentanyl and propofol, used in 94.2 and 92.7% of sedated patients, respectively, were the most prescribed drugs for sedation. Most patients (61.5%) were treated with drugs that act on the CNS without the purpose of sedating. Among those, the most common drug types were anticonvulsants (83.3%), neuroleptics (70.8%), and benzodiazepines (25.0%).

After hospital admission, DAI patients took on average 3.7 days (SD = 7.2) to achieve a score of 6 on the BMR item of the GCS. The median time was 1 day, ranging from less than 24 h to 32 days. However, at the 6-month follow-up, 23 patients (29.5%) did not reach a score of 6 on the BMR item of the GCS; 22 died before reaching that score, and 1 was in a persistent vegetative state until the end of the evaluation period.

As shown in **Table 4**, during hospitalization, most patients (53.8%) underwent surgery, and 19.2% had second surgery. Infections were recorded in 25.6% of cases and other complications in 52.6% of patients. Most of the victims showed early signs of DAI in the CT (70.5%), and ICH after DAI was detected in 24.2% of the patients, although ICP monitoring was performed in only three patients (3.8%).

In this study, the average hospital stay of patients was 19.1 days (SD = 22.9), and the median stay was 11 days (range: less than 1–111 days). Mortality at 6 months after DAI was 30.8%; among those who died, the average survival was 13.5 days (SD = 24.1), and the median survival was 4.5 days (range: less than 1–110 days). Most patients (63.0%) went home after discharge from IC/HCFMUSP; the others required hospitalization in other hospitals for continued care.

Outcome at 6 Months

Six months after the DAI, according to the GOS-E, a large portion (45.1%) of these patients reached upper good recovery, and 25.5% showed lower good recovery. Individuals that were classified as "independent but disabled" amounted to 16.6% of the sample

TABLE 3 | Comparisons between patient conditions (dead or alive, independent or dependent) at 6 months after DAI in relation to characteristics at hospital admission.

	Survival			GOS-E		
Characteristics related to admission	**No** ***n*** **(%)**	**Yes** ***n*** **(%)**	***p*-Value**	**Independent** ***n*** **(%)**	**Dependent** ***n*** **(%)**	***p*-Value**
Sedation						
Yes	22 (91.7)	50 (92.6)	>0.999	41 (91.1)	6 (100.0)	>0.999
No	2 (8.3)	4 (7.4)		4 (8.9)	–	
RR	[a]	[a]		[c]	[c]	
Normal	19 (82.6)	45 (91.8)	0.257	38 (92.7)	5 (83.3)	0.432
Altered	4 (17.4)	4 (8.2)		3 (7.3)	1 (16.7)	
Hypotension						
Yes	11 (45.8)	4 (7.4)	**<0.001**	4 (8.9)	–	>0.999
No	13 (54.2)	50 (92.6)		41 (91.1)	6 (100.0)	
Bradycardia						
Yes	2 (8.3)	–	0.092	–	–	–
No	22 (91.7)	54 (100.0)		45 (100.0)	6 (100.0)	
Tachycardia						
Yes	15 (62.5)	26 (48.1)	0.241	23 (51.1)	3 (50.0)	>0.999
No	9 (37.5)	28 (51.9)		22 (48.9)	3 (50.0)	
Hypoxia by SpO_2 [b]						
Yes	9 (37.5)	3 (5.7)	**0.001**	3 (6.7)	–	>0.999
No	15 (62.5)	50 (94.7)		42 (93.3)	5 (100.0)	
Hypoxia by PO_2	[c]	[c]		[e]	[e]	
Yes	3 (12.5)	8 (16.0)	>0.999	6 (14.3)	2 (33.3)	0.258
No	21 (87.5)	42 (84.0)		36 (85.7)	4 (66.7)	
Hypoglycemia	[d]	[d]		[c]	[c]	
Yes	1 (4.3)	2 (4.2)	>0.999	2 (4.9)	–	>0.999
No	22 (95.7)	46 (95.8)		39 (95.1)	6 (100.0)	
Hyperglycemia	[d]	[d]		[c]	[c]	
Yes	12 (52.2)	10 (20.8)	**0.008**	9 (22.0)	1 (16.7)	>0.999
No	11 (47.8)	38 (79.2)		32 (78.0)	5 (83.3)	
Pupillary abnormalities						
Yes	21 (87.5)	26 (48.1)	**0.001**	21 (46.7)	4 (66.7)	0.419
No	3 (12.5)	28 (51.9)		24 (53.3)	2 (33.3)	

HCFMUSP, 2013–2014.

[a]*Excludes 6 cases without information.*

[b]*Excludes 1 case without information.*

[c]*Excludes 4 cases without information.*

[d]*Excludes 7 cases without information.*

[e]*Excludes 3 cases without information.*

RR, respiratory rate; SpO_2, peripheral oxygen saturation; PO_2, partial pressure of oxygen.

Bold numbers correspond to significant p values.

(9.8% upper moderate disability and 7.8% lower moderate disability); six subjects were classified as disabled and dependent (11.8%), with three ranked as having upper severe disability, two as lower severe disability, and one was in a persistent vegetative state. Thus, the vast majority of patients (88.2%) achieved recovery consistent with independent life at 6 months after DAI, while those severely disabled and dependent constituted a small proportion (11.8%; **Figure 1**).

Factors Associated With Mortality

To identify factors associated with mortality within 6 months of the DAI, the 78 patients admitted to IC/HCFMUSP were grouped according to their vital state (dead or alive) in this period. In the analysis of the variables that reflected the severity of the trauma and TBI, as measured by the ISS, NISS, MAIS-Head, severity of DAI, and the presence of hypotension, statistically significant differences were observed for all variables ($p < 0.001$). **Table 5** shows that death was the most frequent outcome among patients with severe DAI; out of 28 patients with severe DAI, 22 died (78.6%).

In **Table 3**, a statistically significant difference is apparent between the groups (dead and alive) for the presence of hypotension ($p < 0.001$), hypoxia by SpO_2 ($p = 0.001$), hyperglycemia ($p = 0.008$), and pupillary changes ($p = 0.001$). The values of the GCS assigned to DAI patients at admission were similar between the dead and alive groups ($p < 0.137$).

Table 4 shows that there was a statistically significant association between victims who died and those that presented, during hospitalization, with other complications ($p = 0.002$), early signs of DAI in CT ($p = 0.006$), and ICH ($p = 0.003$). The average values for GCS 48 h after the withdrawal of sedation were different between the dead and alive groups ($p < 0.001$).

The multiple logistic regression model tested the variables that achieved a *p*-value <0.05 in the analysis. The results point to the presence of hypoxia by SpO_2 at admission ($p = 0.029$) and

TABLE 4 | Comparisons between patient conditions (dead or alive, independent or dependent) at 6 months after DAI in relation to characteristics related to hospital stay.

	Survival			GOS-E		
Characteristics related to hospital stay	**No** ***n*** **(%)**	**Yes** ***n*** **(%)**	***p*-Value**	**Independent** ***n*** **(%)**	**Dependent** ***n*** **(%)**	***p*-Value**
ICU stay						
Yes	22 (91.7)	50 (92.6)	>0.999	42 (93.3)	6 (100.0)	>0.999
No	2 (8.3)	4 (7.4)		3 (6.7)	–	
Use of continuous sedation						
Yes	22 (91.7)	47 (87.0)	0.713	39 (86.7)	6 (100.0)	>0.999
No	2 (8.3)	7 (13.0)		6 (13.3)	–	
Other treatments with drugs that act on the CNS						
Yes	13 (54.2)	35 (64.8)	0.372	27 (60.0)	6 (100.0)	0.078
No	11 (45.8)	19 (35.2)		18 (40.0)	–	
Surgery						
Yes	13 (54.2)	29 (53.7)	0.970	23 (51.1)	5 (83.3)	0.204
No	11 (45.8)	25 (46.3)		22 (48.9)	1 (16.7)	
Second surgery						
Yes	4 (16.7)	11 (20.4)	>0.999	9 (20.0)	1 (16.7)	>0.999
No	20 (83.3)	43 (79.6)		36 (80.0)	5 (83.3)	
Infection						
Yes	7 (29.2)	13 (24.1)	0.634	8 (17.8)	4 (66.7)	**0.022**
No	17 (70.8)	41 (75.9)		37 (82.2)	2 (33.3)	
Other complications						
Yes	19 (79.2)	22 (40.7)	**0.002**	15 (33.3)	6 (100.0)	**0.003**
No	5 (20.8)	32 (59.3)		30 (66.7)	–	
Early DAI signs in CT						
Yes	22 (91.7)	33 (61.1)	**0.006**	25 (55.6)	6 (100.0)	0.070
No	2 (8.3)	21 (38.9)		20 (44.4)	–	
ICP monitoring						
Yes	2 (8.3)	1 (1.9)	0.223	–	1 (16.7)	0.118
No	22 (91.7)	53 (98.1)		45 (100.0)	5 (83.3)	
ICH						
Yes	11 (45.8)	8 (14.8)	**0.003**	4 (8.9)	4 (66.7)	**0.004**
No	13 (54.2)	46 (85.2)		41 (91.1)	2 (33.3)	
Hypotension						
Yes	7 (29.2)	6 (11.1)	0.096	5 (11.1)	1 (16.7)	0.548
No	17 (70.8)	48 (88.9)		40 (88.9)	5 (83.3)	
Hypertension						
Yes	15 (62.5)	38 (70.4)	0.601	31 (68.9)	6 (100.0)	0.170
No	9 (37.5)	16 (29.6)		14 (31.1)	–	
Hypothermia						
Yes	17 (70.8)	29 (53.7)	0.156	23 (51.1)	5 (83.3)	0.204
No	7 (29.2)	25 (46.3)		22 (48.9)	1 (16.7)	
Hyperthermia						
Yes	18 (75.0)	32 (59.3)	0.181	26 (57.8)	6 (100.0)	0.072
No	6 (25.0)	22 (40.7)		19 (42.2)	–	
Hypoglycemia						
Yes	7 (29.2)	16 (29.6)	0.967	13 (28.9)	3 (50.0)	0.363
No	17 (70.8)	38 (70.4)		32 (71.1)	3 (50.0)	
Hypoglycemia in the first 5 days						
Yes	5 (20.8)	7 (13.0)	0.498	7 (15.6)	–	0.578
No	19 (79.2)	47 (87.0)		38 (84.4)	6 (100.0)	
Hyperglycemia						
Yes	19 (79.2)	31 (57.4)	0.064	26 (57.8)	4 (66.7)	>0.999
No	5 (20.8)	23 (42.6)		19 (42.2)	2 (33.3)	
Hyperglycemia in the first 5 days						
Yes	13 (54.2)	25 (46.3)	0.521	21 (46.7)	4 (66.7)	0.419
No	11 (45.8)	29 (53.7)		24 (53.3)	2 (33.3)	

HCFMUSP, 2013–2014.

ICU, intensive care unit; CNS, central nervous system; CT, computed tomography; ICP, intracranial pressure; ICH, intracranial hypertension.

Bold numbers correspond to significant p values.

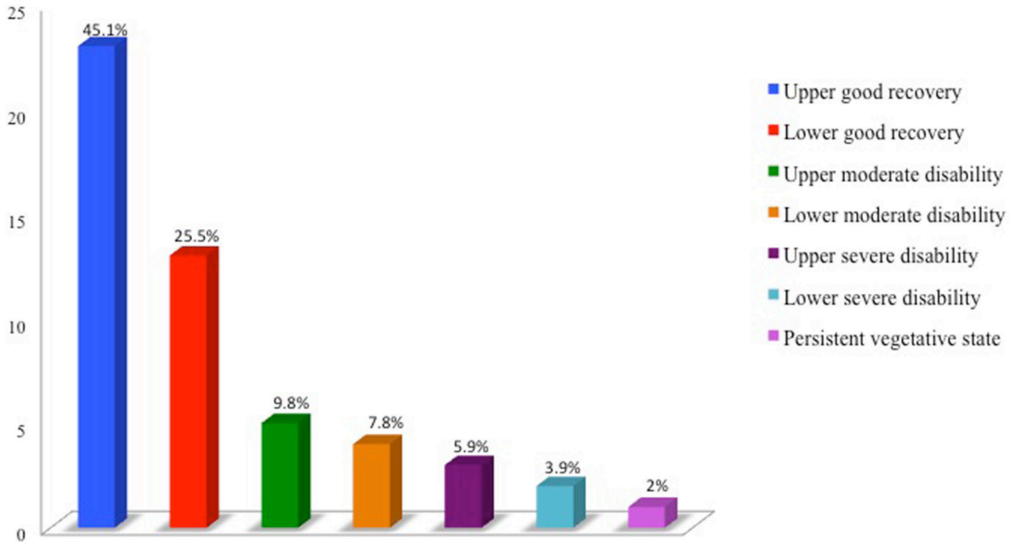

FIGURE 1 | DAI victims at 6 months after trauma: second functional capacity by the Extended Glasgow Outcome Scale (GOS-E). HCFMUSP, 2013–2014.

TABLE 5 | Comparisons between patient conditions (dead or alive, independent or dependent) at 6 months after DAI in relation to severity of DAI.

	Survival			GOS-E		
DAI severity	**No** ***n*** **(%)**	**Yes** ***n*** **(%)**	***p*-Value**	**Independent** ***n*** **(%)**	**Dependent** ***n*** **(%)**	***p*-Value**
Mild/moderate	2 (8.3)	48 (88.9)	**<0.001**	44 (97.8)	1 (16.7)	**<0.001**
Severe	22 (91.7)	6 (11.1)		1 (2.2)	5 (83.3)	

HCFMUSP, 2013–2014.
Bold numbers correspond to significant p values.

severe DAI ($p < 0.001$) as risk factors for death. No other variables reached significance during modeling and thus were not included in the model. The model in **Table 6** was not a good fit for the data because the confidence intervals (CIs) of odds ratios (ORs; 95%) for both variables were large.

To further explore the effects of factors other than DAI severity on the outcomes of this injury, a model excluding this variable was tested. This model showed that the presence of hypotension at admission ($p = 0.016$) and the value of NISS ($p = < 0.001$) were independently associated with mortality at the end of this regression (**Table 7**).

Factors Related to Dependence 6 Months after DAI

Considering only the 51 patients who survived and participated in the 6-month follow-up, we identified two groups of patients with DAI: those needing assistance to carry out their daily activities (dependent) and those living independently 6 months after the trauma.

Analyzing the variables that reflect the severity of the trauma and of TBI, measured by the ISS ($p = 0.003$), NISS ($p = 0.001$), MAIS-Head ($p < 0.001$), ICH ($p = 0.004$; **Table 4**), infection ($p = 0.022$; **Table 4**), other complications ($p = 0.003$; **Table 4**), and severity of DAI ($p < 0.001$), all had shown statistically significant differences between the groups. **Table 5** shows that patients with severe DAI were predominant in the dependent group, whereas among patients living independently, the injury was mild/moderate in almost all cases.

There was a statistically significant difference between the groups regarding the average length of ICU hospitalization ($p < 0.001$), continuous sedation ($p = 0.001$), and length of hospital stay ($p = 0.037$). Furthermore, the average value of GCS 48 h after withdrawal of sedation was different between the independent and dependent groups ($p < 0.001$). In comparing the two groups, the mean periods of time were higher in the dependent group, and the average GCS score was lower.

During modeling, the first variable entered in the model was severity of DAI ($p \leq 0.001$), with which no other variable attained p-value <0.05. Similar to the mortality model, the CI of OR (95%) was quite broad for the result of regression to dependence (**Table 8**). Thus, once more, the severity of DAI was excluded from the model; in the final model (**Table 9**), only the duration of hospital stay was statistically significant in the logistic regression model with a p-value = 0.008.

TABLE 6 | Logistic regression model of risk factors for mortality up to 6 months after DAI.

			CI for OR (95%)	
Variable	***p*-Value**	**OR**	**Lower limit**	**Upper limit**
Hypoxia by SpO_2 at admission (yes)	**0.029**	18.77	1.36	259.27
DAI severe	**<0.001**	125.63	14.02	1,125.95

HCFMUSP, 2013–2014.
OR, odds ratio; CI, confidence interval.
Bold numbers correspond to significant p values.

TABLE 7 | Logistic regression model of risk factors for mortality up to 6 months after DAI excluding severity of injury.

			CI for OR (95%)	
Variable	***p*-Value**	**OR**	**Lower limit**	**Upper limit**
Hypotension at admission (yes)	**0.016**	7.86	1.48	41.78
NISS	**<0.001**	1.14	1.07	1.22

HCFMUSP, 2013–2014.
NISS, New Injury Severity Score.
Bold numbers correspond to significant p values.

TABLE 8 | Logistic regression model of risk factors for dependence at 6 months after DAI.

			CI for OR (95%)	
Variable	***p*-Value**	**OR**	**Lower limit**	**Upper limit**
DAI (severe)	**0.000**	205.00	11.02	3,813.02

HCFMUSP, 2013–2014.
Bold numbers correspond to significant p values.

TABLE 9 | Logistic regression model of risk factors for dependence at 6 months after DAI excluding severity of the injury.

			CI for OR (95%)	
Variable	***p*-Value**	**OR**	**Lower limit**	**Upper limit**
Duration of hospital stay (days)	**0.008**	1.07	1.02	1.12

HCFMUSP, 2013–2014.
Bold numbers correspond to significant p values.

DISCUSSION

Diffuse axonal injury is a microscopic lesion associated with significant mortality and morbidity. Evaluating the risk factors related to its consequences is of great importance for the implementation of appropriate multidisciplinary care and health policies aimed at the prevention and rehabilitation of patients with DAI.

Six months after DAI, 24 patients (30.8%) had died as a consequence of the trauma or of complications. Nevertheless, among those who survived this period (51 patients), 88.2% achieved GOS-E classification consistent with independent living, and 45.1% had shown full recovery from trauma, reporting a return to the pre-injury state. During this period, individuals with disabilities formed 29.4% of the sample, and 11.8% of those were dependent.

The literature reports worse outcomes – frequency of disability (40.0–87.5%) and dependency (20.0–41.3%) – for patients with DAI, evaluated by GOS or GOS-E at 6 months after injury (26–30). However, most participants in our study had mild (44.9%) or moderate (19.2%) DAI, and among these, only one (2.2%) was dependent during the period of the study (**Table 5**). Severe DAI stood out as a risk factor for mortality and dependence in this study, although the multivariate logistic regression analysis did not yield a good fit in the statistical models: CI of OR (95%) ranged from 13.65 to 395.89 for mortality and 11.2 to 3,813.02 for dependence. Though the confidence intervals clearly confirm the important role of the severity of DAI as a risk factor for adverse consequences of this injury, they were quite large because of the small number of deaths (two cases) and dependence events (one case) among patients with mild and moderate DAI, leading to instability in the model.

Diffuse axonal injury is difficult to diagnose in the acute phase. The combination of clinical signs and imaging may suggest the diagnosis, but confirmation is only possible post-mortem (31). Using CT in the emergency room helps in the identification, diagnosis, and location of hemorrhages. Despite having low resolution in soft tissue evaluation after TBI, CT is considered a useful tool for identifying early signs of DAI in the acute phase and is widely employed in severe TBI victims because of the short duration of the exam, its wide availability in trauma centers, and its conduciveness for use in unstable patients (31).

Studies (3, 5, 11, 18, 27, 32) show that the frequency of DAI is higher in severe TBI victims who have indirect signs of injury on CT, such as intraventricular hemorrhage and subarachnoid hemorrhage, rather than a normal CT scan. In this study, for the analyses of association with the consequences of trauma, patients with normal CT scans were analyzed separately from those with signs suggestive of DAI, and the presence of these signs was more frequent among patients who progressed to death.

Studies that have emphasized imaging and biomarkers to estimate the severity and prognoses for patients with DAI corroborate the findings in this study: the greater the severity of DAI, the worse the outcome for the patient (32–34).

In addition to early signs of DAI in CT, other variables were associated with mortality: trauma severity indicators (ISS and NISS) and TBI (MAIS-Head); pupillary changes; hypotension; hypoxia measured by SpO_2 and hyperglycemia on admission; GCS score after withdrawal of sedation; presence of ICH; and complications, other than infection, during hospitalization. Patients who died displayed these signs and clinical changes more often during hospital admission and hospitalization and had higher severity scores and lower GCS scores after withdrawal of sedation than those who survived.

Among trauma victim characteristics, hypoxia by SpO_2 and hypotension on admission, in addition to the NISS, had significant effects in multivariate analysis. Nevertheless, the best fitting statistical model included only the last two variables and showed that patients with hypotension on admission were 7.86 times more likely to die than those without this symptom. Furthermore,

each additional point in NISS value increased the chance of death in the first 6 months after DAI by 14.0%.

