

ORIGINAL WORK



# Serum Levels of HDL Cholesterol are Associated with Diffuse Axonal Injury in Patients with Traumatic Brain Injury

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## Abstract

**Background:** It is well known that lipids are vital for axonal myelin repair. Diffuse axonal injury (DAI) is characterized by widespread axonal injury. The association between serum lipids and DAI is not well known. The purpose of this study was to investigate the associations of serum lipid profile variables (triglycerides, high- and low-density lipoproteins, and total cholesterol) with DAI detected by magnetic resonance imaging (MRI) and with clinical outcome for patients suffering from traumatic brain injury (TBI).

**Methods:** This study included 176 patients with a history of TBI who had undergone initial serum lipid measurements within 1 week and brain MRIs within 30 days. Based on MRI findings, patients were divided into negative and positive DAI groups.

**Results:** Of the 176 patients, 70 (39.8%) were assigned to DAI group and 106 (60.2%) patients to non-DAI group. Compared with the non-DAI group, patients with DAI had significantly lower levels of high-density lipoprotein cholesterol (HDL-C) in serum during the first week following TBI. Multivariate analysis identified HDL-C as an independent predictor of DAI. Patients with lower serum HDL-C levels were less likely to regain consciousness within 6 months in TBI patients with DAI lesions identified by MRI.

**Conclusions:** Plasma levels of HDL-C may be a viable addition to biomarker panels for predicting the presence and prognosis of DAI on subsequent MRI following TBI.

**Keywords:** Traumatic brain injury, Diffuse axonal injury, HDL cholesterol

## Introduction

Traumatic brain injury (TBI) is the leading cause of death and disability in young and middle-aged adults [1–3]. Traditionally, TBI is classified based on biomechanical and neuroradiological characteristics into either focal or diffuse injury [4]. Diffuse axonal injury (DAI) is caused by a rotational acceleration–deceleration force to the brain resulting in damage to white matter axons, which leads to disruption of neuronal networks [5]. Magnetic resonance

imaging (MRI) is the sensitive method to detect DAI, as it is capable to visualize even subtle axonal damage [4].

Myelin is a multilamellar cell sheath surrounding neuronal axons, which is essential for maintenance of axonal integrity and cell survival. As a vital component of the myelin sheath, the importance of cholesterol in the brain has been recognized since at least the early nineteenth century [6]. It has been estimated that up to half of the white matter in the central nervous system (CNS) may be composed of myelin. Lipids, especially lipoproteins, play many important roles in the CNS. There is increasing evidence emerging, for example, that lipoproteins are coupled to the progression of neurodegenerative diseases such as Alzheimer's disease [7]. High-density lipoprotein

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(HDL) plays a vital role in the transport of lipids and cholesterol in human plasma and is present in the CNS due to its ability to cross the blood–brain barrier (BBB) [8, 9]. HDL is also involved in several key functions in the CNS. It has been shown that HDL has both antioxidant and anti-inflammatory properties [10, 11]. In addition, HDL may play a protective role in the BBB injury under pathological conditions [12].

Unfortunately, very limited information is available to date on the possible roles that serum HDL levels may play in TBI. DAI is a common finding in patients suffering from severe TBI, and is often associated with prolonged comas and poor outcomes, making DAI a challenging clinical entity to study [13]. Nonetheless, predicting which DAI patients may eventually regain consciousness remains extremely difficult. Although the mechanisms underlying the outcome following severe TBI are complex, neuroinflammation and oxidative damage have been extensively validated as important contributors to the pathophysiology of secondary damage post-TBI [14]. Over the past few years, BBB disruption has been increasingly recognized as a crucial secondary injury mechanism following TBI [15]. The disruption of the BBB is also responsible for traumatic axonal injury [16]. It is generally thought that there is no net transfer of cholesterol from the periphery into the CNS since the BBB could limit the movement of plasma lipoproteins into the brain. However, it is also generally thought that the brain does not produce apoA-I, which is the major protein component of HDL in the plasma, and that apoA-I in the brain comes from the circulation. In addition, plasma and cerebrospinal fluid HDL cholesterol are reported to be correlated, which suggests that plasma HDL levels can influence brain HDL level [17]. As above-mentioned, HDL may play a beneficial role in the BBB injury [12]. In addition, DAI is associated with microglial/macrophage-mediated immune inflammatory response [18]. Microglial/macrophage activation is induced by DAI-mediated secondary pathologies such as Wallerian degeneration and/or cellular membrane perturbation. The presence of axonal breakdown products may contribute to prolonged activation of immune cell which results in persistently high inflammatory responses [18]. In this study, we focused on measuring serum HDL in TBI patients, because there is an emerging body of data suggesting protective roles of serum HDL on neuroinflammation, oxidative processes, and BBB disruption as above mentioned. An association between serum lipids and DAI, however, has never been established in previous studies to the best of our knowledge.