Physiological changes after TBI, such as hypoxia and hypotension, can result in secondary brain damage. Preserving the airways after trauma may result in favorable outcomes after severe TBI (13). Hypoxia and hypotension in severe TBI victims are common, and their occurrence in the initial hours is significantly associated with increased mortality (13, 35–37). Hypertension may relate to reduced brain perfusion and can worsen ICH and cerebral edema (36). Analysis of hypotension incidence in early resuscitation in TBI patients in the city of San Francisco showed that of 107 victims studied, 26 (24%) had hypotension, averaging 1.5 episodes per patient (mean duration 9.1 min). Of these patients with hypotension, 65.0% died, with the frequency of hypotension episodes directly proportional to the number of deaths (38).

Changes in glucose levels are either caused by metabolic or physiological disorders or are a stress response that reflects the severity of the injury, and they are related to unfavorable outcomes (39–41). Research (39) on 380 victims of TBI in the first 5 days of ICU admission indicated association of high glucose levels (≥160 mg/dL) in the first 24 h after admission with mortality and that mortality was higher in patients with hypoglycemia (<60 mg/dL).

Our results were broadly in line with a previous study of 78 patients diagnosed only with DAI; after an average of 12.3 months, factors significantly associated with mortality were hypotension, plasma glucose >144 mg/dL, low scores on the GCS at hospital admission, increased number of DAI lesions in CT and minor trauma injuries, presence of shock, coagulation disorders, transfusion, and no recovery of consciousness (5). In multivariate analysis, only the absence of recovery of consciousness and the large number of DAIs were identified as independent risk factors for mortality (5).

Studies that analyzed the association of TBI patient characteristics with mortality identified the following factors independently associated with mortality: the Marshall classification in CT (42), severity of the injury measured by CT (43), diffuse head injuries II–IV (44), lower score on the GCS (44, 45), hypotension (43–45), hyperglycemia (45, 46), hypothermia (46), SBP (43, 47), SpO_2 (48), hypoxia (44, 45), shock (45), ICP monitoring (49), a score of 5 on MAIS-Head (47, 49), and a high score in the ISS (43, 45, 47, 49).

In this study, NISS was independently associated with mortality; however, no other reports of NISS application to groups of DAI patients were found. Although literature review indicates that NISS has better performance than ISS in predicting mortality (50), the scientific community is still cautious about replacing ISS with NISS for trauma severity identification, and thus, this indicator is underutilized.

In addition to the severity of the injury, dependence after 6 months following DAI was associated with higher scores in trauma severity indicators (ISS and NISS) and TBI (MAIS-Head), longer duration of sedation and hospitalization in the ICU and in the hospital, the presence of ICH, infection, other complications during hospitalization, and low scores on the GCS 48 h after the withdrawal of sedation.

Excluding the severity of DAI from dependence modeling, hospital stay was a significant predictor in the multiple regression model, with each additional hospitalization day increasing the chance of a patient being dependent 6 months after DAI by 7.0%.

A similar association was previously reported in an analysis of 41 patients with severe TBI in Hong Kong, which showed that prolonged hospitalization and advanced age were independent predictors of poor outcome 42 months after trauma (51). In another study (52) of 60 patients with severe TBI in the US, the length of the hospital stay and the length of the stay in the ICU were statistically associated with outcome 6 months after trauma. Moreover, in Brazil, a survey of patients with TBI conducted between 6 months and 3 years after trauma determined that individuals hospitalized for 12 days or more were 5.76 times more likely to become dependent than those with shorter hospital stays (24). According to Calvi et al. (33), factors associated with dependence or mortality in patients with TBI evaluated 3 months after trauma were lower GCS score on admission, higher ISS score, longer hospital stay, and longer stay in the ICU.

In a study (53) of 30 patients with DAI, severe TBI, pupillary abnormalities, higher ISS score, and lesions in the knee of the corpus callosum were associated with dependence 1 year after trauma. In multivariate analysis of these patients, only the corpus callosum lesions increased the risk of dependence 1 year after DAI. Assessment of patients with DAI 3 months after trauma in Japan showed that the lowest GCS score, the number of brain lesions identified on MRI, and the highest average value in ICP were significantly associated with dependence after trauma (54). In studies with patients with DAI and moderate TBI, greater severity of brain lesions identified by Marshall rating on CT (34) and higher number of DAIs in this exam (5) predicted dependence or mortality 6 months after trauma. However, the recovery of consciousness and lesions in the corpus callosum (5) were predictors of positive results after an average of 12 months after DAI. In addition, the assessment of factors related to dependence 6 months after trauma in 102 DAI patients showed that age, bilateral absence of pupillary reactivity to light, and multiple lesions observed in the corpus callosum and brainstem by MRI were associated with dependence (33).

Research on factors associated with DAI consequences typically uses imaging results for analysis (5, 34, 53, 54) and shows a relationship between changes in the images and consequences of DAI, although these results have been contradictory. In this study, only the presence of early signs of DAI in CT was tested as an independent variable, making it difficult to compare the findings.

The limitations of this study include the lack of MRI data, as the combination of imaging data with clinical findings might have made the diagnosis of DAI more reliable and allowed the comparison of clinical data with the brain lesions detected. Another limitation was the lack of available resources to perform this test on all participants; only 20 patients underwent this exam, and the difficulties of subjecting patients in a severe condition to this evaluation prevented considering MRI results in this study.

Applications of the results of this research must take into account some limitations. The sample included patients from a single institution, a referral center for the treatment of highly complex cases, which limits the generalization of the results.

Moreover, the lack of hospital records on the patient's clinical condition at the trauma scene and during transport limited the identification of pre-hospital stage factors that might be associated with the outcomes of DAI.

It should also be noted that the rehabilitation treatments of the patients were listed but not included in the association analyses, although it has been observed that all patients who were not independent at the last evaluation (6 patients) were seen by health-care experts to support their recovery.

CONCLUSION

At 6 months after DAI, 24 patients (30.8%) died due to trauma or complications. However, among the survivors that were evaluated over this period (51 patients), 88.2% achieved GOS-E classification consistent with independent living, and 45.1% had full recovery from trauma. According to the GOS-E, six patients (11.8%) remained dependent at that point in time.

The sociodemographic characteristics of the study participants, related to trauma and PHC, were not associated with mortality and dependence. On the other hand, trauma severity indicators (ISS and NISS), TBI (MAIS-Head), and severity of DAI showed statistically significant associations with those consequences. Some clinical features observed at hospital admission were associated with mortality: pupillary changes, hypotension, hypoxia by SpO_2, and hyperglycemia. During hospitalization, the GCS score after withdrawal of sedation, presence of ICH, and complications other than infection were associated with mortality and dependence. Early signs of DAI were associated only with mortality. Infection, continuous sedation time, and length of stay in the ICU and hospital were factors related to dependence.

Severe DAI stood out as a risk factor for mortality and dependence in the multivariate logistic regression analysis, although without good fit in the final model and with large CI of the OR (95%). Patients with severe DAI ($n = 28$) in almost all cases died or were dependent at 6 months after DAI. Along with severe DAI, the presence of hypoxia by SpO_2 was a risk factor for mortality; however, variables of admission hypotension and NISS value showed the best fit for the model. Individuals who presented with hypotension on admission were 7.86 times more likely to die than victims without this change. The probability of dying was 14.0% higher for each additional point in the NISS.

Excluding the severity of DAI from dependence modeling, hospital stay was the variable that stood out in the multiple regression model, with each additional day of hospitalization increasing the chance of a patient remaining dependent 6 months after DAI by 7.0%.

AUTHOR CONTRIBUTIONS

RV contributed to the study design, manuscript development, and data analysis, wrote the manuscript, and performed the final review. WP contributed to data analysis and reviewed the manuscript. DO contributed to the writing and review of the manuscript. MT contributed to the manuscript review. AA contributed to the manuscript review. RS contributed to the study design, manuscript development, data analysis, review of the tables, writing of the manuscript, and the final review.

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Functional, Structural, and Neurotoxicity Biomarkers in Integrative Assessment of Concussions

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Concussion is a complex, heterogeneous process affecting the brain. Accurate assessment and diagnosis and appropriate management of concussion are essential to ensure that athletes do not prematurely return to play or others to work or active military duty, risking re-injury. To date, clinical diagnosis relies primarily on evaluating subjects for functional impairment using instruments that include neurocognitive testing, subjective symptom report, and neurobehavioral assessments, such as balance and vestibular-ocular reflex testing. Structural biomarkers, defined as advanced neuroimaging techniques and biomarkers assessing neurotoxicity and immunoexcitotoxicity, may complement the use of functional biomarkers. We hypothesize that neurotoxicity AMPA, NMDA, and kainite receptor biomarkers might be utilized as a part of comprehensive approach to concussion evaluations, with the goal of increasing diagnostic accuracy and facilitating treatment planning and prognostic assessment.

Keywords: concussion, mild TBI, neuropsychological evaluations, advanced MRI sequences, neurotoxicity and neuroplasticity biomarkers

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INTRODUCTION

Concussion is a heterogeneous injury that requires a multifaceted and comprehensive approach for assessment, diagnosis, and management. Several clinical tools are routinely used, including symptom report, neurocognitive testing, and postural stability/vestibular and oculomotor assessments (1). However, these tools, which represent functional biomarkers, have practical limitations and may not possess adequate sensitivity in diagnosing all concussions. More objective means of assessment, including advanced neuroimaging techniques and neurotoxicity biomarkers, have gained research attention. These may facilitate diagnosis and could potentially assist with monitoring recovery and determining prognosis. Preliminary studies suggest neurotoxicity biomarkers, in conjunction with neurocognitive testing, might improve diagnostic certainty of suspected concussions and may provide valuable information on what domains of brain function are affected (e.g., vestibular system) or the severity of a concussion (2). This may be particularly valuable, since the grading scales for concussion severity are not scientifically validated, lack prognostic utility, and are largely considered antiquated or outdated (3).

Laboratory chemistry tests confirm systemic changes of energy metabolites in concussion (e.g., ATP, creatine, lactate, as well as blood gases and minerals) (4). Quantitative values of brain-borne neurotoxicity degradation fragments of ionotropic glutamate receptors (GluRs) and antibodies to excitotoxicity biomarkers in blood are important early markers of injury (5). Therefore, these biomarkers, when added to a comprehensive evaluation of concussion, may improve diagnosis and prognosis. The purpose of this review is to highlight the potential role of neurotoxicity biomarkers in a comprehensive evaluation of sports-related concussion.

CLINICAL DEFINITIONS: CONCUSSION

Concussion is defined as a "complex pathophysiological process affecting the brain, induced by biomechanical forces," with associated traumatically induced alteration in mental status with or without loss of consciousness, as specified in a recent consensus statement on concussion in sports (6). Several physical, cognitive, emotional, somatic, and sleep-related symptoms may be present for days to weeks following injury. These are linked to cognitive, vestibular, and oculomotor dysfunction of various brain systems or domains (6). The terms "concussion" and "mild traumatic brain injury" (mTBI) are proposed to be used interchangeably, since much of the clinical symptomology overlaps and as many as 80% of concussions are diagnosed as mTBI. Neither mTBI nor concussive injuries show gross abnormalities on standard neuroimaging, and most patients recover without permanent impairment (7, 8). Typically, about 80% of post-concussive symptoms resolve spontaneously within 7–10 days of the acute phase (6).

BIOMECHANICS OF INJURY

Rotational forces can cause a transient disruption of function in the reticular activating system, resulting in the loss of consciousness associated with concussion (9). Prior biomechanical data have demonstrated that a simple impact of the head with a solid surface (rotational acceleration >5,000 rad/s^2) leads to the greatest stress–strain to the frontotemporal regions, connecting limbic structures as well as the corpus callosum and cortical–spinal tract (10). As a result, rotational acceleration, diffuse shear, and strain forces cause variable degrees of injury to neurons, glia, the blood–brain barrier (BBB), and vascular structures, leading to transitory ionic functional disturbances with clinical manifestations. These include sudden confusion, lack of balance or coordination, vision abnormalities, and memory impairment (11). Despite using advanced techniques, such as the head impact telemetry system (HITS), researchers have not been able to diagnose concussion reliably by quantifying a specific threshold (12). Researchers argue that the validity of head impact metrics has not been adequately addressed for sports, and clinicians have been cautioned to not rely on impact magnitude or location to predict acute clinical outcomes, symptom severity (**Table 1**), neuropsychological function, or balance abnormalities (10, 13).

FUNCTIONAL BIOMARKERS: CLINICAL ASSESSMENT

Clinical assessment of concussion is structured around the various domain-specific impairments exhibited following

TABLE 1 | Biomechanical attempts to assess severity of concussion.

Severity	Characteristics		Transitory disturbances		Reference
	Impact force in gravity force (g) and radian per seconds (rad/s^2)	**Impact location**	**Frequent symptoms**	**Symptom duration**	
Mild	**Direct impact**				
	Linear acceleration ~30–65 g Time ~ 1 ms	Frontal, parietal, and temporal lobes	Often no symptoms, no functional changes	About 24 h	(14)
	Rotational acceleration – 4,000–5,000 rad/s^2	Brainstem, spinal tract			(15)
	Coup-countercoup (AIS[a] = 1)				(16)
	linear acceleration ~ 50–100 g ICP[b] < 173 kPa	Frontal lobe and upper end of brainstem			
Moderate	**Direct impact**				
	Linear acceleration ~70–90 g Time ~ 1–3 ms	Frontal, parietal, and temporal lobes	No outward symptoms but substantial functional alterations	1–3 days	(17)
	Rotational acceleration – 5,000–6,500 rad/s^2	Brainstem, spinal tract			(10)
	Coup-countercoup (AIS = 2)				
	linear acceleration ~ 100–150 g ICP ~ 140–190 kPa	Frontal lobe and upper end of brainstem			(18)
Severe	**Direct impact**				
	Linear acceleration >100 g Time – 4 ms	Frontal, parietal, and temporal lobes	Often but not always clinically observed functional impairment	Up to 2–3 weeks	(19)
	Rotational acceleration ~ 7,000–13,000 rad/s^2	Brainstem, spinal tract			(20)
	Coup-countercoup (AIS = 3–4)				
	Linear acceleration ~ 150–250 g ICP ~ 201–282 kPa	Frontal lobe and upper end of brainstem			

[a]*AIS – Abbreviated Injury Scale.*
[b]*ICP – intracranial pressure.*

injury – cognitive, vestibular, somatic, and emotional – adapted for various settings, such as on the "sideline" at sporting events as well as during recovery following acute, subacute, and chronic injury (**Table 2**).

Sideline Assessment

The Standardized Concussion Assessment Tool and its revisions (SCAT2, SCAT3) are the most widely used tools on the sideline (31). These comprise a number of empirically validated measures adapted across several domains of assessment, including symptom report, mental status, attention/memory, and balance. The SCAT is "likely to identify the presence of concussion in the early stages of post-injury," according to the 2013 American Academy of Neurology concussion guidelines (32). Others argue that this tool is insensitive to identifying mild deficits, and is subject to practice effects (33). Furthermore, it should not be relied on for decisions regarding whether an athlete should be allowed to return to play (34). The Display Enhanced Testing for Concussions and mTBI (DETECT) system has been developed in response to the need for a fast and efficient sideline neuropsychological test (35). It is a 7-min battery of tests that allows for real-time cognitive testing in situations previously deemed impractical or unavailable for patients with concussion, such as the sideline. However, it is still being examined for validity and reliability.

Neurocognitive Testing

Neurocognitive testing has been coined a "cornerstone" of concussion management since the initial Concussion in Sport Group meeting in 2001. Typically, neurocognitive testing is completed in an office or quiet setting, and has been shown to be sensitive both to acute deficits (e.g., <24 h following injury) as well as those detected after an athlete is symptom free (36, 37). Of all the commercially available computerized test batteries, the Immediate Post-concussion and Cognitive Testing Test Battery (ImPACT) is the most widely used and researched and the only one formally approved by the FDA; others are summarized in **Table 2**. Although largely reserved for those with protracted recoveries, for some athletes, traditional paper and pencil neuropsychological testing may be a component of concussion protocols. While neurocognitive evaluation is essential in assessing and managing concussion in a majority of cases, it is important to recognize that neurocognitive deficits are occasionally absent following concussion. A recent meta-analysis of computerized neurocognitive tests (CNTs) suggests an overall low-to-moderate magnitude of effects size, attributing these findings to the heterogenic nature of concussion (38). There are other limitations in using neurocognitive testing following concussion. Several extraneous factors may influence neurocognitive test performance, including motivation, effort, sleep, pain, and anxiety (39). Neurocognitive testing

TABLE 2 | Test battery for concussion assessment.

Tools	Intended use	Properties	Limitations	Reference
Sideline				
Sports concussion assessment tool (SCAT3)	Sideline assessment diagnostic	Sensitivity 80–94% Specificity 76–91%	Should not be used for return to play decisions	(21)
Computerized neurocognitive tests				
Immediate post-concussion and cognitive testing (ImPACT)	In office, diagnosis and management	Sensitivity 82–91% Specificity 69–89% Test–retest reliability 0.25–0.85	Must have a trained professional for interpretation; potential for misuse (e.g., poor control on environment, use as a standalone measure)	(22, 23) http://www.impacttest.com/
Automated Neurocognitive Assessment Metrics (ANAM)	In office, diagnosis and management	Test–retest reliability 0.14–0.81		(24) http://www.vistalifesciences.com/index.php/anam-intro.html
Axon Sports Computerized Cognitive Assessment Tool	In office, diagnosis and management	Test–retest reliability 0-0.94 (53% of ICC's failing minimum standards)		www.axonsports.com/
Concussion Vital Signs	In office, diagnosis and management	Test–retest reliability 0.07–0.87		http://www.concussionvitalsigns.com/
Postural Stability/Vestibular				
Balance error scoring system (BESS)	Sideline/Acute	Specificity up to 91% Sensitivity 34–64%	Practice effects, poor intra/inter/after season reliability	(25, 26)
Sensory organization test (SOT)	In office	Sensitivity 48–61% Specificity of 85–90%		(27)
Vestibular/oculomotor screening test (VOR, VMS)	In office		Relies heavily on symptom report	(28)
Oculomotor tests				
Eye-Track Advance (ETA)	Sideline assessment	Sensitivity 54–77% Specificity of 67–92%	Depends on past medical history, medications, and drugs used	(29)
King–Devick (K–D) test	Sideline	Test–retest reliability 0.90	Practice effects, requires baseline	(30)
Vestibular/oculomotor screening test (saccades, near point convergence)	In office	Internal consistency (Cronbach's alpha = 0.92)	Relies heavily on symptom report	(28)

can be challenging to interpret among subpopulations, such as individuals with learning disabilities or attention deficits in the absence of baseline testing (40).

Postural Stability/Vestibular

Assessing balance and postural stability may be useful in the acute stages of injury (25). The Balance Error Scoring System (BESS) is the most widely used balance assessment following concussion, due to its rapid ease of administration and low cost. However, the system's sensitivity has been criticized because of the influence of other factors, such as fatigue, type of sport, and history of ankle injury or instability (41). The Sensory Organization Test (SOT) evaluates dynamics in postural stability using a force plate, and has superior reliability compared to the BESS (27). However, it is not feasible to use on the sideline due to the required equipment. Furthermore, similar to neurocognitive testing, deficits in postural stability are not always present after concussion or may resolve rapidly. Another tool developed for concussion assessment is the vestibular/ocular motor screening (VOMS) (28), which uses several tests for screening central vestibular functioning, including the vestibular–ocular reflex and visual-motion sensitivity.

Oculomotor Assessment

Both subjective and objective measures of oculomotor functioning have utility in diagnosis and management of concussion. The VOMS measures near point of convergence and provocation of symptoms on saccades, both sensitive to concussion (28), and correlates with neurocognitive impairment (42). The King–Devick (K–D) is a 1-min test in which the athlete is asked to read, quickly and accurately, several pages of single-digit numbers that are arrayed left to right in columns that do not vertically align (43). Preliminary evidence suggests K–D is easily administered, can differentiate concussed from non-concussed athletes, and has high test–retest reliability ($r = 0.90$) (44). However, there is concern regarding practice effects (45). The objective eye-tracking technology concussion test, used in both sports and combat settings, records the subject visually tracking a target as it moves through a circular trajectory (46). Gaze error variability has been found to correlate significantly with attention and working memory measures on neurocognitive testing (29).

NEUROIMAGING

Concussion is generally regarded as a functional, rather than a structural, brain injury, with conventional neuroimaging findings considered normal and adding little value to clinical management of this injury beyond ruling out more severe pathology, such as a skull fracture or intracranial hemorrhage (47).

However, advanced magnetic resonance imaging (MRI) methods have demonstrated the ability to detect and localize pathophysiologic consequences of concussion or mTBI (48). Functional MRI (fMRI) data have shown a strong link between brain region functioning vs. concussion severity and time to recovery; however, this procedure is used primarily in the research setting (49). Diffusion tensor imaging (DTI)/DTI-based tractography is used to evaluate axonal tearing after the impact. Quantitative high-resolution imaging techniques, such as susceptibility-weighted imaging (SWI) or angiography (SWAN), depict iron deposits in brain microvessels, while cerebrovascular dysfunction in areas of hyper- or hypoperfusion can be detected by high-resolution diffusion-weighted imaging (DWI).

Recent studies that used SWI or perfusion-weighted imaging (PWI) with DTI and magnetic resonance (MR) spectroscopy found abnormalities in 80% of patients with mTBI when these three imaging modalities were collated (50, 51).

Diffusor Tensor Imaging

Diffusion tensor imaging (3T DTI)-based tractography evaluates diffuse axonal injury (DAI). Additionally, 3T fluid-attenuated inversion recovery (FLAIR) imaging allows detection of non-hemorrhagic DAI (47) and could potentially assist in assessment of structural abnormalities in concussions. Due to success in assessing a population with moderate-to-severe TBI, DTI has gained popularity for use in mTBI because of its enhanced technical resolution. DTI can reveal white matter alterations affecting the corticospinal tract (CST); the internal capsule; the corpus callosum (52); the inferior/superior, longitudinal, and fronto–occipital fasciculi; and the posterior thalamic and acoustic radiations in athletes following sports concussions (53). While increased fractional anisotropy (FA) numbers indicate reversible changes in white matter, reduced FA values reflect a loss of white matter integrity in a region of interest (54–56). Overall, data from DTI studies of mTBI have been contradictory, however, showing both increased FA values in acute and chronic studies (57, 58) as well as decreased FA levels (59). Recently, Davenport et al. (60) demonstrated that a single football season can produce alterations in DTI parameters in the absence of clinical concussion.

Diffusion-Weighted Imaging

Microvessel dysfunction can be detected by high-resolution DWI. DWI allows for differentiation between cytotoxic edema (restricted diffusion) and vasogenic edema (increased diffusion), and has been shown to identify shearing injuries not evident on T2/FLAIR or gradient echo sequences (61). Significant involvement of the neurovascular unit has also been shown in pediatric patients with sports-related concussions who experienced persistent symptoms (50). Recent MRI-based studies assessing cerebral flow alterations due to concussions and mTBI are indicating the emerging concept of concussion as a form of cerebrovascular disease (62).

Susceptibility Weighted Imaging

Small hemorrhages in white matter have been detected by high-resolution SWI in retrospective studies of retired NFL players (63), youths with sports-related concussion (64), and, in amateur boxers, more severe cases of mTBI (65). Significant SWI hypointensity alterations in brains of adult male hockey players within a single season have been observed in the subacute stage of injury compared to baseline images (66). However, SWI does not provide quantitative measures of magnetic susceptibility (67).

Susceptibility-Weighted Angiography

As with SWI, SWAN focuses on intracerebral small vessels for identifying cerebral hemorrhage and calcifications (68) by visualizing measurable changes in cerebral veins due to hypoxia (69). It is particularly useful in visualizating micro-hemorrhages and lesions near the skull base (70), and has contributed to a proposed MRI grading scale (71, 72). Application of susceptibility-weighted imaging mapping (SWIM) for quantitative assessment (67) of venous blood oxygenation in the acute stage of mTBI has shown an excess of oxygen in impacted areas that may reflect a neuroprotective response following the injury (73).

In summary, several MRI sequences (DTI, DWI, FLAIR, SWI, SWAN) may be valuable in determining the location and level of microstructural abnormalities (micro-lesions) and edema following concussion. However, to date, MRI studies for mTBI are limited by study design, including consensus in methodology; e.g., 1.5 or 3T, sequence, slice thickness, and spatial resolution (74). Furthermore, there is often variability in timing after injury, age of subjects, severity of injury, and brain region analyzed. Additionally, use of advanced imaging-derived techniques, such as DTI, with multi-shell diffusion and high-definition tractography modeling might be useful in identifying subtle structural changes due to mild concussion (52). The development of universal and more inclusive protocol(s) for mTBI studies might help overcome current limitations in pursuit of comparable, reproducible data.

GLUTAMATER RECEPTOR BIOMARKERS

Biomarkers for concussion can be categorized as diagnostic or prognostic/monitoring (75, 76). Prospective, diagnostic biomarkers provide information about a disease or condition and may assess the severity of concussion. Prognostic biomarkers (risk assessment) are defined as indicators that may provide the anticipated natural history of a disorder in the absence of a therapeutic intervention. Monitoring biomarkers (recovery) are used to follow a previously diagnosed or established disease or condition; for example, in making a decision whether a person should be returned to play in sports or to work or active military duty.

Diagnostic Potential

Ideal biomarker(s) for the diagnosis of concussion should (i) reflect the origin of transient subtle brain injury resulting from the impact and show a specific location of subtle brain injury, (ii) include detectable biological markers at an early stage of brain damage (within minutes to hours after event), (iii) correlate with performance on clinical measures, and (iv) demonstrate functional deficits or metabolic disturbances concurrent with advanced neuroimaging (fMRI, DTI, and SWI modalities). Several candidate brain biomarkers are associated with concussion severity (**Table 3**).

Neurotoxicity biomarkers include the ionotropic GluRs. The AMPA receptors (AMPAR; GluR1 subtype) are located

TABLE 3 | Candidate biomarkers for identification of concussions severity.

Biomarker	Performance characteristics			Strengths (intended use)	Limitations (biomarker studies)	Reference
	Cut off ng/ml	Sensitivity %	Specificity %			
AMPAR peptide	0.4	89–91	91-92	Associated with diffuse axonal injury (DAI)	Need data assessed within 24 h after concussion	(77)
NMDAR peptide	1.0	83	91	A biomarker of microvessel damage and correlates with development of cortical vasogenic edema	Need to be assessed within 24 h after concussion	(5)
Calpain-derived αII-spectrin N-terminal fragment (SNTF)	Not established	100	75	Associated with DAI and shows white matter abnormalities	Low levels in biological liquids for mTBI. There are no concussion studies	(78)
Breakdown products of glial fibrillary acidic protein (GFAP-BDP)	0.03	60–93	75–97	Detects hemorrhage/hematoma and might be used to reduce unnecessary CT/MRI	GFAP-BDP also releases during intracerebral and subarachnoid hemorrhagic strokes. There are no concussion studies	(79)
GFAB autoantibody	2.9–3.0	–	–	Shows dynamic interactions between post-injury and specific autoimmune response	Small sample size of the study	(80)
Kainate receptor peptide	0.5	83–90	83–92	Might be associated with brainstem injury and regulates venous circulation (development of cytotoxic edema)	Need to be assessed within 24 h after concussion	(81)
S-100B	0.06 0.12	94 29	50 96	A marker of compromised blood–brain barrier	Lack of specificity	(82)
Total Tau protein	–	Area under curve: 0.91 (95% CI, 0.81-1.00)		Correlates to severity of concussions and predicts longevity of recovery	Small sample size of the study	(83)
UCHL1	–	Area under curve: 0.62 (95% CI, 0.57–0.71) 0.72		A potential diagnostic assessment of acute TBI Associated with outcome	UCHL1 found in more severe TBI. Limited concussion studies	(84, 85)

exclusively in synaptic terminals (77) and could indicate diffuse dendritic–axonal injury (86). AMPAR is primarily distributed in the forebrain and subcortical pathways, including the hippocampus, amygdala, thalamus, hypothalamus, and brain stem (87, 88). These regions of the brain are predictable sources of biomarkers given the functional spatial-temporal coherence, developmental pathways, and cerebral plasticity affected by mild brain injury (89). The NMDA receptors (NMDAR: NR2 subtypes) are localized on the epithelial surface of microvessels that form the BBB and regulate cerebral arterial microvascular function (90). The biomechanical forces that lead to concussion may cause mechanical damage and energy failure in parenchymal cells and endothelia that comprise the BBB. Furthermore, concussion drives neurotoxicity biomarker peptides to be released continuously into the bloodstream through the compromised BBB within hours to days after impact. During the acute phase of concussion, a massive release of glutamate upregulates excitotoxic AMPAR (55, 91). The GluR1-subunit of N-terminal AMPAR fragments are rapidly cleaved by extracellular proteases and released into the bloodstream, where this degradation product, identified as a biomarker of neurotoxicity, can be directly detected (peptide fragment of 5–7 kD).

A feasibility study examining the diagnostic potential of the AMPAR peptide assay was conducted by administering neuropsychological testing (ImPACT) and neuroimaging to concussed athletes (20.8 ± 1.8 years old, 56M/28F, 1–2 weeks post-concussion, GCS = 14–15) and age-, gender-matched healthy controls (21M/19F) in conjunction with measurements of the biomarker (86). The sensitivity (91%) and specificity (92%) of AMPAR peptide to assess acute and semi-acute concussions (preliminary cut off of 0.4 ng/mL) in college athletes was established. Additionally, in athletes with multiple concussions, worse ImPACT scores on processing speed, reaction time, and cognitive efficiency correlated with abnormal levels of AMPAR peptide (2.0–12.0 ng/mL) and DAI changes apparent on MRI (2).

Kainate receptors (KAR, GluR5/6), which are located mostly in the hippocampus, subcortical areas, spinal cord tract, and brainstem (92), might potentially affect cerebral venous circulation. Glutamate serves as a neuromediator for the medulla involved in regulation of involuntary life sustaining functions, such as breathing, swallowing, heart rate, and consciousness (92), primarily through KAR (2). In patients with mTBI, the decrease of venous function due to a rise in venous oxygenation in the affected thalamostriate and right basal areas (93) might involve KAR. As a component of post-military deployment mTBI screening, KAR peptide detection in active duty military personnel (37M/16F, 23.0 ± 1.2 years old, 1 week after blast injury, GCS = 13–15) with impaired venous circulation in cervical areas defined by dopplerography yielded an optimal cut-off value of 1.0 ng/mL (90% sensitivity, 83% specificity), at which a positive predictive value of 93% was achieved. A clinical study conducted with civilians who sustained mTBI (25M/20F, 30.1 ± 3.0 years old, GCS = 13–15) and admitted to ED within 24 h after the impact due to violence-related events, motor vehicle crashes, and incidental falls showed KAR peptide sensitivity of 83% and specificity of 92% (cut-off of 0.5 ng/mL), with a significant positive likelihood ratio of 10.5 to assess severe concussions (unpublished data). Notably, AMPAR and NR2 peptides were also abnormal in these cohorts.

Prognostic/Monitoring Approaches

Biomarkers intended to measure recovery following concussion should potentially (i) reflect neurological symptoms, (ii) identify the immune system response to inflammation, (iii) further predict outcomes, and (iv) assist with treatment/therapy recommendations.

Disruption of the BBB due to biomechanical force is believed to cause instantaneous increase in brain vasculature permeability, with both immediate and delayed pathogenic consequences (7, 94). The effects of a compromised BBB are exacerbated when accompanied by an immunological response initiated by entry of peripheral anti-CNS autoantibodies (95–97), which may be prognostic biomarkers of brain injury (98). Due to the nature of the normal immune response, antibodies to brain antigens detected in serum are typically associated with advanced or chronic conditions of brain damage (99, 100), whereas neuronal antigens measured in plasma are more likely to reflect acute injuries (101).

Early experimental and clinical research of antibodies to GluR1 subtype of AMPA receptors as an immunoexcitotoxicity biomarker has demonstrated their diagnostic value in detecting pathological brain-spiking activity and epileptic seizures (102, 103) as a consequence of traumatic brain injury, thereby representing a prognostic risk factor. Above threshold increases of AMPAR antibodies measured in serum of children ($n = 60$, 7–16 years old) with chronic posttraumatic headache within 6–12 months after sustained single or multiple concussions were correlated with abnormal brain-spiking activity on EEG (104). Clinical studies of GluR1 antibodies in adult patients with different chronic neurological pathology ($n = 1866$) performed in Russia, Europe, and the USA have demonstrated diagnostic potential (sensitivity of 86–88%, specificity of 83–97%) in assessment of seizures (epilepsy) as consequence of sustained single or multiple concussions (105, 106).

One potential prognostic biomarker is a marked increase in the level of NR2 antibodies. These increased levels are evidence of persistent brain changes related to delayed cerebral ischemia (107), which can follow concussion. In three subjects with prior concussion, NR2 antibody levels remained elevated beyond the cut-off (2.0 ng/mL) throughout the 1.5-year study duration (2). Interestingly, visual memory composite scores on ImPACT were impaired in two of the three athletes. Furthermore, reduction in NR2 peptide and antibodies threshold levels corresponded with improvement on ImPACT scores and reduction of post-concussive symptoms.

An AMPAR peptide assay has been evaluated as a tool for management and decision making for return to play, work, and active military duty following concussion. In a longitudinal study, initial assessment of AMPAR peptide in athletes showed that of 84 subjects (20.8 ± 1.8 years old, 56M/28F, 1–2 weeks post-concussion, GCS = 14–15), 18 (21%) had increased biomarker levels after concussion (108). In the subsequent 15 months, AMPAR peptide values decreased in 11 of the 18 subjects (61%), with levels close to normal (0.07–0.9 ng/mL). These athletes also had

normal ImPACT scores, and were allowed to return to play. After an additional 3 months, the seven remaining athletes remained symptomatic and had higher AMPAR peptide values compared to controls. Consequently, they were advised to postpone their return to playing sports and directed to neuroimaging (2).

A recent prospective off-season study assessed AMPAR peptide levels in a professional American football team. Among players (43M, 27.8 ± 3.1) who had no concussions reported in the prior season, AMPAR peptide levels in plasma (within 0.1–1.0 ng/mL) cleared 40 players (93%) to play. The remaining three players (7%) were advised to postpone return to play due to higher AMPAR peptide levels (1.2–2.3 ng/mL). Interestingly, two out of the three players with elevated AMPAR peptide levels reported concussion a week before blood draw, suggesting a cumulative effect. Preliminary assessment of assay performance characteristics in semi-acute (within 2 weeks after the concussion) assessment yielded an 89% sensitivity and 91% specificity (unpublished data).

Impaired Neuroplasticity Biomarkers for Concussion (Acute and Chronic Conditions)

Due to breakdown of the BBB, most proteins responsible for impaired neuroplasticity enter the bloodstream, fluids, and tissues relatively late (109). Among these are the calpain-derived αII-spectrin N-terminal fragment (SNTF) (78, 110), αII-spectrin breakdown products (SBDPs) for axonal injury (111), S100 associated with compromised BBB (112), astrocyte-specific protein glial fibrillary acidic protein (GFAP), a biomarker of hematoma/hemorrhage (113) and GFAP autoantibody as a post-injury autoimmune response (80), tau protein, a microtubule-associated structural protein located within axons, and ubiquitin carboxy-terminal hydrolase L1 (UCHL1), a cysteine protease expressed in neurons as well as neuroendocrine cells (114) (**Table 3**).

Following TBI, SNTF accumulates in axons and blood. In a study of patients with mTBI admitted to an emergency room, blood SNTF levels correlated with white matter abnormalities on DTI, as well as cognitive dysfunction and persisted for at least 3 months (110). Among professional ice hockey players, serum SNTF increased at 1 h after concussion and remained significantly elevated from 12 h to 6 days, before declining to preseason baseline. By contrast, serum SNTF levels were unchanged after training in healthy controls (78). Serum SNTF exhibited sufficient diagnostic accuracy for delayed return to play in this study (area under the curve = 0.87).

S100 proteins are small acidic proteins with diverse functions that range from cell cycle progression, transcription protection from oxidative cell damage, and apoptosis (115, 116). A study of collegiate and semi-professional athletes who completed S100B testing at baseline and following concussion demonstrated that relative and absolute increase in serum S100B could accurately distinguish concussion from sport-related exertion (82). A correlation between S100B levels and number of sub-concussive head hits has also been reported (117). Other studies have suggested that low levels of S100B were able to rule out mTBI (118, 119). Recent studies have demonstrated that measurement of S100B would assist in indicating if a CT scan would be needed in the acute setting (120–122). The transient disruption of the BBB from multiple sub-concussive and/or concussive events may allow accumulation of S100B autoantibodies that could be associated with predisposition to neurological disease, including chronic traumatic encephalopathy (CTE). Despite prolonged rest, repetitive head impacts sustained in contact sports have been shown to cause persistent white matter alterations (123). Thus, S100B autoantibodies may be useful biomarker after acute concussion and in evaluating more chronic consequences of head trauma.

Two studies have shown that serum GFAP and its breakdown products (GFAP-BDP), markers of hemorrhage, were increased in patients with an abnormal CT compared to those with a normal CT after mTBI (124, 125). GFAP remained elevated in axonal injury on MRI in a subset of mTBI patients at 3 months' post-injury (124). Similarly, serum GFAP-BDP was significantly higher in patients with intracranial lesions on CT, compared with those without lesions, and was able to predict which patients required neurosurgical intervention (113). A recent study of 396 patients with mild-to-moderate TBI established that GFAP out-performed S100B in detecting intracranial CT lesions, particularly in the setting of extracranial fractures (126). GFAP-BDP demonstrated very good predictive ability (AUC = 0.87) as well as significant discrimination of injury severity (125). Recent study detecting GFAP autoantibodies found three to five times higher biomarker values in chronic and acute cases accompanies with prior TBI (80). However, it is unclear if serum GFAP would be useful in detecting a more mild form of TBI, such as a sports-related concussion.

Ubiquitin carboxy-terminal hydrolase L1 levels in CSF and serum demonstrated predictive value for severe TBI in adult and children (127, 128) and were also increased after seizures (129). It was suggested that UCHL1 is potentially reporting the BBB integrity (94). Assessments of UCH-L1 after sport-related sub-concussive head hits displayed lack of clinical significance (117).

Tau protein is a microtubule-associated structural protein located within axons. The deposition of hyperphosphorylated tau (p-tau) protein in clusters around small blood vessels of the cortex is indicative of tauopathy and is related to CTE (130). Although the factors that contribute to CTE are poorly understood, both professional athletes (131) and military veterans (132) have been diagnosed with this disease. To date, CTE is primarily diagnosed postmortem (133); however, the first attempt to detect total tau protein in plasma using digital array technology (**Table 3**) has recently been reported (83).

DISCUSSION

Clinical assessment tools for concussion all represent functional biomarkers. These include symptom report, neurocognitive testing, and postural stability/vestibular and oculomotor assessments. While widely used to guide clinical care, concern regarding the sensitivity of these functional biomarkers as well as their feasibility for use in the sports arena exists. For example, sideline testing is brief and, therefore, may not be sensitive enough to identify mild concussion. Comprehensive neurocognitive

TABLE 4 | Proposed functional, metabolic, and structural biomarkers to assess severity of acute concussions and mTBI.

Neuroanatomical area of injury	Clinical and Neuropsychological scores			Neurotoxicity markers[c]	Proposed scores		
	GCS	ImPACT[a]	SCAT3[b]		3T (or higher) MRI[d]		GCS/MRI/biomarkers
					Preferable modality	Scores	
Subcortical dendritic-axonal injury (DAI)	15	0.3–0.4	19.3	AMPAR (A)	DTI	1	15A1
Cortical–subcortical, subcortical–cervical area, or cortical–cervical area, transient events (vasogenic edema)	14	0.2–0.3	40.7	AMPAR (A) NR2(N) KAR(K)	DWI, DTI	2	14 AN2 –cortical – subcortical 14AK2 – subcortical –cervical 14NK2- cortical–cervical
Cortical-subcortical and cervical area (cytotoxic edema)	12–13	0.1–0.2	46.2	Overall involvement of ionotropic GluRs ANK	FLAIR, SWI/SWAN, DTI	3	12ANK3 13ANK3

[a]*Considered only cognitive efficacy score.*
[b]*SCAT3 data were considered from (21).*
[c]*Considered only abnormal values of AMPAR, AMPA receptor peptide fragment (candidate biomarker of DAI); NR2, peptide fragment of NMDA receptor subtype (arterial microvascular marker candidate); KAR, kainite receptor peptide fragment (venous circulation biomarker candidate).*
[d]*MRI scores are suggested analog to that proposed by Potapov et al. (71) based on location/level of brain lesions for TBI.*

testing may appear normal, leading to premature return to play. More objective means of assessment, including advanced neuroimaging techniques and neurotoxicity biomarkers, have gained research attention. These measures may facilitate diagnosis and potentially assist with monitoring a subject's recovery. Preliminary studies suggest neurotoxicity biomarkers, in conjunction with neurocognitive testing, may improve diagnostic certainty of suspected concussions as well as provide valuable information on prognosis, allowing a clinician to modify treatment recommendations.