In order to clarify potential associations of serum HDL levels with DAI in TBI patients, we sought to examine: (1) the relationship between serum HDL level and the

presence of DAI, as seen on MRI, and (2) the clinical significance of HDL for predicting the recovery of consciousness in patients with DAI.

## Methods

### Study Population

We retrospectively screened all patients with TBI admitted to the neurosurgery, emergency, or rehabilitation department of Nanfang hospital from 2007 to 2017. Patients were considered eligible for this study if they met the following criteria: (1) aged 18–75 years old; (2) absence of previous neurologic disorders; (3) absence of a previous history of hyperlipidemia requiring statins. Exclusion criteria included concurrent liver cirrhosis, terminal stage cancer, and patients who had received total parenteral nutrition at the time of blood sampling.

### Parameters

Demographic data and other clinical information were collected from the electronic patient record system. A standardized case collection form included the causes of trauma, age, sex, injury severity score (ISS), and Glasgow coma scale (GCS) scores at baseline. The time interval from injury to initial MRI was also calculated. We also evaluated initial computed tomography (CT) obtained within 24 h after the onset regarding the injury severity validated in the Rotterdam CT scoring system.

Serum analyses for lipids and lipoproteins were performed within 1 week after the onset of trauma. Serum samples were collected in the morning (~7:00 a.m.). Total cholesterol and triglyceride levels were measured enzymatically using diagnostic reagent kits (Denka Seiken, Tokyo, Japan). HDL and low density lipoprotein (LDL) cholesterol concentrations were measured by the homogeneous method. These assays were conducted on an automated chemistry analyzer.

### Evaluation of MRI

A consecutive 176 patients with TBI underwent MRI within 30 days following trauma. All MRIs were performed with a 3.0 T scanner (Siemens Avanto, Siemens Medical, Erlangen, Germany).

Based on the MRI findings, patients were divided into DAI and non-DAI groups. The DAI group included hemorrhagic and non-hemorrhagic DAI. DAI-associated lesions were defined as having hypointense/decreased signals for T2 \* gradient echo and susceptibility weighted imaging sequences, and/or restricted diffusion for diffusion weighted imaging (DWI) sequence in white matter localization not extending to the cortex. A hemorrhagic DAI was defined as having hypointense foci noted on T2 \* weighted imaging (WI), that was not compatible with vascular, bony, or artifactual structures and was

located in the consistent brain regions [19]. A non-hemorrhagic DAI was defined as having a hyperintense focus noted on DWI, T2 weighted imaging, or fluid attenuated inversion recovery located in the consistent brain regions without association of hypointense foci on the corresponding T2 \* WI [19, 20]. Patients were then assigned to one of the four DAI stages according to Adams et al. [21]: stage 0, no DAI; stage 1, DAI lesions confined to the lobar white matter or cerebellum; stage 2, DAI lesions located in the corpus callosum with or without lesions of stage 1; and stage 3 DAI lesions located in the brainstem with or without lesions of stages 1 and/or 2. All brain MR images were blindly analyzed by neuroradiologists experienced in the field of central nervous system diseases.