This review highlights potential structural and neurotoxicity biomarkers that could be integrated into the current clinical concussion evaluation that, at present, relies primarily on functional biomarkers. Research to date supports a move toward administering a more comprehensive panel of biomarkers; namely, neurotoxicity markers. These biomarkers represent a second generation of brain "tracers" that could be used to decipher the microstructural abnormalities observed on advanced neuroimaging. For example, abnormal values of one (15A1), two (14AN2, 14NK2, 14 AK2), or three GluRs (12-13ANK3) may assist in making a decision to return a subject to play, extended rest, or further medical assistance (**Table 4**). Those athletes or patients with abnormal scores using these biomarkers should be directed to neuroimaging assessment (DTI, DWI, SWI, FLAIR), and may benefit from an individualized treatment plan. In the future, it may be possible to classify concussion severity with neuroimaging, similar to proposed MRI classification for TBI (71). Furthermore, the use of neurotoxicity biomarkers may assist radiologists with additional information concerning the area of interest, where microstructural changes might be located (cortical, subcortical, or brainstem injuries).

Accurate diagnosis of concussion and appropriate management of the injury to prevent premature return to play is vital to protecting the long-term brain health of athletes and others at risk of re-injury. Structural and neurotoxicity biomarkers can be used to complement the functional biomarkers currently used to assess concussion to facilitate diagnosis and treatment.

HUMAN SUBJECTS

This review presents retrospective analyses of neurotoxicity biomarkers measured in subjects with concussions and mild TBI previously disclosed in peer-reviewed scientific publications.

All retrospective analyses of human data concerning GluR biomarker studies conducted by the authors were performed in accordance with institutional and national guidelines and regulations. The institutional review protocol governing the college and professional athletes' enrollment was approved by the respective Institutional Review Boards at Kennesaw State University, the University of Pittsburgh, and by the USAMRMC ORP HRPO under proposal DM090557 (award W81XWH-11-2-0081). The research protocol for using samples collected from active duty personnel and civilians who suffered mild TBI in the studies described herein were approved by the Ethics Committee at Burdenko Institute of Neurosurgery (Moscow, Russia). Written informed consent was provided by all research subjects. The privacy of participants was protected using global unique identifiers.

AUTHOR CONTRIBUTIONS

SD and JDM are joint senior authors. AMS, JDM, EVA, and AAP are contributed equally to this work.

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CASE REPORT
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Restoration of Upper Limb Function in an Individual with Cervical Spondylotic Myelopathy using Functional Electrical Stimulation Therapy: A Case Study

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Non-traumatic spinal cord pathology is responsible for 25–52% of all spinal cord lesions. Studies have revealed that spinal stenosis accounts for 16–21% of spinal cord injury (SCI) admissions. Impaired grips as well as slow unskilled hand and finger movements are the most common complaints in patients with spinal cord disorders, such as myelopathy secondary to cervical spondylosis. In the past, our team carried out couple of successful clinical trials, including two randomized control trials, showing that functional electrical stimulation therapy (FEST) can restore voluntary reaching and/or grasping function, in people with stroke and traumatic SCI. Motivated by this success, we decided to examine changes in the upper limb function following FEST in a patient who suffered loss of hand function due to myelopathy secondary to cervical spondylosis. The participant was a 61-year-old male who had C3–C7 posterior laminectomy and instrumented fusion for cervical myelopathy. The participant presented with progressive right hand weakness that resulted in his inability to voluntarily open and close the hand and to manipulate objects unilaterally with his right hand. The participant was enrolled in the study ~22 months following initial surgical intervention. Participant was assessed using Toronto Rehabilitation Institute's Hand Function Test (TRI-HFT), Action Research Arm Test (ARAT), Functional Independence Measure (FIM), and Spinal Cord Independence Measure (SCIM). The pre–post differences in scores on all measures clearly demonstrated improvement in voluntary hand function following 15 1-h FEST sessions. The changes observed were meaningful and have resulted in substantial improvement in performance of activities of daily living. These results provide preliminary evidence that FEST has a potential to improve upper limb function in patients with non-traumatic SCI, such as myelopathy secondary to cervical spondylosis.

Keywords: functional electrical stimulation, upper extremity rehabilitation, grasping, spinal cord disorders, myelopathy

INTRODUCTION

Although cervical spondylotic myelopathy (CSM) is categorized as a degenerative disorder most frequently seen in older individuals, this type of spinal cord dysfunction is influenced by genetic, environmental, and occupational factors as well. Hence, CSM affects a spectrum of patients across different age groups (1, 2), is the most common cause of spinal cord dysfunction in modern society worldwide (3), and is a major source of disability (4) that negatively affects quality of life (5, 6). Adults older than 55 years of age, who have cervical spondylosis are more predisposed to CSM as the aging process results in spondylotic changes, and these, in turn, result in direct compressive and/or ischemic dysfunction of the spinal cord (5, 6). The most characteristic CSM syndrome consists of varying combinations of the following triad: (a) painful stiff neck, (b) brachialgia and numb hands, and (c) spastic leg weakness with unsteadiness of gait (7). Upper extremity dysfunction is, in essence, one of the classic sign of CSM. This may have a variable presentation in the form of numbness or paresthesia, clumsiness, or weakness of the hand, hand and/or forearm muscle weakness, and atrophy, and occasionally there may be severe atrophy of the hand muscles. As a result, the individuals with CSM syndrome typically experience difficulty performing motor tasks, such as buttoning buttons, using keys, using a computer keyboard, pushing buttons on a cellular phone, or text messaging. These are considered as common early signs of CSM (8).

In a prospective study of CSM (8), it was observed that the earliest change indicative of CSM was the inability to adduct the little finger. This condition further progressed to affect the ring and middle fingers. As the disease became more severe, this loss of adduction in the fingers innervated by the ulnar nerve was followed by the inability to extend them (8). There are many underlying causes for this condition. In most cases, CSM is a progressive condition (9). Development of moderate to severe signs and symptoms of CSM usually indicate poor prognosis. In those cases in which the clinical status and imaging findings corroborate CSM diagnosis, surgical intervention may play a key role in managing CSM (9). Given the progressive nature of CSM, many clinicians advocate surgical treatment of patients with this condition.

In this article, we are presenting a case study in which functional electrical stimulation therapy (FEST) (explained in detail in the following section) was applied to an individual who had a CSM-related surgical treatment 22 months prior to taking part in the study. At the time, the participant enrolled in the study, he presented with progressive right hand weakness that resulted in his inability to voluntary open and close the hand and manipulate objects with his right hand. In what follows, we will present results that suggest that the FEST may be an effective intervention to manage CSM.

BACKGROUND

Functional Electrical Stimulation

Functional electrical stimulation (FES) is a method that is able to produce functional movements in paralyzed muscles after an injury or damage to the central nervous system, including, but not limited to, stroke, spinal cord injury (SCI), and traumatic brain injury (10–19). By delivering low energy electrical pulses to nerves that are innervating muscles of interest (targeted muscles), the FES is able to induce controlled movements in the limbs and body (16, 18, 19). Careful application of highly controlled stimulation sequences is able to generate synergistic muscle activations that can produce complex movements, such as reaching, grasping, and walking.

Functional Electrical Stimulation Therapy

Originally, FES was envisioned to be used as an orthotic device (also known as neuroprosthesis), intended to be worn permanently with users activating it whenever he/she required to reach or grasp an object, or to walk. Our team and others have been developing an alternative method for using FES technology. In this embodiment, FES technology is used to deliver a short-term therapeutic intervention, where the patient is expected to undergo a finite number of FEST sessions. Upon completion of the FEST program, the patient will have recovered partial or complete voluntary motor function in the targeted extremity, i.e., upper or lower extremity (20–30). The FEST program is designed to "retrain" the injured neuromuscular system, through repetitive performance of task-specific exercises. The FES during these training sessions is used to provide assistance with the components of the task that the individual is unable to perform independently. The assistance provided by the FES system to accomplish each task during the training session is determined based on day-to-day performance. At the completion of the 40–60 1-h-long FEST sessions, the individual is usually able to perform the tasks unassisted or with minimal assistance (i.e., without the help of FES device).

We have successfully used our FEST program to assist (i) adults with incomplete and complete SCI, and (ii) adults and children with severe upper limb deficit following stroke, to recover sustained reaching and/or grasping motor functions (22, 24, 25, 27–30). In our upper limb FEST program, the participant must attempt to initiate or execute the specific motor task unassisted, such as pinch grasp. Once a brief (10- to 20-s long) attempt to perform the specific task has been made, the therapist delivers FES-induced electrical pulses to the muscles to assist the individual to complete the task. Multitudes of different reaching and grasping tasks are trained. Each task is slightly different, and each task is trained on average between 5 and 7 min. During the early stages of FEST, performance of the entire task may be supported by FES, if required. As the therapy progresses, FES assistance is slowly reduced and eventually phased out. We believe that the combination of (i) active participation of the patient during therapy, (ii) the way in which FES system generates the movement, (iii) the fidelity of the movement performed using FES, (iv) the accuracy with which FES system mimics the natural limb movements, and (v) repetitive FES-induced movements are critical ingredients of this therapy. Previous experiments in SCI and stroke populations, conducted by our team (2, 22–24, 26–28, 30–32) and others (18, 33–35), indicate that improvements in grasping function, as a result of FEST, are meaningful and clinically relevant.

Currently, the mechanisms responsible for recovery of function using FEST are unknown. However, few physical and neurological mechanisms that may explain the effects of

this therapeutic intervention have been already identified. The "peripheral" mechanisms are (i) muscle strengthening, (ii) increased flexibility and range of motion in the affected limb, and (iii) reduced muscle spasticity. The "neurological" mechanisms are (i) cortical reorganization, (ii) neuroplastic changes in the central nervous system, (iii) strengthening of the synaptic connection *via* Hebb's rule, and (iv) functional reorganization and retraining of unaffected, but functionally related, areas of the central nervous system, allowing them to take control over the damaged parts of the central nervous system. Presently, the FEST experts are inclined to explain the improvements using the above listed and few additional neurological mechanisms (36).

Motivation for This Study

In conclusion, there is strong evidence to support the use of FEST as an effective tool for retraining of reaching and grasping functions in neurological populations, such as stroke or SCI. Since CSM is a degenerative disorder of the cervical spine, which can be grouped among non-traumatic SCIs, it is very likely that the FEST for reaching and grasping will help this patient population improve voluntary reaching and grasping. Furthermore, it has been shown that strengthening muscles with the help of FES may as well bring about considerable improvements in walking and standing functions in the individuals with CSM (37). Therefore, this study was conducted to test if similar outcomes can be achieved in individuals with CSM following FEST. To the best of our knowledge, this is the first reported case of a chronic CSM patient treated with FEST for grasping.

DISCUSSION

Diagnosis

The study participant was a 61-year-old male who underwent C3–C7 posterior laminectomy and instrumented fusion in November 2012, for cervical myelopathy. Cervical spine X-ray showed degenerative changes more prominent at C4–C7 levels. Following surgery, the participant experienced progressive weakness in the right hand. In November 2013, he underwent a redo cervical decompression and extension of fusion down to T2 level. Cervical spine MRI revealed moderate foraminal stenosis at C5–T1, and it was recommended that he undergoes re-exploration of his previous fusion and extension down to T2, bilateral foraminotomies, at C5–C6, C6–C7, and C7–T1. Postoperatively, the study participant developed right upper extremity numbness and progression of hand weakness. A cervical spine MRI was obtained demonstrating no acute findings or evidence of epidural hematoma and otherwise expected postoperative findings. The numbness and progression of hand weakness were thought to be related to intraoperative nerve root manipulation, which are typically transient in nature. At 22 months post initial surgery, the study participant continued to have right upper extremity numbness and weakness of the hand, as well as wasting of the intrinsic muscles of the right hand. The participant was unable to extend the lateral three fingers, namely, the middle, ring, and little fingers, despite relatively well-preserved function of the wrist. He was also unable to perform rapid extension of the fingers.

Typically, following the surgery for CSM, one can expect significant functional recovery, which should plateau at 6 months following the intervention (5, 6). Since the patient did not experience any significant improvement in hand function at 22-month mark, and since his hand function continued to deteriorate, he was invited to participate in this FEST case study.

Treatment

The patient was screened to confirm that he did not have any contraindications (i.e., pacemaker, spinal stimulation, rash or open wound at any potential electrode site) to FEST. Prior to participating in the study, FEST was explained to the patient, including the risks and benefits of this treatment. After he received all pertinent information, the patient signed an informed consent form that was approved by the Research Ethics Board of the Toronto Rehabilitation Institute – University Health Network.

Our team has developed a hardware platform that can be used to deliver the FEST for improving upper limb function. This system is called Compex Motion (38), and it has four current regulated stimulation channels. The system can be used to deliver customized and diverse reaching and grasping FES protocols. In this particular case, the participant received FES protocols that enabled him to perform hand opening, palmar grasp, and lateral pinch grasp. The stimulation was delivered using standard self-adhesive surface stimulation electrodes, which were placed on the subject's skin above the following muscles:

- flexor digitorum superficialis muscle and the flexor digitorum profundus muscle (finger flexion);
- flexor pollicis brevis (thumb flexion);
- extensor digitorum muscle (finger extension);
- lumbrical muscles (finger extension).

Stimulation parameters used to stimulate the above muscles were

- balanced, biphasic, current regulated electrical pulses;
- pulse amplitude from 6 to 30 mA (muscle dependent);
- pulse duration from 0 to 250 μs;
- pulse frequency 40 Hz.

The stimulation sequences (protocols) for this patient were tailored to his clinical presentation and his goals of therapy. The protocols allowed the participant to grasp and manipulate objects with the help of FES delivered by the Compex Motion system. The command for activating the stimulation sequence was initiated by a pushbutton that was triggered by the therapist who administered the therapy. Typical activation process was as follows. The participant was instructed on a particular grasping or hand opening task that he was supposed to perform. Then, the therapist instructed the participant to try to perform that task. The participant was given 10–20 s to try to execute the task on his own. Only after the participant completed as much of the task as he could by himself, the FES was triggered by the therapist to assist the participant in completing the entire task/movement. The participant received FEST three times per week for 5 weeks, i.e., 15 treatments sessions in total.

The subject was trained to perform (i) hand opening using finger extensors only, (ii) hand opening using a combination of

lumbrical muscles and finger extensors, (iii) palmar grasp, and (iv) lateral pinch grasp. Throughout FEST sessions, the participant was taught how to approach, reach, grasp, and manipulate various objects without a fear that he will drop them during the object manipulation process. The FES-assisted grasping ensured that all objects were grasped robustly and could be manipulated with ease. With time, this training allowed the participant to become more confident in reaching, grasping, and manipulating objects. As a result, with time, the participant started to perform the same reaching, grasping, and object manipulation tasks, without using FES.

Outcome Measures

At baseline and following completion of 15 FEST sessions, the participant was assessed using the following standardized assessments. The assessments were performed by a researcher who was not involved in the therapy delivery process.

Toronto Rehabilitation Institute's Hand Function Test

Toronto Rehabilitation Institute's Hand Function Test (TRI-HFT) (39) was used to assess unilateral gross motor function of the hand. The TRI-HFT consists of two sub-tests. The first one evaluates object manipulation skills using palmar grasp and lateral or pulp pinch grasp, and the second one evaluates the strength of both palmar and lateral pinch grasps. Note: TRI-HFT has been found to be very responsive to FEST.

Action Research Arm Test

Action Research Arm Test (ARAT) (40, 41) is a test developed to assess the upper limb function in stroke patients, and it consists of four sub-tests: grasp, grip, pinch, and gross movement.

Functional Independence Measure

Functional Independence Measure (FIM) (42) is an assessment specially developed to measure the degree of independence. The FIM is commonly used by both rehabilitation institutions and insurance companies to monitor patients' improvements during rehabilitation.

Spinal Cord Independence Measure

Spinal Cord Independence Measure (SCIM) (43) is a disability scale that has been specifically developed to evaluate the functional outcomes of patients with traumatic and non-traumatic SCI.

RESULTS

The participant completed 15 therapy sessions, as well as baseline and discharge assessments. Following 5 weeks of FEST, the participant was able to pick up small objects, which he was unable to do prior to FEST (see **Figure 1**). **Table 1** summarizes all the clinical assessment scores at baseline and at discharge (i.e., after completion of therapy).

FIGURE 1 | TRI Hand Function Test – object manipulation. **(A–C)** Object manipulation before FES therapy, i.e., patient unable to grasp and manipulate objects, and **(D–F)** Object manipulation after FES therapy, i.e., patient able to grasp and manipulate objects.

TABLE 1 | Participant's scores on all outcome measures at baseline and at discharge.

Outcome measures	Scores		
	Baseline	Discharge	Change
ARAT			
• ARAT – total score (MCID = 5.7)	37	44	7[a]
• ARAT – grasp	13	17	4
• ARAT – grip	10	10	0
• ARAT – pinch	5	8	3
• ARAT – gross movement	9	9	0
TRI-HFT			
• TRI-HFT – object manipulation (MCID not available)	54	65	11[a]
• TRI-HFT – wooden blocks (MCID not available)	48	62	14[a]
• TRI-HFT – instrumented cylinder (Nm)	0.2	0.5	0.3
• TRI-HFT – credit card (N)	0	5	5
• TRI-HFT – wooden bar thumb direction	3	10	7
• TRI-HFT – wooden bar little finger direction	30	30	0
FIM			
• FIM – total score (MCID = 22)	109	119	10
• FIM – self-care sub-score (MCID not available)	25	35	10
SCIM			
• SCIM – total score (MCID not available)	92	95	3
• SCIM – self-care sub-score (MCID not available)	12	15	3

ARAT, Action Research Arm Test; TRI-HFT, Toronto Rehabilitation Institute's Hand Function Test; FIM, Functional Independence Measure; SCIM, Spinal Cord Independence Measure; MCID, minimum clinically important difference.
[a]Change scores represent clinically meaningful change.

This case study examined the efficacy of FEST as a clinical intervention for treating severe upper extremity impairment due to myelopathy secondary to cervical spondylosis. We found that the participant experienced considerable and clinically meaningful improvements in hand function as measured by TRI-HFT and ARAT. The pre/post differences in scores on all measures clearly demonstrated improvement in voluntary hand function following FEST. It is noteworthy that, in subacute stroke and traumatic SCI individuals, at least 20 and preferably 40 1-h FEST sessions are required to achieve clinically meaningful change. The fact that we were able to elicit clinically meaningful change in a chronic patient with CSM after only 15 1-h sessions suggests that the FEST for upper limb may be a viable therapeutic intervention for this patient population. These results provide preliminary evidence that rehabilitation treatment consisting of repetitive FEST designed to improve arm and hand function has the potential to restore voluntary grasping function in patients with CSM. The results also suggest that FEST leads to subsequent improvements in the quality and complexity of tasks that the participant was able to execute before and after study completion. These improvements in hand function subsequently lead to improvements in activities of daily living and increased level of independence. Following therapy completion, the participant subjectively reported (i) that he has observed improvements in his arm and hand function, and that he felt that he gained additional independence in activities of daily living; (ii) that the FEST allowed him to "awake his hand" when his fingers felt "dead" and "glued together"; (iii) that he experienced "more normal" movements in his hand and greater articulation in his fingers especially with respect to fine motor skills; and (iv) that he is now functioning at a "higher level."

CONCLUDING REMARKS

The results of our study are very encouraging; however, the study has certain limitations. This is a single case study with no control group, which makes it difficult to comment on comparative improvement that could have been achieved if the participant was administered a same dose of conventional occupational therapy alone. However, we have to acknowledge that the participant decided to participate in this study because he had already reached a plateau in recovery following many sessions of conventional occupational therapy. Based on the results of this case study, we believe that a study with a larger number of CSM patients is warranted, where the effects of FEST for reaching and grasping can be compared against current best practices for treating this patient population.

AUTHOR CONTRIBUTIONS

The work presented here was carried out in collaboration between all authors: MP, VZ, and TV. MP contributed to the design of the study, interpreted the results, and wrote the paper. VZ co-worked on associated data collection, interpreted the results, and wrote the paper. TV co-worked on data interpretation and revising paper for important intellectual content. All authors have seen and approved the manuscript.

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Conflict of Interest Statement: Dr. MP cofounded the company MyndTec, Inc. (www.myndtec.com), which is presently manufacturing the product MyndMove®. MyndMove® delivers Dr. MP's multichannel FEST for reaching and grasping for stroke and SCI patients. Although Dr. MP is the Chief Technology Officer of the company, the company is operating independently from his research program at the University of Toronto and Toronto Rehabilitation Institute – University Health Network. The study was conducted independently of MyndTec, Inc. MyndTec, Inc. did not participate in the study design and execution.

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REVIEW
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Diffusion Tensor Imaging Findings in Post-Concussion Syndrome Patients after Mild Traumatic Brain Injury: A Systematic Review

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Objectives: To review the evidence for the use of diffusion tensor imaging (DTI) parameters in the human brain as a diagnostic tool for and predictor of post-concussion syndrome (PCS) after a mild traumatic brain injury (mTBI).

Design: Systematic review.

Data sources: All relevant studies in AMED, Embase, MEDLINE, Ovid, PubMed, Scopus, and Web of Science through 20 May, 2016.

Study selection: Studies that analyze traditional DTI measures [fractional anisotropy (FA), mean diffusivity (MD), radial diffusivity (RD), and axial diffusivity (AD)] and the severity of PCS symptoms or the development of PCS in humans after an mTBI.

Data extraction: Population studied, patient source, mTBI diagnosis method, PCS diagnosis method, DTI values measured, significant findings, and correlation between DTI findings and PCS.

Data synthesis: Ten studies investigated correlations between DTI values and PCS symptom severity or between DTI values and the development of PCS in mTBI patients. Decreased FA and increased MD and RD were associated with the development and severity of PCS. AD was not found to change significantly. Brain regions found to have significant changes in DTI parameters varied from study to study, although the corpus callosum was most frequently cited as having abnormal DTI parameters in PCS patients.

Conclusion: DTI abnormalities correlate with PCS incidence and symptom severity, as well as indicate an increased risk of developing PCS after mTBI. Abnormal DTI findings should prompt investigation of the syndrome to ensure optimal symptom management at the earliest stages. Currently, there is no consensus in the literature about the use of one DTI parameter in a specific region of the brain as a biomarker for PCS because no definite trends for DTI parameters in PCS subjects have been identified. Further research is required to establish a standard biomarker for PCS.

Keywords: diffusion tensor imaging, mild traumatic brain injury, post-concussion syndrome, biomarker, systematic review

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INTRODUCTION

Traumatic brain injury (TBI) is an important global health issue, with the incidence of TBI reported to hospitals in developed countries being approximately 200 per 100,000 people annually (1). Globally, approximately 10 million TBIs are serious enough to result in death or hospitalization each year (2). TBI is more common in adolescents and young adults; a Canadian study found that 20% of students in grades 7–12 had sustained a TBI (3). Classification of TBI as a mild traumatic brain injury (mTBI) is primarily based on an initial Glasgow Coma Scale (GCS) score of 13–15 (4). Other mTBI classification criteria consider the duration of loss of consciousness (LOC) and duration of posttraumatic amnesia (PTA), if present (5). mTBIs are the most frequent TBIs, accounting for 70–90% of all brain injuries treated at hospitals. However, because a majority of mTBI cases are not reported to hospitals, the true incidence of mTBI is estimated to be above 600 per 100,000 people per year (6).

Post-concussion syndrome (PCS), also referred to as post-concussional disorder (PCD), refers to a set of somatic, affective, and cognitive symptoms that manifests days after the initial head injury. Although these symptoms usually resolve within 3 months, they can persist for longer (7). Patients whose symptoms persist for less than 3 months are referred to as having experienced post-concussion symptoms, while those with symptoms persisting for longer than 3 months are diagnosed with PCS (8, 9). PCS often has a significant impact on quality-of-life, but currently there are no validated treatments for PCS beyond patient monitoring and symptom management.

The first step toward developing an effective treatment is to understand the pathophysiology and anatomical basis of the development of PCS and establish dependable biomarkers of the syndrome. Unfortunately, the current definition of PCS is vague because as a *syndrome*, it is only a set of signs and symptoms. Diagnosing PCS depends solely on clinical criteria, the judgment of the physician or healthcare professional, the patient's self-reporting of symptoms, and the diagnostic assessment selected. For this reason, PCS diagnosis is unreliable and poorly defined.

The Rivermead Post-Concussion Symptoms Questionnaire (RPCSQ) (10) is often used to quantify PCS symptoms. However, it has been shown that these criteria do not meet modern psychometric standards, and it was suggested that the usual practice of summating the RPCSQ into a single score is unreliable (11). Other assessments used in the diagnosis of PCS include the World Health Organization (WHO) International Classification of Diseases (ICD) guidelines (12), the British Columbia Postconcussion Symptom Inventory (BC-PSI) (13), and the Neurobehavioral Symptom Inventory (NSI) (14). Unfortunately, PCS symptoms are not specific to TBI patients (7, 15–18), further complicating PCS diagnosis *via* these assessments. mTBI patients have been found to report a greater number and increased severity of PCS symptoms when compared with moderate or severe TBI patients (19, 20). Identifying a biomarker specific to TBI patients with PCS would greatly improve diagnosis and treatment.

Axonal damage can cause impaired network function (21, 22) and may explain the symptoms experienced by patients after a TBI (23). Diffusion tensor imaging (DTI) is a non-invasive, *in vivo* imaging technique that measures the quantity and direction of water molecule diffusion (24). It is well-documented and validated for use in mapping microstructural changes, such as axonal damage, in the brain (24–28). The most commonly measured DTI parameter in brain research is fractional anisotropy (FA) (28), a measure of the directionality of diffusion (25). Other common DTI parameters include mean diffusivity (MD), a scalar measure of the total diffusion within a voxel (25), radial diffusivity (RD), a scalar measure of the diffusion in two directions perpendicular to the length of an axon, and axial diffusivity (AD), a scalar measure of the diffusion along the length of an axon (29). Through these measurements, DTI can detect microstructural changes in the brain's white matter tracts; abnormalities in these measurements indicate axonal damage, which may correlate with PCS symptoms (28, 30).

Diffusion tensor imaging has been used in the PCS population to study axonal damage that may be the underlying cause of the syndrome. A specific DTI biomarker for PCS would help identify and characterize these patients, providing the basis for treatments that target the anatomical deficits that cause the syndrome. A number of studies have looked at the classic DTI parameters in the PCS population (31–40). These studies offer information that can be helpful for clinicians and patients managing PCS, but often differ in the DTI parameters analyzed and in the brain regions found to have abnormal DTI values. A review to summate the current literature will help to design future studies to address gaps in the field. This paper reviews the use of DTI parameters as biomarkers for diagnosing and predicting PCS after mTBI. By summarizing the current literature on the use of DTI parameters in patients with PCS after an mTBI, this paper aims to assist future researchers, clinicians, and patients in determining the role of DTI as a diagnostic tool for and predictor of PCS.

METHODS

Literature Search

A comprehensive literature search was conducted on AMED, Embase, MEDLINE, Ovid, PubMed, Scopus, and Web of Science for all relevant articles reporting on the use of DTI in subjects who developed PCS post-mTBI, through 20 May, 2016. The databases were searched with the following search phrase using the Boolean logic operators "OR" and "AND": "(DTI OR diffusion tensor imaging OR diffusion tractography) AND (mTBI OR mild traumatic brain injury OR concussion) AND (postconcussive syndrome OR post-concussive syndrome OR post concussive syndrome OR postconcussion syndrome OR post-concussion syndrome OR post concussion syndrome) AND human." To ensure maximal article capture, these search terms also encompassed the following Medical Subject Headings (MeSH) terms: "diffusion tensor imaging," "brain injuries," and "post-concussion syndrome." Manual searching of relevant journals and reference lists of studies found in the above search provided additional articles.

The search terms identified above yielded 205 studies. For this review, the PCS population was defined as patients who experience persistent symptoms for 3 months or longer post-injury. Inclusion criteria were studies published in English; use of human

participants; and studies that analyzed changes in measured DTI parameters in patients with PCS following an mTBI. Exclusion criteria were studies that did not report method of diagnosing PCS; studies that did not report method of diagnosing mTBI; studies that were not original research; and studies with fewer than six participants. Two readers independently screened all 205 studies, removing 79 duplicates, 16 conference abstracts, and 1 foreign language paper. Six additional studies were identified from relevant reference lists and journals. Of the 115 studies remaining, 92 were excluded for containing one or more exclusion criterion. A further 13 studies were removed after rescreening because they included patients who were assessed for PCS symptoms within 3 months of injury. This resulted in 10 studies that were included in this review. The flow diagram for the paper selection process is presented in **Figure 1**.

Data Extraction and Analysis

Each study was assessed by two reviewers for quality using STROBE guidelines (41). A point was awarded for each required criterion that was met. The two reviewers performed individual assessments, and scores were compared, with discrepancies resolved by discussion. The two reviewers also performed data extraction for the population studied, patient source, number of PCS patients, patient demographics (age and gender), mechanism of injury, mTBI diagnosis method, PCS diagnosis method, time interval between injury and PCS diagnosis, time interval between injury and imaging, control group characteristics (screening process, number, and matching process), DTI values measured, DTI analysis method [voxel-wise or region of interest (ROI)], significant findings, conclusions (correlations between DTI and PCS), and study limitations for all nine studies. DTI studies most often do not quantify changes in DTI parameters because there are no established values for healthy patients, so only qualitative changes (increases and decreases) in these parameters were extracted for this review. Results of data extraction were compared between the reviewers, with discrepancies resolved with help from a third reader.

RESULTS

A total of 205 studies were screened for eligibility, with 10 studies, published within the last 6 years, qualifying for review based on the inclusion and exclusion criteria. All studies scored between 17 and 20 points out of a possible 22 in STROBE quality assessment. The 10 studies included PCS patients from 8 to 65 years of age, with the mean age across all studies being 29.58 years. One study analyzed a pediatric population (31), one study analyzed a mixed pediatric and adult population (35), and the rest analyzed adult populations. Also, 235 PCS patients were included in the 10 studies reviewed, with 141 male and 44 female PCS patients tested in the 8 studies that reported sex breakdown. All studies included control groups. One study (36) used a combination of patients with orthopedic injuries and healthy patients as controls, two studies (34, 39) used uninjured military members as controls, and the remaining seven studies used healthy controls. Nine studies (31–36, 38–40) performed a group-wise

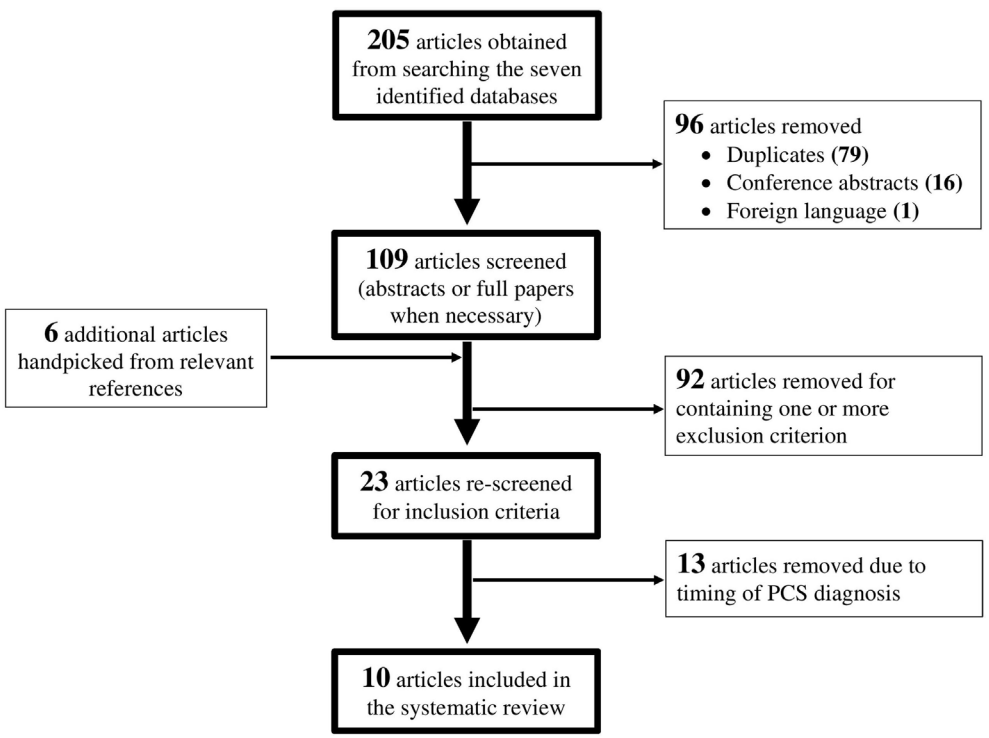

FIGURE 1 | Flow chart depicting the paper selection process.

comparison between controls and PCS subjects, while the last study (37) directly matched PCS patients to controls. Matching was achieved on the bases of age, gender, and years of education.

For the DTI analysis, four studies (33, 38–40) performed a whole brain voxel-wise analysis, four studies (31, 32, 34–36) used an ROI analysis, and two studies (37) used a voxel-wise approach to identify ROIs for subsequent ROI analysis. In the 10 studies reviewed, the time interval between injury and imaging ranged from 7 days to 259 months, with a median imaging time of 20.5 months post-injury. The time interval between injury and PCS assessment ranged from 3 to 259 months, with a median assessment time of 23.2 months post-injury.

Seven of the ten studies analyzed DTI parameters in imaging conducted after PCS was diagnosed, thereby assessing the value of DTI as a biomarker of PCS (**Table 1**). The remaining three studies analyzed imaging conducted at the subacute or pre-PCS stage of injury in patients who were later diagnosed with PCS (**Table 2**). The importance of these studies is in the prediction of PCS development in mTBI patients.

A decrease in FA and increase in MD and RD were commonly observed in PCS patients post-mTBI. The most common finding across all studies was that FA decreased in patients with PCS following mTBI compared to controls, although three studies included in the review found no significant changes in FA (**Table 3**). An increase in MD was also found, although three of the six studies that analyzed MD did not find a significant change (**Table 4**). Messé et al. (38) compared mTBI patients with PCS to mTBI patients not experiencing PCS and found higher MD values in the PCS-present group. An increase in RD was also found in three of five studies that analyzed RD (**Table 5**). No changes in AD were observed in the five studies that analyzed this parameter. These changes in DTI parameters also had a positive correlation with PCS symptom severity. The corpus callosum was most frequently reported as being affected in PCS, with reduced FA and increased MD and RD (**Table 6**).

DISCUSSION

Findings in DTI Parameters

Decreased FA and increased MD and RD were reported in PCS patients compared to healthy controls or PCS-absent trauma patients. Abnormal DTI values were also reported to be correlated with an increase in number and severity of PCS symptoms, suggesting that greater axonal damage causes more severe PCS symptoms. Decreased FA is a well-documented finding in brain injury (42–46), as is increased MD (42, 44, 45) and RD (43). In addition, regions within the corpus callosum were most often found to be affected in PCS patients. Two additional articles reporting on case studies also found decreased FA, specifically in PCS patients (47, 48). The corpus callosum is involved in inter-hemispheric integration of motor, sensory, and cognitive information, and damage to this area might lead to extensive behavioral, emotional, and cognitive impairments, as observed in the PCS groups. Although the effects of damage to the corpus

TABLE 1 | Studies looking at DTI parameters as a biomarker for PCS.

Article	Population studied	Diagnosis method (mTBI; PCS)	DTI analysis approach	DTI values measured
Bartnik-Olson et al. (31)	Pediatric patients, sustained a sports-related mTBI in an organized athletic event	International Conference on Concussion in Sport; self-reported symptoms	ROI analysis	FA, MD, RD, AD
Bouix et al. (32)	Patients with persistent PCS, sustained an mTBI in an MVC, blast exposure, sports-related event, or assault	Emergency department triage; headaches, emotional dysregulation, cognitive, or memory impairments	ROI analysis	FA, MD, RD, AD
Dean et al. (33)	Patients who sustained an mTBI but did not report to the hospital, no reported mechanism of injury	WHO (ICD-10); RPCSQ	Voxel-wise analysis	FA
Delano-Wood et al. (34)	Military veterans with a closed head TBI from blast exposure or blunt force trauma	US DoD and the Department of Veterans Affairs TBI Task Force; NSI	ROI analysis	FA
Levin et al. (36)	Post-deployment veterans and service members, sustained mTBI in a blast exposure	Physician diagnosis; NSI	ROI analysis	FA, MD
Maruta et al. (37)	Patients with a single, isolated concussive injury to the head, no reported mechanism of injury	Physician diagnosis; self-reported symptoms	Voxel-wise and ROI analysis	FA, MD, RD, AD
Miller et al. (39)	Military veterans, sustained an mTBI in a blast exposure	American Congress of Rehabilitation Medicine; RPCSQ	Voxel-wise analysis	FA

TABLE 2 | Studies looking at DTI parameters in prospective PCS patients.

Article	Population studied	Diagnosis method (mTBI; PCS)	DTI analysis approach	DTI values measured
D'Souza et al. (35)	Patients from a neurosurgery clinic, no reported mechanism of injury	American Congress of Rehabilitation Medicine; RPCSQ	ROI analysis	FA, MD
Messé et al. (38)	Patients presenting to the emergency department, sustained an mTBI in an MVC, pedestrian injury, or aggression incident	American Congress of Rehabilitation Medicine; self-reported symptoms	Voxel-wise analysis	MD
Polak et al. (40)	PCS patients referred from a concussion clinic, sustained an mTBI in a sports-related event, fall, or when struck by an object	Physician diagnosis *via* the Buffalo Concussion Treadmill Test; WHO	Voxel-wise analysis	FA, MD, RD, AD

TABLE 3 | Studies that analyzed fractional anisotropy.

Article	Affected region	Change
Studies that found changes in FA		
Bouix et al. (32)	Whole brain	↓
Dean et al. (33)	Right anterior corona radiata, internal capsule (anterior limb), corpus callosum (splenium), fornix, frontal medial superior gyrus	↓
Delano-Wood et al. (34)	Pontine tegmentum	↓
D'Souza et al. (35)	Corpus callosum, left uncinate fasciculus, bilateral superior thalamic radiations	↓
Levin et al. (36)	Corpus callosum	↓
Miller et al. (39)	Greater number of white matter clusters	↓
Polak et al. (40)	Corpus callosum (genu)	↓
Studies that found no changes in FA		
Bartnik-Olson et al. (31)		
Maruta et al. (37)		
Messé et al. (38)		

TABLE 4 | Studies that analyzed mean diffusivity.

Article	Brain region	Change
Studies that found changes in MD		
Bartnik-Olson et al. (31)	Corpus callosum (genu)	↑
D'Souza et al. (35)	Left uncinate fasciculus	↑
Messé et al. (38)	Corpus callosum (forceps major and minor), inferior fronto-occipital fasciculus, inferior longitudinal fasciculus, superior longitudinal fasciculus, corticospinal tract, left anterior thalamic radiation	↑
Polak et al. (40)	Corpus callosum (genu)	↑
Studies that found no changes in MD		
Bouix et al. (32)		
Levin et al. (36)		
Maruta et al. (37)		

TABLE 5 | Studies that analyzed radial diffusivity.

Article	Brain region	Change
Studies that found changes in RD		
Bartnik-Olson et al. (31)	Internal capsule (right anterior limb)	↑
Bouix et al. (32)	Whole brain	↑
Polak et al. (40)	Corpus callosum (genu)	↑
Studies that found no changes in RD		
Maruta et al. (37)		
Messé et al. (38)		

TABLE 6 | Studies that found changes in the corpus callosum.

Specific region	DTI value	Change	Article
Genu	MD	↑	Bartnik-Olson et al. (31)
			Polak et al. (40)
	RD	↑	Polak et al. (40)
Splenium	FA	↓	Dean et al. (33)
Whole corpus callosum	FA	↓	Levin et al. (36)
			D'Souza et al. (35)
Forceps major	MD	↑	Messé et al. (38)
Forceps minor	MD	↑	Messé et al. (38)

callosum are not fully understood, it is reasonable to expect that these effects would include the symptoms of PCS patients.