### Study Outcome

The primary outcome measured was recovery of consciousness. Patients were considered to have recovered consciousness if they met at least one of the following demonstrations: (1) functional interactive communication, (2) functional use of one or more objects, or (3) clearly discernable behavioral manifestation of a sense of self. The judgment on the unconscious state during the follow-up period was evaluated by the Coma Recovery Scale-Revised (CRS-R) [22]. All patients were followed for at least 6 months. Clinical outcome raters were blind to laboratory data and MRI results.

### Statistical Analysis

All statistical analyses were performed using SPSS software version 13.0 (SPSS Inc., Chicago, IL, USA). A  $p$  value of  $<0.05$  was considered statistically significant.

A number of conditions have defined normal HDL ranges for their specific populations, but this varies according to different diagnoses. Given the absence of normative values for TBI, we divided our cohort into quartiles to objectively define a measurable threshold. Continuous HDL quartiles were defined as less than 0.77 mmol/L for quartile 1 (Q1); 0.77–0.97 mmol/L for quartile 2 (Q2); 0.97–1.22 mmol/L for quartile 3 (Q3); and more than 1.22 mmol/L for quartile 4 (Q4).

Normally distributed data are presented as the mean  $\pm$  standard deviation (SD) and compared using Student's  $t$  test. Non-normally distributed continuous data are presented as median (range) and compared by the Mann–Whitney  $U$  test. Chi-square or Fisher's exact tests were performed to compare categorical data. Logistic regression analysis was used to determine which variables independently predicted the presence of DAI by MRI and clinical outcome. The times to recovery of consciousness for patients with DAI were illustrated with Kaplan–Meier curves, with adjustment for baseline characteristics. The predictive power of serum HDL level

was analyzed using the receiver operating characteristic (ROC) curve method.

## Results

### Enrollment and Characteristics of the Patients

Of 3411 patients with TBI screened for eligibility, 176 patients with TBI met all the inclusion criteria and were enrolled in this study. The clinical, injury-related variables and mean serum lipids levels of the cohort are shown in Table 1. Of the 176 patients, 70 (39.8%) had DAI as identified on MRI. The GCS scores at admission were significantly lower in patients with DAI ( $p < 0.001$ ), as were the Glasgow Outcome Scale–Extended (GOSE) score at 6 months after injury ( $p < 0.001$ ). No statistically significant differences were found between the DAI and non-DAI groups regarding age, sex, cause of injury, or time interval from injury to initial MRI.

The patients' serum lipid and lipoprotein levels were measured within 1 week following injury. The mean of serum HDL cholesterol levels in patients with DAI was found to be significantly lower than those of patients without DAI ( $p < 0.001$ ). However, no significant difference was found between the DAI and non-DAI groups in

**Table 1 Demographic and clinical characteristics of patients**

	DAI group	Non-DAI group	$p$ value
Demographic variable			
No. of patients	70	106	NS
Age	42.0 $\pm$ 16.6	44.3 $\pm$ 16.13	0.372
Sex			0.104
Male	61 (87.1%)	82 (77.4%)	
Female	9 (12.9%)	24 (22.6%)	
Median GCS score	8 (5–13)	14 (9–15)	<b>&lt;0.001</b>
Cause of accident			
Traffic accident	36 (51.4%)	55 (51.9%)	0.953
Fall	29 (41.4%)	34 (32.1%)	0.205
Others	5 (7.1%)	17 (16.0%)	0.081
Median time from onset until initial MRI scan (interquartile range)	24 (18–27)	23 (12–26)	0.090
Median GOSE score 6 months later	4 (2–6)	6 (4–7)	<b>&lt;0.001</b>
Lipid profile variables (mmol/L)			
HDL-C	0.8 $\pm$ 0.2	1.1 $\pm$ 0.3	<b>&lt;0.001</b>
LDL-C	2.5 $\pm$ 0.9	2.7 $\pm$ 0.8	0.069
Total cholesterol	4.2 $\pm$ 1.0	4.4 $\pm$ 0.9	0.351
Triglycerides	1.8 $\pm$ 0.5	1.9 $\pm$ 0.9	0.830

Bold values indicate statistical significance ( $p < 0.05$ )

GCS Glasgow coma score, GOSE Glasgow Outcome Scale–Extended, HDL-C high-density lipoprotein cholesterol, LDL-C low density lipoprotein cholesterol

the other component of the lipid profile including total cholesterol, triglyceride levels, and LDL cholesterol.