Studies included in this review assessed different patient populations, including pediatric, adult, and military. Although age- and education-related differences in structural and functional neuroanatomy have been documented (49, 50), all of the studies had a case–control design. Patients were matched to controls by age, gender, and education level, therefore ensuring that changes detected in DTI parameters were not a result of comparisons made between inherently distinct populations. However, consistent findings in FA, MD, and RD across the studies suggest that the microstructural white matter damage detected is consistent in all patient groups. This possibility increases the value of DTI as a universal biomarker of PCS. In addition, 8 of the 10 studies (31–35, 38–40) excluded patients with abnormal CT or routine clinical MRI findings. This exclusion criterion indicates that there is damage identified by DTI, which is not detected *via* more commonly used modalities, further emphasizing the utility and importance of DTI in the clinical setting. It is often acknowledged that the more widely available structural neuroimaging modalities have limited value in the mTBI and PCS populations (8, 51, 52).

Post-concussion syndrome has been found to be more prevalent in the mTBI population than the moderate or severe TBI populations (19, 20). However, it has been noted that axonal injury in these more severely injured groups usually presents with focal lesions and so is detectable *via* clinical MRI (53). Therefore, not all axonal injury is indicative of PCS. This evidence suggests that generalized axonal injury does not correlate well with PCS, but diffuse axonal injury (DAI), which presents as widespread axonal injury limited to the microstructure scale, may be more specific to PCS. This is also supported by a recent study using magnetic resonance spectroscopy (MRS), which found significant correlations between DAI and PCS (54). However, PCS may not only be a result of neurological damage but may also develop due to psychological distress (55–57). For this reason, a patient who has not sustained an mTBI may still experience PCS because it is not unique to the mTBI or TBI population. Although patients with DAI may develop PCS, not all PCS patients have DAI.

ROI versus Voxel-Wise Analysis

Two studies (37, 39) that applied a voxel-wise analysis of the whole brain reported no significant findings in the corpus callosum. Maruta et al. (37) reported no significant findings in any brain region but was the only study to have no significant findings in all four DTI parameters in a whole brain analysis. Miller et al. (39) did find a decreased FA in several white matter clusters in the whole brain but did not identify specific brain regions where the clusters were located. Voxel-wise analysis requires intersubject registration of subjects' brains and normalization to standard atlases. Both of these processes involve smoothing and hence may result in masking small differences between subjects or groups. All studies that used ROI analysis found significant changes in DTI parameters, suggesting that ROI analysis might be more proficient at identifying changes in specific brain regions. However, ROI analysis has some limitations: it requires a brain structure to be predefined for analysis, and manual selection of

the region to draw an ROI may lead to intersubject differences in ROI location. Despite these limitations, the success of ROI analysis in the studies reviewed is encouraging; once a particular region has been identified as most commonly damaged in PCS, diagnosis *via* DTI will be faster than if a voxel-wise approach was necessary.

Data Synthesis

Although DTI can detect axonal damage and possibly predict PCS onset or be used to diagnose PCS, there is insufficient evidence supporting the observed results to validate any parameter in a specific brain region as a biomarker for PCS. The trends in the parameters are a decrease in FA and an increase in MD and RD, but there is no uniformity in the brain areas investigated for these changes. These findings suggest that there may be more than one DTI biomarker for PCS and that axonal damage does contribute to PCS symptoms. The subjective nature of PCS and the possibility of a large number of brain regions being involved in PCS may have led to the indefinite results. Each patient has a unique illness experience due to their baseline for many of the symptoms, such as fatigue, feelings of depression, feelings of frustration, forgetfulness, poor concentration, and restlessness. Patients have varying pain tolerances and emotional fortitudes that could either magnify or diminish the severity of PCS symptoms being reported. In addition, a researcher may focus on one or more brain regions or on the entire brain, depending on his or her research interests and image processing preferences.

Other Potential PCS Biomarkers

Other imaging modalities have been used to study PCS. In addition to the poor value of CT and MRI in the PCS population, studies have shown conflicting results in positron emission tomography (PET) in the PCS population, with some finding correlations between PET results and PCS (58–60), while others do not (51). A more promising imaging modality may be MRS, which has been used to detect DAI that is significantly correlated with PCS (54), although there is unsubstantial evidence that it is a reliable PCS biomarker. Evoked potential (EP) studies have concluded that significant results in the EP data correlate with PCS (61, 62), but there is not enough concrete evidence to support these measurements as a PCS biomarker. Biochemical markers of PCS may also be viable. A review paper (63) identified S100 proteins, neuron-specific enolase (NSE), and cleaved Tau protein (CTP) as potential serum biochemical markers for predicting PCS after an mTBI. The review concluded that none of the three compounds are well-validated for use, although S100 was most widely studied in the mTBI population and appears to be the most promising. It is possible that a combination of DTI, clinical factors, and biochemical markers may be needed to accurately and objectively diagnose PCS or predict its development after an mTBI.

Future Research Directions

Further research into this topic is necessary. All of the studies included in this review are cross-sectional. Future studies should consider a longitudinal cohort study design to track changes in DTI parameters during PCS progression and resolution, which would provide more concrete evidence of a specific biomarker for PCS. Recruiting a larger PCS patient population is required to reduce sample size biases. Research on DTI parameters in the brain is also required to establish "normal" values for FA, MD, RD, and AD, so that significant differences are based on a universal standard as opposed to being derived from each study's controls. This will ensure that significant results are not misrepresented. Future case–control studies should try to use an orthopedic or other non-head trauma control group to help remove error in PCS reporting due to factors other than the sustained mTBI. Case–control studies may also consider comparing PCS-present mTBI patients to PCS-absent mTBI patients to eliminate most external confounding factors. These studies would be more likely to observe changes in DTI parameters specifically due to PCS. The large range of time intervals between injury and imaging reported in the ten reviewed articles is also a concern. In addition, 13 articles were excluded from this review because researchers conducted PCS assessment within 3 months of injury. It is suggested that future studies include patients whose PCS symptoms persist for 3 months or longer, as the literature supports PCS diagnosis when symptoms persist for this length of time. Finally, a standard PCS assessment should be administered to ensure consistency across all future studies. Consulting the Common Data Elements identified by the National Institutes of Health would help establish assessment standards.

CONCLUSION

To our knowledge, this is the first systematic review that examines the use of DTI parameters in the human brain as a diagnostic tool for patients with PCS and a predictor of PCS in mTBI patients. DTI abnormalities indicate axonal damage, which leads to an increased risk of developing PCS after an mTBI. However, no DTI biomarker for PCS is identified due to the small body of research conducted on the topic and the heterogeneity of results reported. Further research is required to establish a standard DTI biomarker for PCS diagnosis and prediction.

AUTHOR CONTRIBUTIONS

EK and NO – study concept, study design, literature search, quality assessment, data extraction and analysis, manuscript draft, manuscript critical revision, and final approval. EH and MC – study concept, study design, manuscript critical revision, and final approval. All authors agree to be accountable for all aspects of the work.

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Role of Anticonvulsants in the Management of Posttraumatic Epilepsy

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Posttraumatic seizures (PTS) have been recognized as a major complication of traumatic brain injury (TBI). The annual incidence of TBI in the United States is 1.7 million. The role of anticonvulsants in the treatment of posttraumatic epilepsy (PTE) remains uncertain. Based on current studies, however, anticonvulsants have been shown to reduce early PTS occurring within the first 7 days, but little to no benefits have been shown in late PTS occurring after 7 days. In this paper, we provide a mini review of the role of anticonvulsants and current advances in the management of PTE.

Keywords: posttraumatic seizures, traumatic brain injury, epilepsy, anticonvulsants, management

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POSTTRAUMATIC EPILEPSY

Posttraumatic epilepsy (PTE) due to traumatic brain injury (TBI) has many causes, including wartime combat, vehicle accidents, work-related injuries, and sports injuries. Wartime combat injuries, especially blast injuries and penetrating head injuries (PTI), have shown to increase the risk of seizures, as that of blast models of TBI (1–4). The annual incidence of TBI is estimated to be 1.7 million in the United States, and seizures have been recognized as one of the major complications of this condition (5). The incidence of PTE was described by Annegers and colleagues who conducted a retrospective study in order to identify the characteristics of brain injuries that are associated with the development of seizures for 50 years. The results showed that the severity of the injury was correlated with the interval during which the risk of seizures was increased, even after more than 20 years post injury (6). The other study of interest was the Vietnam Head Injury Study (VHIS) that was a prospective, longitudinal follow-up of 1,221 Vietnam War Veterans who had PTI. The prevalence of PTE in this cohort was 45–53%. Patients with PTI carry a high risk of PTE even for decades; so, long-term medical follow-up is required (7). Similarly, the prospective study by Salazar and colleagues showed that seizure frequency in the first year predicted future severity of seizures. A higher seizure frequency was seen in the first year and was also associated with subjects having a longer duration of epilepsy and persistent seizures (8).

ROLE OF ANTICONVULSANTS IN THE MANAGEMENT OF POSTTRAUMATIC EPILEPSY

The seizures after head injury result in secondary brain damage, which involves increased intracranial pressure, increased metabolic brain demands post head injury, and excessive release of neurotransmitters,

which result in further complicating the existing damage. The main goal of anticonvulsants is to minimize the brain damage by preventing early seizures (9).

The other role of anticonvulsants apart from antiseizure activity is the neuroprotective effect, which has been demonstrated in animal models. Phenytoin, which is still considered as an agent of choice, has been shown to have neuroprotective properties in animal models. Vartanian and colleagues showed that phenytoin has been linked with decreased neuronal damage in neonatal rats following hypoxia (10). Another study by Tasker and colleagues showed similar results in rat hippocampal structures (11). Researchers suggested that neuroprotective effects were related to a blockage of voltage-dependent sodium channels during hypoxia, which decreased the spread of calcium-induced neurotoxicity following hypoxic brain injury (10, 11).

Posttraumatic seizures (PTS) are divided into two subgroups, early and late PTS. Early seizures occur within the first 7 days after brain injury, and late seizures occur after 7 days of injury. These definitions are important in terms of management and predicting prognosis of PTE (12).

The prospective randomized trials did not show promising results of the role anticonvulsants in the management of PTS. The randomized clinical trials are summarized in **Table 1**. No significant differences were seen in the treatment versus the non-treatment groups (13–20). Summary of selected non-randomized trials for posttraumatic seizure prevention was shown in **Table 2**, which also did not show a significant difference between groups (21–28).

Temkin and colleagues showed that phenytoin was considered effective in preventing provoked seizures and promising at preventing unprovoked seizures. Carbamazepine was considered effective in preventing provoked seizures after TBI, although its status was considered uncertain in preventing unprovoked seizures. Phenobarbital was considered promising at preventing provoked seizures and uncertain at preventing unprovoked seizures. Finally, the combination of phenytoin and phenobarbital was considered promising to prevent provoked and unprovoked seizures. It was also shown that provoked seizures showed promising results, but for unprovoked seizures, no drugs were shown to be effective. AEDs prescribed to prevent epileptogenesis should be avoided until clinical trials have found a drug for this purpose (29). Similarly, Chang and Lowenstein conducted a literature review of the evidence of AED prophylaxis in patients with severe TBI in order to guide better practice recommendations. Patients given phenytoin prophylaxis compared to controls had a significantly lower risk of early PTS in

TABLE 1 | Summary of selected randomized controlled trials (RCT) for posttraumatic seizure prevention.

Reference	Study design	Number of patients randomized (*N*)	Methods	Outcome
Dikmen et al. (13)	RCT	124	Phenytoin versus placebo Patients were randomized to receive either PHT or placebo for 1 year and observed one more year without medication	No significant differences seen in neuropsychological examinations in 1 year between the 2 groups
Temkin et al. (14)	RCT	123	Phenytoin versus placebo Treatment was started within 24 h of injury for 1 year and then 2 groups were followed for 2 years	Early seizures: improvement seen in the PHT GROUP Late seizures: no difference between the 2 groups
Young et al. (15)	RCT	244	Phenytoin versus placebo Treatment was started within 24 h of injury	Early seizures: no difference between the 2 groups Late seizures: study was not designed to determine late seizure outcome
Young et al. (16)	RCT	179	Phenytoin versus placebo Treatment was started within 24 h of injury and 2 groups were followed for 18 months to determine late seizure outcome	Early seizures: study was not designed to determine early seizure outcome Late seizures: no difference between the 2 groups
McQueen et al. (17)	RCT	164	Phenytoin versus placebo Two groups were followed for 2 years Occurrence of seizures was used as outcome measure	Early seizures: study was not designed to determine early seizure outcome Late seizures: no difference between the 2 groups
Szaflarski et al. (18)	RCT	52	Phenytoin versus levetiracetam Treatment was started within 24 h of injury between the 2 groups	Early seizures: no difference between the 2 groups Late seizures: study was not designed to determine late seizure outcome
Temkin et al. (19)	RCT	379	Phenytoin for 1 week versus valproate for 1 month versus valproate for 6 months Treatment was started within 24 h of injury Follow-up of these groups continued for 2 years	Early seizures: no difference among 3 groups Early seizures: no difference among 3 groups
Manaka (20)	RCT	191	Phenobarbital versus no treatment Treatment was started within 4 weeks post head injury They received full dose for 2 years and tapered off in third year Follow-up in 5 years	Early seizures: study was not designed to determine early seizure outcome Late seizures: no difference among 3 groups

TABLE 2 | Summary of selected non-randomized trials for posttraumatic seizure prevention.

Reference	Study design	Number of patients randomized (*N*)	Methods	Outcome
Servit and Musil (21)	Non-RCT	167	Treatment group (n = 143) were administered phenytoin or phenobarbital Control group (n = 24) where conventional treatment was used Duration: 2 years	Early seizures: not applicable Late seizures: 25% in the control group and 2.1% in the treatment group
Inaba et al. (22)	Prospective controlled trial	813	Participants were administered either levetiracetam or phenytoin for 7 days	Early seizures: no difference between the 2 groups Late seizures: not applicable
Kruer et al. (23)	Retrospective cohort	109	Retrospective review of patients who received levetiracetam or phenytoin	Early seizures: no difference between the 2 groups Late seizures: not applicable
Gabriel and Rowe (24)	Cohort	19	Participants were divided based on levetiracetam and phenytoin prophylaxis Follow-up interview conducted to assess seizure outcome	Early seizures: no difference between the 2 groups Late seizures: no difference between the 2 groups
Jones et al. (25)	Cohort	27	Phenytoin versus levetiracetam administered during first 24 h post severe TBI	Early seizures: no difference between the 2 groups Late seizures: not applicable
Bhullar et al. (26)	Case-control	93	Phenytoin versus no treatment to determine occurrence of early seizures	Early seizures: no difference between the 2 groups Late seizures: not applicable
Formisano et al. (27)	Retrospective and prospective	137	Anticonvulsants versus no treatment Study 1: prospective Study 2: retrospective	Study 1 – No difference between the 2 groups Study 2 – Late seizures higher in the treated group
Watson et al. (28)	Cohort	404	Glucocorticoids administered within 1 day versus no glucocorticoids	Early seizures: not applicable Late seizures: no difference between the 2 groups

pooled class I studies. There were no significant differences in the risk of late PTS patients receiving phenytoin, carbamazepine, or valproate prophylaxis versus controls in pooled class I and class II studies. In these studies, adverse effects were frequent, but mild and serum AED levels were suboptimal. The authors concluded that phenytoin prophylaxis is effective in decreasing the risk of early PTS in adult patients with severe TBI. However, late PTS are not decreased by AED prophylaxis (30). Current guidelines issued by the Brain Trauma Foundation and the American Academy of Neurology (AAN) for the management of severe TBI recommend seizure prophylaxis only for 7 days post injury. Phenytoin still remains the desired treatment because it has been extensively studied and there is proven evidence of its efficacy. The other antiepileptic agents, such as phenobarbital, valproate, and carbamazepine, have gone through limited trials as compared to phenytoin and their adverse effect profiles and pharmacodynamics properties still make phenytoin the desired antiepileptic for early prophylaxis of PTS (30).

The new anticonvulsants were favored over the older agents because of their unique pharmacokinetic properties, fewer serious side effects, and fewer drug–drug interactions (31). A second generation anticonvulsant that has generated particular interest is levetiracetam (23). Jones and colleagues conducted a retrospective study, which showed that levetiracetam can be used as an alternative to phenytoin, but the study was limited due to a small sample size (25). Szaflarski and colleagues conducted the first prospective randomized comparative trial of intravenous levetiracetam versus phenytoin for seizure prophylaxis in the neurointensive care unit setting, which showed that levetiracetam can be used as an alternative to phenytoin for seizure prophylaxis in the neuroscience intensive care setting. This study had limitations, including a small sample size and a lack of data reported on the concomitant use of sedating agents for induction of pharmacologically-induced coma (18). Kirmani and colleagues also conducted a literature review on the role of intravenous levetiracetam in seizure prophylaxis of severe TBI patients, which showed that levetiracetam can be used as an option in acute PTS (32). Gabapentin (GBP) is another anticonvulsant that acts at the α2δ-1 submit of the L-type calcium channel. It has been shown that chronic administration of GBP after cortical injury is antiepileptogenic in the undercut model of PTE. The results suggest that it may have a neuroprotective effect and may also decrease excitatory synapse formation. These results suggest the potential use of GBP as an anticonvulsant following TBI (33). A meta-analysis by Zafar and colleagues also concluded that there is no particular drug that is superior in preventing early seizures (34).

Wroblewski and Joseph reported 10 case studies of TBI patients treated with intramuscular midazolam for acute seizure cessation after other benzodiazepine drugs had failed. Slight to moderate sedation was the only reported side effect. The study was limited due to small sample size and was conducted to treat rather than prevent PTS (35).

The role of anticonvulsants in early PTS seems favorable as compared to late PTS. Anticonvulsants are found to be effective in patients who develop PTE.

Phenytoin remains the most commonly used anticonvulsants, but the side effects do favor the use of newer anticonvulsants, e.g., levetiracetam because of lack of drug–drug interactions and availability in parenteral form. The cognitive side effects and non linear kinetics limit the use in certain patient populations (13). Carbamazepine has shown to be effective but drug–drug interactions and unavailability in parenteral form limits the use

of this agent. Neurocognitive side effects were also seen in other older anticonvulsants, including Phenobarbital, which may mask the mental status findings in TBI patients because of the sedating effects. Valproate can cause coagulopathy which may result in intracranial hemorrhage (30, 31).

Unfortunately, limited scientific data exist, which are specific to PTE with other anticonvulsants, and there is a need for additional controlled randomized clinical trials to explore more options.

NEW DIRECTIONS IN THE MANAGEMENT OF POSTTRAUMATIC EPILEPSY

The PTE can be differentiated from PTS that are sequelae from TBI. The term PTE signifies recurrent seizure disorder due to TBI or any surgery on the brain (36).

Posttraumatic epilepsy remains a challenge despite new medications that have come in the last decade. We still face problems with effective seizure control. TBI is the most common cause of acquired focal epilepsy (37–40). The model that was commonly used to assess antiepileptogenic interventions is the rostral parasagittal fluid percussion injury model (rpFPI) (41–43). This model helps in mimicking closed head injury by reproducing destructive processes as well as regenerative inflammatory processes (44). This is now considered as an excellent model for PTE (41–44). This model may progress to intractable multifocal epilepsy after a few months post injury (45). Interestingly, this model has shown to represent a severe form of PTE which is not controlled by carbamazepine, valproic acid, and carisbamate (41, 42). One study using this model showed mild cooling of epileptogenic focus and prevention of recurrent seizures. Based on the above studies, prolonged and mild cooling has been tried in these subgroups of patients and was found to be safe and improve functional recovery (46–49).

The animal model data also show that it is possible to target anti-inflammatory agents that are used for other indications as alternative to anticonvulsants. Progesterone has been shown to have promising effects in several brain injury models (50). The smaller sample size in humans did show some positive results (51, 52). However, the current evidence is insufficient to support the use of progesterone in the management of TBI (53).

CONCLUSION

Anticonvulsants have proven to be beneficial in the first 7 days post injury. Phenytoin still remains the anticonvulsant of choice because it is widely studied and researched as compared to other anticonvulsants. Levetiracetam seems to be a viable alternative because of its unique pharmacodynamics properties; however, more head-on prospective clinical trials are needed regarding phenytoin in order to prove its efficacy as a first line agent in PTE. Clinical trials are needed to study the efficacy of second and third generation anticonvulsants in the treatment of PTE. Clinical trials are also needed to prove the role of mild selective cooling in patients with PTE.

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All authors listed, have made substantial, direct, and intellectual contribution to the work and approved it for publication.

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REVIEW
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Effects of Soccer Heading on Brain Structure and Function

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Soccer is the most popular sport in the world, with more than 265 million players worldwide, including professional and amateur ones. Soccer is unique in comparison to other sports, as it is the only sport in which participants purposely use their head to hit the ball. Heading is considered as an offensive or defensive move whereby the player's unprotected head is used to deliberately impact the ball and direct it during play. A soccer player can be subjected to an average of 6–12 incidents of heading the ball per competitive game, where the ball reaches high velocities. Moreover, in practice sessions, heading training, which involves heading the ball repeatedly at low velocities, is common. Although the scientific community, as well as the media, has focused on the effects of concussions in contact sports, the role of subconcussive impacts, as it can occur during heading, has recently gained attention, considering that it may represent an additional mechanism of cumulative brain injury. The purpose of this study is to review the existing literature regarding the effects of soccer heading on brain structure and function. Only in the last years, some investigations have addressed the impact of heading on brain structure, by using neuroimaging techniques. Similarly, there have been some recent studies investigating biochemical markers of brain injury in soccer players. There is evidence of association between heading and abnormal brain structure, but the data are still preliminary. Also, some studies have suggested that subconcussive head impacts, as heading, could cause cognitive impairment, whereas others have not corroborated this finding. Questions persist as to whether or not heading is deleterious to cognitive functioning. Further studies, especially with longitudinal designs, are needed to clarify the clinical significance of heading as a cause of brain injury and to identify risk factors. Such investigations might contribute to the establishment of safety guidelines that could help to minimize the risk of possible adverse effects of soccer on brain structure and function.

Keywords: sports, soccer, heading, injury, brain, cognition

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INTRODUCTION

Soccer is the most popular sport in the world, with more than 265 million players worldwide, including professional and amateur ones (1). Although it is a sport not traditionally identified as high-risk for concussions (2–4), soccer players are prone to traumatic brain injury (5–7), and up to 22% of all soccer injuries are concussions (8). Furthermore, several studies have shown that concussion rates in

soccer are comparable to, and often exceed, those of other contact sports traditionally considered as inherently more violent, such as football and ice hockey (9). A prospective study investigating female middle-school soccer players (10) reported that heading the ball accounted for 30.5% of concussions. Similarly, a recent retrospective analysis involving high-school soccer players (11) showed that heading was responsible for 30.6% of concussions among boys and 25.3% among girls, although the most frequent mechanism of injury in heading-related concussions was player–player contact during the ball dispute.

Head injury during soccer is usually the result of either direct contact (e.g., head vs. head, head vs. knee, and head vs. the ground) or contact with the ball while heading it. In this sense, soccer is unique in comparison to other sports, as it is the only sport in which participants purposely use their head to hit the ball. Heading is considered an offensive or defensive move whereby the player's unprotected head is used to deliberately impact the ball and direct it during play. Players may head the ball to pass to another player, move the ball down the field, or score a goal. To counter to external forces to the head during impact, players must prepare for impact by bracing the neck musculature and properly execute the technique by moving the entire body in one motion (12). A soccer player can be subjected to an average of 6–12 incidents of heading the ball per competitive game, where the ball reaches high velocities (13). Moreover, in practice sessions, heading training, which involves heading the ball repeatedly at low velocities, is common. Although the scientific community, as well as the media, has focused on the effects of concussions in contact sports, the role of subconcussive impacts, as it can occur during heading, has recently gained attention, considering that it may represent an additional mechanism of cumulative brain injury. The term "subconcussive" was proposed to describe the impact to the head that may cause neuronal dysfunction in the absence of concussive symptoms (14).

Heading involves repeated impact, acceleration–deceleration of the brain inside the skull, and possibly rotation of the brain. Moreover, cumulative effects of repetitive minor injury may not manifest for many years, as in chronic traumatic encephalopathy (15). Therefore, pathological evidence of traumatic brain injury, if detectable, is likely to present prior to onset of overt symptoms or disability. Importantly, the possible negative effects of heading may depend on the rate of exposures, the time between exposures, and the vulnerability of individual players (15).

The purpose of this study is to review the existing literature regarding the effects of soccer heading on brain structure and function. These investigations have explored the consequences of immediate (e.g., after a soccer match), short-term (e.g., after one or few soccer seasons), and long-term (e.g., after many soccer seasons) heading exposure. Only in the last years, some studies have addressed the impact of heading on brain structure, by using neuroimaging techniques. Similarly, there have been some recent studies investigating biochemical markers of brain injury in soccer players. There is evidence of association between heading and abnormal brain structure, but the data are still preliminary. Also, some studies have suggested that subconcussive head impacts, as heading, could cause cognitive impairment, whereas others have not corroborated this finding.

We searched three databases – such as PubMed, LILACS, and Scopus – for articles published until December 2015, by using the terms "soccer," "heading," and "brain." The search retrieved 92 articles published from 1981 to 2015. Considering the inclusion criteria, which involved papers with original research design, English language, and focus on the effects of soccer heading on brain structure and/or function, 29 articles were selected for the present review. We had full access to all these studies, except to two of them.

EFFECTS OF SOCCER HEADING ON BRAIN STRUCTURE

Some investigations have demonstrated changes in brain structure of soccer players and suggested an association between these changes and soccer heading. However, the data are still preliminary, as summarized in **Table 1**.

Neuroimaging

A small number of studies have investigated the impact of heading on brain structure by using neuroimaging techniques. An early study by Sortland and Tysvaer (16) evaluated male former professional soccer players, aged between 39 and 68 years, who were submitted to cerebral computer tomography (CCT) with assessment for brain atrophy, visually and by linear measurements. The results showed that, by visual grading, about one-third of the players had slight to moderate central atrophy with widening of the lateral ventricles, which was strongly supported by linear measurements compared to normal controls. The authors assumed that the brain damage is a result of playing soccer for years, therefore a consequence of long-term heading exposure, with multiple small head injuries mainly connected with heading.

More recently, Lipton et al. (15) investigated white matter microstructure in male and female amateur soccer players, with a mean age of 30.9 years, by using the diffusion tensor magnetic resonance imaging (MRI) technique. The primary imaging outcome was fractional anisotropy (FA), which provides assessment of the degree of anisotropic diffusion, defined as the presence of diffusional movements in different directions, occurring within a brain region. FA tends to be high in regions of high cellular organization, and low in regions where the cells are not specifically oriented (17). Therefore, FA is often used to measure the integrity of white matter, since it reflects the degree of myelination and axonal density. The participants completed a questionnaire to quantify heading in the prior 12 months, which characterizes a short-term heading exposure, and were also submitted to a computerized neuropsychological evaluation, which aimed to assess psychomotor speed, attention, executive function, and memory. Each soccer player headed approximately 432 times over the previous year. High frequency of heading was associated with lower FA at three locations in temporo-occipital white matter, and also with poorer performance in the memory test. Interestingly, these associations were not linear, but there were thresholds in terms of number of headings needed to trigger FA reduction (between 885 and 1550 headings per year, depending on the brain region) and cognitive impairment (1800 headings per year). The results,

TABLE 1 | Summary of the studies investigating the effects of soccer heading on brain structure.

Reference	Samples	Methods	Heading exposure	Main results
Neuroimaging				
Sortland and Tysvaer (16)	Male former professional soccer players	CCT	Long-term exposure	One-third of the players had slight to moderate central atrophy with widening of the lateral ventricles
Lipton et al. (15)	Male and female amateur soccer players	Diffusion tensor MRI	Short-term exposure	High frequency of heading was associated with lower FA at three locations in temporo-occipital white matter
Koerte et al. (18)	Male former professional soccer players and non-contact sport athletes	MRI	Long-term exposure	Greater cortical thinning with increasing age in the right inferolateral–parietal, temporal, and occipital cortex was demonstrated in soccer players compared to controls
Jordan et al. (21)	Male professional soccer players and track athletes	MRI	Long-term exposure	No differences were verified in brain structure between soccer players and controls
Biochemical markers of brain injury				
Mussack et al. (22)	Male amateur soccer players and patients after minor traumatic brain injury	Analysis of serum S-100B levels	Immediate exposure	S-100B serum levels were elevated after heading when compared to normal exercise. None of the soccer players reached S-100B serum levels verified in subjects showing traumatic brain injury
Stålnacke et al. (24)/Stålnacke et al. (25)	Male professional soccer players/female professional soccer players	Analysis of serum S-100B and NSE levels	Immediate exposure	Serum levels of S-100B and NSE increased after a game. Increases in S-100B were positively correlated to the number of headings and of other trauma events
Straume-Naesheim et al. (26)	Male professional soccer players	Analysis of serum S-100B levels	Immediate exposure	Serum levels of S-100B increased after a regular league match, with or without head impact, after a high-intensity training session without heading, and after a low-intensity training session with heading exercises. The increase for the match groups was higher than for the training groups, but no differences were seen between the two match groups or the two training groups
Bamaç et al. (27)	Male professional soccer players	Analysis of serum NGF and BDNF levels	Immediate exposure	Serum levels of NGF and BDNF were elevated in response to heading exercises
Koerte et al. (29)	Male former professional soccer players and non-contact sport athletes	Magnetic resonance spectroscopy	Long-term exposure	Increases in choline and myo-inositol were verified in soccer players when compared with controls. Myo-inositol and glutathione were positively correlated with lifetime estimate of headings
Zetterberg et al. (30)	Male amateur soccer players and non-athletic subjects	Analysis of serum and/or cerebrospinal fluid concentrations of NF-L, T-tau, GFAP, S-100B, and albumin	Immediate exposure	There were no differences in concentrations of biomarkers of brain injury between soccer players who performed 10 or 20 headings or between either of these two groups and the control group. Biomarker levels did not correlate with the number of headings
Stålnacke and Sojka (31)	Male amateur soccer players	Analysis of serum S-100B levels	Immediate exposure	There were no increases in serum levels of S-100B after a heading session. No differences were seen in S-100B between players who performed or not headings, either before or after the session

CCT, cerebral computed tomography; MRI, magnetic resonance imaging; FA, fractional anisotropy; NSE, neuron-specific enolase; NGF, nerve growth factor; BDNF, brain-derived neurotrophic factor; NF-L, neurofilament light protein; T-tau, total tau protein; GFAP, glial fibrillary acidic protein.

which suggest an association between heading and abnormal white matter microstructure and also poorer cognitive performance, were not explained by lifetime concussion history and demographic features. Nonetheless, a limitation of this study is its cross-sectional nature, which avoids the establishment of a clear causal relationship between heading and brain changes.

Koerte et al. (18), in a recent investigation, evaluated cortical thickness in male former professional soccer players, with a mean age of 49.3 years, compared to age- and gender-matched former professional non-contact sport athletes, by using high-resolution structural MRI. All individuals, in both groups, were still actively participating in their respective sports at the time of the study. Soccer players were asked to inform how many headings they performed per week during the past 12 months prior to the investigation, and such a number was multiplied by the total years of formal training in soccer in order to obtain a lifetime estimate of headings, that is, an approximate calculation of long-term heading exposure. The results demonstrated greater cortical thinning with increasing age in the right inferolateral–parietal, temporal, and occipital cortex in soccer players compared to controls. In addition, cortical thinning in soccer players was associated with lower cognitive processing speed in the *Trail Making Test A* (19), which measures visual search and psychomotor speed, as well as with estimated exposure to repetitive subconcussive head impact. Also, a cognitive comparison between groups revealed decreased memory performance in soccer players, in relation to controls, in the *Rey–Osterrieth Complex Figures Test* (20), which measures visuoconstruction, planning and organization, and visual memory, although findings were in the normal range for both groups. According to the researchers, despite the limitations of the study, which include the small sample size and athletes self-report about history of headings, the results suggest that repetitive subconcussive head impact may play a role in age-related cortical thinning that may lead to early cognitive decline in soccer players.

On the other hand, a previous study by Jordan et al. (21) failed to find any evidence of brain structure damage in soccer players. Male professional soccer players, with a mean age of 24.8 years, were compared with age- and gender-matched elite track athletes with respect to a questionnaire regarding symptoms of head and neck injuries, as well as to MRI abnormalities. A heading exposure index was developed to assess a dose–response effect of chronic heading over the player's career. Thus, the authors aimed to examine the consequences of long-term heading exposure. Questionnaire analysis and MRI results demonstrated no significant differences between groups. Among the soccer players, there was no correlation between the outcome variables and heading exposure parameters. However, reported head injury symptoms correlated significantly with histories of prior acute head injuries. According to the researchers, these findings suggest that any evidence of brain trauma in soccer players relates more to acute head injuries than repetitive heading.

Biochemical Markers of Brain Injury

Some studies have specifically investigated biochemical markers of brain damage in soccer players, as well as their relationship with head impacts. Mussack et al. (22) measured serum levels of S-100B, a calcium-binding protein that is present in the astroglial cells of the central nervous system, in male young amateur soccer players, aged between 12 and 17 years, before and after controlled heading and normal exercise, as well as in older patients after minor traumatic brain injury. Previous studies have demonstrated that increased serum concentrations of this biomarker may reflect the presence and severity of brain tissue damage (23). The results of this investigation, which explored the effects of immediate heading exposure, demonstrated that the increases in S-100B serum levels 1 h after a session of heading and also after a session of normal exercise were insignificant and that these concentrations returned to the starting values 6 h after both training sessions. However, S-100B serum levels were significantly elevated, at the three measurement points, after heading when compared to normal exercise. Importantly, none of the young amateur soccer players reached S-100B serum levels verified in subjects showing traumatic brain injury with visible brain damage.

Stålnacke et al. (24) analyzed serum concentrations of two biochemical markers – S-100B and neuron-specific enolase (NSE), a cytoplasmatic enzyme that occurs predominantly in neurons and is also considered as a biomarker of brain tissue damage (23) – in male professional soccer players with a mean age of 26 years. Blood samples were taken from the participants before and after a competitive game and the numbers of headings and of trauma events during soccer play were obtained by video recordings. The results showed that serum concentrations of both S-100B and NSE significantly increased after the game in comparison with the pre-game values. Also, increases in S-100B were significantly and positively correlated to the number of headings and to the number of other trauma events. However, as emphasized by the authors, although heading may have contributed to these increases, the mechanisms involved in the rise of serum concentrations of the biochemical markers are not known yet. A further study by Stålnacke et al. (25), which also examined the consequences of immediate heading exposure, aimed to investigate serum levels of S-100B and NSE in female professional soccer players, with a mean age of 23 years. The results were very similar to that verified in male players, that is, the game induced increases in serum concentrations of S-100B and NSE, and there were significant correlations between the number of headings and of other trauma events and S-100B level increase.

In a study involving male professional soccer players aged between 19 and 35 years, Straume-Naesheim et al. (26) explored the effects of immediate heading exposure, by comparing serum levels of S-100B under four different conditions: after a head impact occurring during a regular league match, after a regular league match with no recorded head trauma, after a high-intensity training session without heading, and after a low-intensity training session with heading exercises only. Blood samples were taken at baseline, within 1 h after the match or the training session (B1), and the next morning (B12). All groups had a significant increase in serum concentrations of S-100B between baseline and B1 and a similar significant decrease from B1 to B12. The increase for the match groups was significantly higher than for the training groups, but no significant differences were seen between the two match groups or the two training groups for any of the sampling time points. In the match group without head trauma, there was no correlation between serum level of S-100B and number of headings. Furthermore, in the training group with heading exercises, no relationship was detected between serum concentration of S-100B and perceived heading intensity. The results suggest that both soccer matches and soccer training cause a transient increase in S-100B. According to the researchers, there is a possible additive effect of high-intensity exercise and heading, but minor head impacts do not seem to cause an additional increase.

Bamaç et al. (27) investigated the effects of immediate heading exposure on serum levels of nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) in male professional soccer players with a mean age of 24 years. NGF and BDNF, members of the neurotrophin family, are argued to be reliable markers of brain damage when released into the circulation (28). Each player completed a series of 15 headings and blood samples were obtained just before and after the training. The results showed that NGF and BDNF serum levels were significantly elevated in response to heading exercise. The authors speculate that the microtrauma caused by repetitive heading and/or the course of survival of the injured neurons may lead to increased NGF and BDNF levels. However, they acknowledge that measurements in peripheral blood can reflect only a limited view of the whole metabolism of these neurotrophins.

Another recent study by Koerte et al. (29) evaluated brain neurochemistry, by using magnetic resonance spectroscopy, in male former professional soccer players, with a mean age of 52 years, without a known history of concussion, in comparison to age- and gender-matched former professional non-contact sport athletes. As in their more recent investigation (18), which involved nearly the same groups of subjects, all participants were still actively participating in their respective sports at the time of the study and, in the group of soccer players, lifetime estimate of headings, an approximate calculation of long-term heading exposure, was also based on the athlete's self-report. The results showed significant increases in choline, a marker of membrane

disruption, and myo-inositol, a marker of glial activation, in soccer players when compared with controls. In addition, myo-inositol and glutathione, an anti-oxidant, were significantly and positively correlated with lifetime estimate of headings in the soccer group. A brief cognitive and balance evaluation revealed no significant differences between groups. This study suggests, as pointed out by the authors, a possible association between heading and altered brain neurochemistry in soccer players. It is possible that even subconcussive head impacts may affect brain chemical concentrations and precede cognitive alterations, although these data are still preliminary.

It is important to emphasize that research on biochemical markers of brain damage in soccer players has also produced controversial findings. Zetterberg et al. (30), who aimed to investigate the consequences of immediate heading exposure, examined serum and cerebrospinal fluid concentrations of some biomarkers of brain injury in male amateur soccer players aged between 19 and 32 years and age- and gender-matched non-athletic control subjects. The players participated in a training session that involved heading a ball kicked from a distance of 30 m. Some players were instructed to perform 10 headings, whereas others were required to perform 20 headings. The participants underwent lumbar puncture – for measurement of neurofilament light protein (NF-L), total tau protein (T-tau), glial fibrillary acidic protein (GFAP), S-100B, and albumin – and serum sampling – for measurement of S-100B and albumin – 7–10 days after the training session. There were no significant differences in serum and cerebrospinal fluid concentrations between soccer players who had performed 10 or 20 headings or between either of these two groups and the control group. Also, biomarker levels did not correlate with the number of headings performed. Therefore, the results suggest that standardized headings in soccer are not associated with known biochemical signs of acute brain injury.

Another study, conducted by Stålnacke and Sojka (31), also explored the effects of immediate heading exposure and aimed to analyze whether the controlled heading of soccer balls was associated with increased serum concentrations of S-100B. Male amateur soccer players, with a mean age of 22 years, were randomly divided into two groups. Players in the experimental group were instructed to perform 5 headings of a ball which was dropped from a height of 18 m, while players in the control group performed no headings. Blood samples were taken before and 30 min, 2, and 4 h after the heading session. The results showed no significant increases in serum concentrations of S-100B in the experimental group at any time point after headings, in comparison with baseline measures. Also, there were no significant differences in serum levels of S-100B between groups, either before or after the heading session. The researchers argue that, in this investigation, the impact probably was not sufficient to cause biochemically discernible damage of brain tissue.

EFFECTS OF SOCCER HEADING ON BRAIN FUNCTION

Similar to the research addressing the effects of soccer heading on brain structure, the literature investigating the effects of these subconcussive impacts on brain function has produced contradictory findings, as summarized in **Tables 2** and **3**. Tysvaer and Løchen (32) examined the consequences of long-term heading exposure, by investigating neuropsychological performance of male former professional soccer players aged between 35 and 64 years. The players were compared with a control group of age- and education-matched hospitalized patients suffering from a variety of disorders, but having no history of head or neck injuries and no evidence of brain damage. The neuropsychological examination, which included tests of attention, concentration, memory, and judgment, demonstrated that 81% of the soccer players had some degree of impairment, compared to 40% of the controls with only a mild degree of impairment. The authors hypothesize that their findings probably reflect the cumulative result of repeated traumas from heading the ball. However, the study offers no evidence of a clear association between heading and cognitive impairment.

A study conducted by Matser et al. (7) compared male professional soccer players (mean age 25.4 years) with a group of age- and gender-matched elite non-contact sport athletes for level of cognitive functioning, by using an extensive neuropsychological test battery. Professional soccer players reported a median of 800 headings during competitive matches in one soccer season, a period of time that can be considered as involving short-term heading exposure, and 54% of them experienced one or more soccer-associated concussions with or without loss of consciousness. The results showed that soccer players exhibited more cognitive impairment when compared with controls, as they had poorer performance on verbal and visual memory, planning, and visuoperceptual processing tasks. Moreover, an increasing number of headings and concussions incurred during soccer participation were associated negatively with cognitive functioning. Field position also influenced performance on neuropsychological testing, as forward and defensive players performed significantly poorer than midfield players and goalkeepers on some tasks. According to the researchers, the results suggest that participation in professional soccer may affect adversely some aspects of cognition, which appears to be attributed to increased frequency of heading the ball and soccer-related concussions, although more investigations are needed to allow extrapolating the findings to amateur or lower exposure soccer players.

Downs and Abwender (33) compared male and female soccer players with swimmers on neuropsychological tests assessing motor speed, attention, concentration, reaction time, and conceptual thinking. Each group of participants was composed of college and professional athletes, with a mean age of 19.6 and 42.1 years, respectively. The results showed that soccer players performed worse than swimmers on measures of conceptual thinking. In particular, the subgroup of older soccer players performed poorer than all other subgroups on measures of conceptual thinking and reaction time. The neuropsychological test scores did not vary as a function of reported history of concussion. Moreover, in the group of soccer players, estimates of career exposure to brain trauma, based on length of career and level of play, an approximate calculation of long-term heading exposure, predicted significantly poorer performance on measures of conceptual thinking, even after statistically

TABLE 2 | Summary of the studies with evidence of brain function impairment in soccer players.

Reference	Samples	Methods	Heading exposure	Main results
Tysvaer and Løchen (32)	Male former professional soccer players and patients with no evidence of brain injury	Extensive neuropsychological test battery	Long-term exposure	81% of the soccer players had mild-to-severe deficits in tests of attention, concentration, memory, and judgment, compared to 40% of the controls with a mild degree of impairment
Matser et al. (7)	Male professional soccer players and non-contact sport athletes	Extensive neuropsychological test battery	Short-term exposure	Soccer players had poorer performance on verbal and visual memory, planning, and visuoperceptual processing tasks, compared with controls. An increasing number of headings and concussions were associated negatively with cognitive functioning. Forward and defensive players performed poorer than midfield players and goalkeepers on some tests
Downs and Abwender (33)	Male and female amateur and professional soccer players and swimmers	Four tests measuring motor speed, attention, concentration, reaction time, and conceptual thinking	Long-term exposure	Soccer players performed worse than swimmers on conceptual thinking. Older players performed poorly than all other subgroups on conceptual thinking and reaction time. Estimates of heading predicted poorer performance on conceptual thinking
Webbe and Ochs (34)	Male amateur and professional soccer players	Extensive neuropsychological test battery	Immediate and long-term exposure	Soccer players with the highest self-reported estimates of heading who experienced headings within the previous 7 days scored lower on tests of verbal learning, verbally based conceptual performance, planning and attention, and information processing speed than other combinations of heading and recency
Rutherford et al. (35)	Male amateur soccer players and rugby and non-contact sport players	Extensive neuropsychological test battery	Long-term exposure	Performance of soccer players was worse than that of rugby and non-contact sport players on divided attention. Cumulative head injury and cumulative heading were marginal predictors of poorer performance on some tests
Zhang et al. (38)	Female amateur soccer players and non-soccer players	One test measuring executive functioning	Immediate exposure	Soccer players were slower than controls. There was an association between slower reaction times and increased hours of soccer per week and years of soccer experience

controlling for age. As pointed out by the authors, these results suggest that playing soccer may be associated with cognitive impairment, although, due to the cross-sectional design of the investigation, heading may not be specifically implicated as a cause.

In a study which explored the effects of immediate heading exposure, by investigating the interaction between recent heading activity and current heading frequency, as well as the consequences of long-term heading exposure, Webbe and Ochs (34) evaluated the cognitive performance of male amateur and professional soccer players aged between 16 and 34 years. Participants were administered a battery of 6 neuropsychological tests and provided a report of their heading practices by answering a structured interview. Players with the highest self-reported estimates of current heading who also experienced headings within the previous 7 days scored significantly lower on tests that measured verbal learning, verbally based conceptual performance, planning and attention, and information processing speed than the other groups, characterized by heading frequency (high, moderate, or low) and recency (presence or absence of heading practice within the previous 7 days). On the other hand, the basic comparison of neuropsychological performance of soccer players vs. age- and gender-matched control athletes, as well as the comparison across the heading groups showed, at most, a weak heading effect. Also, no significant effect was found for estimates of lifetime heading on neuropsychological performance. The researchers argue that although it is not possible to isolate heading from other sources of head impacts, the results suggest that heading the ball may be a factor sufficient to depress, at least temporarily, cognitive functioning in some players.

Rutherford et al. (35) compared male university soccer players (mean age 20.5 years) with age- and gender-matched rugby and non-contact sport players, on a range of 16 neuropsychological tests. Cumulative head injury incidence and cumulative heading, an approximate calculation of long-term heading exposure, were estimated by using self-reports and a combination of observation and self-reports, respectively. The only significant difference between groups was in accuracy scores of *Test of Attention Performance (TAP) – Divided Attention* (36). After control for the influence of the number of head injuries sustained, performance of soccer players was significantly worse than that of rugby and non-contact sport players. Cumulative head injury was a marginal predictor of *Trail Making Test B*, which measures executive control, and *TAP – Divided Attention* latencies in a positive fashion. Also, cumulative heading was a marginal predictor of the number of category shifts in the *Wisconsin Card Sorting Test* (37), which measures executive functioning abilities, in a negative fashion. The authors emphasize that, as consequence of the exploratory analysis, it would be inappropriate to interpret their results as clear evidence of an association between soccer practice, including heading, and neuropsychological impairment. As they point out, this study is limited to identifying relations worthy of further confirmatory examination.

Zhang et al. (38) aimed to investigate if frequent head-to-ball contact could cause cognitive dysfunctions and brain injury to soccer players and compared two groups of high-school female

TABLE 3 | Summary of the studies without evidence of brain function impairment in soccer players.

Reference	Samples	Methods	Heading exposure	Main results
Tysvaer and Storli (39)	Male professional soccer players	Questionnaire elaborated to record the incidence of head injuries due to heading	Long-term exposure	50% of the soccer players reported acute symptoms (e.g., disorientation), 16.4% related protracted symptoms (e.g., headache), and only 4.7% described prolonged symptoms (e.g., weakened memory) due to heading
Putukian et al. (40)	Male and female amateur soccer players	Four tests measuring reaction time, concentration, attention span, speed of information processing, divided attention, and active problem solving	Immediate exposure	There were no differences in pre-test or in post-test scores between athletes who participated in a session of heading practice and athletes who abstained from heading during exercises
Janda et al. (12)	Male and female amateur soccer players	Four tests measuring verbal learning, attention, tracking, information processing speed, and memory	Short-term exposure	After 1 year, no differences were found when comparing pre-season with post-season testing scores. There was no difference between the scores in this study and the standardized norms. There was no correlation between the number of ball impacts and cognitive performance, with the exception of a weak inverse association involving verbal learning in the second year
Stephens et al. (41)	Male amateur soccer players and rugby and non-contact sport players	Extensive neuropsychological test battery	Long-term exposure	There was no difference between groups in scores of all tests. There was no relationship between either cumulative head injury or cumulative heading and cognitive functioning. The only exception was a marginal prediction of poorer performance on divided attention by cumulative heading
Straume-Naesheim et al. (42)	Male professional soccer players	Extensive neuropsychological test battery	Long-term exposure	There was no association between estimated match or lifetime heading exposure and cognitive performance. Only 1.5% of the players qualified as outliers for one or more subtests when compared with the normal range
Kaminski et al. (44)	Female amateur high-school and college soccer players and non-athletes	Two tests measuring concentration, immediate memory recall and verbal memory	Short-term exposure	In both the college and high-school soccer groups, there were no correlations between the total number of headings and the change in scores of all outcome measures from pre-season to post-season. There were no differences between the three groups
Kaminski et al. (45)	Female amateur soccer players	Extensive neuropsychological test battery	Short-term exposure	There was no relationship between the number of headings and neuropsychological performance. None of the cognitive measures demonstrated decreases in performance over a soccer season
Kontos et al. (46)	Male and female amateur soccer players	Extensive neuropsychological test battery and symptom report	Short-term exposure	There were no differences in cognitive performance or symptoms among low, moderate, and high heading exposure groups
Vann Jones et al. (48)	Male retired professional soccer players	One test measuring memory	Long-term exposure	10.9% of the soccer players scored positively for possible mild cognitive impairment or dementia. There was no association between low-risk and high-risk playing positions, respectively, associated with reduced and greater frequency of heading, as well as length of playing career, and a positive screening result

students aged between 15 and 18 years – soccer and non-soccer players – by using a tablet-based approach designed to evaluate executive functioning. This study involved a task in which a visual target appeared randomly at one of the four locations in the screen. In the first situation, the participant was instructed to touch the response box containing the target and, in the second situation, the subject had to touch the response box opposite to the target location. Every soccer player performed head balls during the practice session before the testing, with median 6 head balls per session based on self-reports. No participant in the non-soccer group performed a head ball before testing. Therefore, the authors explored the consequences of immediate heading exposure. Although there were no differences between groups in reaction times in the first situation, soccer players were slower than controls in the second situation, which indicates a disruption specific to voluntary responses. Also, the data showed an association between slower reaction times and increased hours of soccer per week and years of soccer experience. According to the researchers, the results suggest that even subconcussive impacts could be associated with cognitive function changes that are consistent with mild traumatic brain injury of the frontal lobes. However, further studies are needed to evaluate soccer players for longer periods to investigate if these changes are transient or longer lasting.

Although some studies have suggested some degree of association between heading and brain function impairment, others have not corroborated this result. An early study by Tysvaer and Storli (39), which addressed the effects of long-term heading exposure, aimed to examine to what extent heading produced discomfort or permanent head trouble. Male professional soccer players, aged between 18 and 34 years, answered a questionnaire elaborated to record the incidence of head injuries due to heading. None of the players had been operated on for epi- or subdural hematoma or other brain damage an only a few had suffered

concussion. The results also showed that 50% of players reported acute symptoms (e.g., disorientation), 16.4% related protracted symptoms (e.g., headache), and only 4.7% described prolonged symptoms (e.g., weakened memory) due to heading. According to the authors, the questionnaire's data suggest that there seems to be a low percentage of serious head injuries associated with heading practice, although they acknowledge that heading can be dangerous and attention should be paid to teach young players how to head correctly.

Putukian et al. (40) investigated the consequences of immediate heading exposure, by assessing cognitive function of male and female college soccer players before and after typical training sessions. The neuropsychological battery measured reaction time, concentration, attention span, speed of information processing, divided attention, and active problem solving. In one session, the athletes participated in heading practices and, in the other session, they abstained from heading during the exercises. The results showed no differences in pre-test or in post-test scores between heading and non-heading groups. A practice effect was found, since there was an increase in post-test scores compared with pre-test ones, which was consistent between groups. There were also significant differences between males and females in some cognitive test variables. As argued by the researchers, this study was exploratory in nature and only examined the acute effect of heading on a limited number of neuropsychological tests and with a limited sample.

In a study involving a younger population, Janda et al. (12) evaluated the effect of repetitive head impacts due to heading in male and female amateur soccer players with a mean age of 11.5 years, by using a neuropsychological testing protocol and documentation of concussive symptoms. The players were followed over a period of three seasons during the first year and a subgroup of male players was followed for an additional year. Thus, a short-term heading exposure was investigated in this study. The number of times a player headed the ball was monitored throughout the seasons. When comparing pre-season with post-season testing scores, the authors found no significant difference. Also, there was no evidence of difference between the scores in this study and the standardized norms. The data showed no significant correlation between the number of ball impacts and cognitive performance, with the exception of a weak inverse association involving verbal learning in the second year. Of note, however, is the fact that, in the first year, 49% of the players complained of headaches after heading the ball. As pointed out by the authors, it is unclear whether the reported headaches were consequence of mild head injuries or rather localized pain in the region of the impact.

Stephens et al. (41) compared neuropsychological test scores of male school team soccer players with those of male rugby players and non-contact sport players, all aged between 13 and 16 years. Cumulative head injury incidence and cumulative heading, an approximate calculation of long-term heading exposure, were estimated by using self-reports and a combination of observation and self-reports, respectively. The results showed no significant difference between groups in scores of 13 neuropsychological tests. Also, there was no relationship between either cumulative head injury or cumulative heading and cognitive functioning. The only exception was a marginal prediction of *TAP – Divided Attention* accuracy scores by cumulative heading, consistent with the study hypothesis of poorer neuropsychological test performance with increasing cumulative heading. According to the researchers, although this variable should be considered in further research, an interpretation of no heading effects in these adolescent soccer players is more appropriate until results of confirmatory analyses are known.

Another study, conducted by Straume-Naesheim et al. (42), examined the association between long-term heading exposure and previous concussions with performance on neuropsychological tests among male professional soccer players with a mean age of 25.6 years. The athletes completed a questionnaire assessing heading exposure and previous concussions, and a subgroup of players were observed in two to four matches for direct counting of heading actions. All participants were submitted to the computer-based neuropsychological test battery *CogSport* (43), which measures motor function, decision-making, simple, divided and complex attention, working memory, and learning and memory. The results showed no association between estimated match or lifetime heading exposure and cognitive performance. Importantly, self-reported number of headings correlated well with the observed values. Only 1.5% of the players qualified as outliers for one or more subtasks when compared with the normal range. The number of previous concussions was positively associated with lifetime heading exposure, but there was no association between previous concussions and cognitive performance. Although this study has some important limitations, it reveals no evidence of cognitive impairment caused by subconcussive and concussive trauma in soccer.

Kaminski et al. (44) investigated whether there was a relationship between number of headings taken in a season, which characterizes a short-term heading exposure, and scores on cognitive function and balance in female high-school and college soccer players (mean age 15.1 and 19.1 years, respectively). The study also involved an age- and gender-matched college control group, whose members were not participating in any organized sport. Prior to and immediately following the soccer season, all participants were given a battery of neuropsychological and postural stability tests. Heading data were documented by counting the number of times each player headed the ball during sanctioned games. In both the college and high-school groups, there were no significant correlations between the total number of headings and the change in scores of all outcome measures from pre-season to post-season. Moreover, the authors found no significant differences between the three groups in post-season scores of the neuropsychological tests, which measured concentration and immediate memory recall and verbal memory. The only significant difference was noted in post-season balance scores between the college players and the other two groups, as the first group had a worse performance. However, while the high-school and control groups were slightly improved from pre-season to post-season, the college group did not change, which suggests that one season of soccer participation did not have a detrimental effect on postural control. Therefore, overall, the results showed no evidence of cognitive or balance deficits in female soccer players.

In a further study, also involving female high-school soccer players and exploring the effects of short-term heading exposure, Kaminski et al. (45) evaluated computerized neuropsychological test performance before and after a competitive soccer season and measured the number of headings per match. There was no relationship between purposeful heading and neuropsychological performance. The results indicated that none of the cognitive measures deteriorated. Interestingly, two measures showed small but significant improvements over baseline, which the authors attribute to a test–retest practice effect. Although this investigation involved a large number of participants and provided a perspective over one playing season, studies involving longer time periods are needed to help answer the question about the potential long-term effects of heading practice.

Aiming to compare the effects of low, moderate, and high short-term heading exposure, Kontos et al. (46) investigated male and female amateur soccer players, aged between 13 and 18 years, on computerized cognitive performance and symptoms. Participants completed the *Immediate Postconcussion Assessment and Cognitive Testing* (*ImPACT*), a previously validated test battery used to assess and manage concussions in sports (47), which includes a report of concussive symptoms and measures verbal memory, visual memory, motor processing speed, reaction time, and impulse control. The researchers recorded observed numbers of headings for each player during two randomly selected practices and games for each of the soccer teams during a season. The results showed no differences in computerized cognitive performance or symptoms among low, moderate, and high heading exposure groups. Moreover, the sample of soccer players scored significantly higher across all cognitive tasks and reported fewer symptoms than the age- and gender-matched 10th percentile (i.e., unusually low) norms. A comparison between genders revealed that males headed the ball more frequently and showed lower verbal and visual memory and motor processing speed scores than females. According to the researchers, the findings do not support a relationship between soccer heading exposure and cognitive impairment and symptoms in male and female youth soccer players. They suggest that if any association exists, it is subtle and may affect only a small number of athletes, which deserves future investigation.

In a recent study, Vann Jones et al. (48) questioned whether long-term heading exposure was associated with persistent cognitive decline. Male retired professional soccer players were required to complete a self-assessed test of cognition, the *Test Your Memory* questionnaire, a previously validated tool (49). Further information was collected in order to analyze the potential effect of a number of variables on cognition. The mean age of the participants and the mean length of the professional playing career were 67.4 and 13.8 years, respectively. The results showed that 10.9% of the responders scored positively for possible mild cognitive impairment or dementia. There was no association between low-risk and high-risk playing positions, respectively, associated with reduced and greater frequency of heading, as well as length of playing career, and a positive screening result. As expected, age was a risk factor, although this was not significantly different from the local population prevalence for mild cognitive impairment across age groups over 65 years (50). Therefore, the results demonstrated no evidence of association between chronic subconcussive head injury in soccer and accelerated cognitive decline. The authors suggest that the short-term and medium-term cognitive impairment caused by heading may only be transient. Thus, once the players end their playing careers, their risk of cognitive decline would fall in line with the population. However, future longitudinal studies involving larger samples of professional soccer players are needed to support these findings.

FINAL COMMENTS

The research about the effects of heading on brain structure and function has produced intriguing results, but the findings are still inconclusive. There are very few studies involving neuroimaging techniques in order to investigate possible associations between heading practice and brain structure abnormalities in soccer players. Also, a small number of studies have evaluated biochemical markers of brain injury in these individuals. Both neuroimaging and biomarker technologies are promising areas of research that should be more explored in future investigations for assessing the effects of heading.

Although the number of studies addressing the effects of subconcussive impacts on brain function is relatively greater, when compared to the amount of investigations focused on brain structure, questions persist as to whether or not heading is deleterious to cognitive functioning. Many of these studies have methodological limitations, which should be taken into account when considering discrepancies in results, including lack of a suitable control group, failure to control for history of concussion, lack of screening for alcohol use, estimates of heading exposure based on self-reports, small sample size, low or unknown response rates, inappropriate statistical methods, among others. It is also important to note that most research in this area has concentrated on male soccer players. The growth of the female soccer population at all levels of competition (44) calls attention to the need of including these athletes in further studies.

It is noteworthy, although not focused in this review, that some investigations [e.g., see Ref. (51–55)] have addressed the effects of heading practice specifically on postural stability and oculomotor control, which ultimately also reflect brain functioning. However, their results are still inconclusive. Moreover, other relevant studies [e.g., see Ref. (56–60)] have investigated biomechanical aspects of heading, by exploring measurements of head impact, which can help to better understand the risk and safety of heading a soccer ball.

It has been estimated that professional soccer players play approximately 300 games and head the ball more than 2000 times during their careers (39). The technique of heading is complex and varies for different game situations. Proper heading technique, which involves stabilization of the neck musculature as well as the torso to reduce rotational forces, may protect soccer players from possible deleterious effects (5). In this sense, younger players may be at a great risk for head and neck injury from heading, since their technique is not yet fully developed. While learning this skill, several impacts will probably occur using an improper technique. In addition, a study involving female high-school soccer

players (61) demonstrated significant, albeit moderate, negative correlations between neck strength and head acceleration from heading. Thus, as pointed out by the authors, the results suggest that athletes with weaker necks cannot tolerate headings as well as athletes with stronger necks. Therefore, more studies involving individuals in the formative years of development of soccer skills, including investigations about validity of protection equipment as well as age-appropriated soccer balls, could be valuable.

Considering that soccer extends its reach throughout the world, and it is currently the most played sport, the long-term consequences of soccer-related concussive and subconcussive brain trauma may represent a major public health problem. As emphasized by some authors (62), although a spectrum of chronic neurological injuries may occur, a primary concern involves chronic traumatic encephalopathy. All reported neuropathologically confirmed cases of this neurodegenerative disease have history of repetitive brain trauma, although not all individuals with such a history develop the pathology (63, 64). The prevalence of chronic traumatic encephalopathy, originally reported in boxers by Martland (65), is still unknown and an *in vivo* diagnosis is not currently possible, which may enhance the concern about potential consequences of heading practice.

Further studies, especially with longitudinal designs, are needed to clarify the clinical significance of heading as a cause of brain injury, which remains controversial and unexplored, and to identify risk factors. Such investigations might contribute to the establishment of safety guidelines that could help to minimize the risk of possible adverse effects of soccer on brain structure and function.

AUTHOR CONTRIBUTIONS

All authors gave substantial contributions to conception and design of the article. AR drafted the work, and RL and PC revised it critically. All authors approved the final version of the review and are accountable for all aspects of the study.

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