#### Relationship Between Initial Serum HDL-C Quartiles and Presence of DAI on Subsequent MRI

Due to the absence of defined normative values of serum HDL-C for TBI patients, we divided our cohort into quartiles to objectively define a measurable threshold.

To assess the relative importance of HDL-C to the associations with DAI after injury, we constructed a multivariable binary logistic regression model (Table 2). All variables were considered as candidate variables for the analysis, including HDL-C quartile, age, sex, GCS score, Rotterdam CT score, and cause of accident. When all other factors had been adjusted for, HDL-C level in Q1 was found to be significantly associated with an increased risk of having DAI on MRI (odds ratio (OR) 44.355, 95% confidence interval (CI) 10.204–192.811;  $p < 0.001$ ), along with GCS score at admission. In addition, patients presenting with lower GCS score at baseline were more likely to suffer from DAI after TBI.

In addition, the power of initial serum HDL-C to predict DAI on MRI was further assessed with ROC analysis, as shown in Fig. 1. This ROC analysis showed

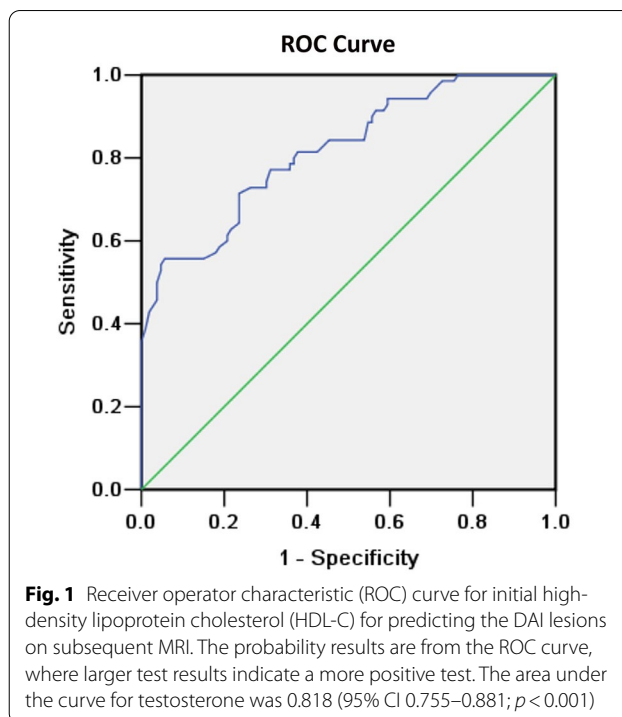
**Table 2 Relationship between HDL cholesterol quartiles at initial visit postinjury and presence of DAI on subsequent MRI on logistic regression**

Variable	Adjusted odd ratio	95% CI	<i>p</i> value
Multivariate analysis			
Age	0.999	0.972–1.026	0.999
Sex			0.839
Male	1		
Female	1.125	0.360–3.519	0.839
GCS	0.877	0.784–0.981	<b>0.022</b>
Rotterdam CT score	1.047	0.706–1.552	0.821
Cause of accident			0.518
Traffic accident	1		
Fall	0.984	0.395–2.448	0.972
Others	0.105	0.105–1.855	0.265
HDL-C quartiles			<b>&lt; 0.001</b>
Q4	1		
Q3	2.525	0.687–9.281	0.163
Q2	3.203	0.847–12.118	0.086
Q1	44.355	10.204–192.811	<b>&lt; 0.001</b>

Bold values indicate statistical significance ( $p < 0.05$ )

The HDL-C quartile boundaries were: the lowest quartile (Q1) corresponds to HDL-C  $\leq 0.77$  mmol/L,  $0.77$  mmol/L < quartile 2 (Q2)  $\leq 0.97$  mmol/L,  $0.97$  mmol/L < quartile 3 (Q3)  $\leq 1.22$  mmol/L, and the highest quartile (Q4)  $> 1.22$  mmol/L

DAI diffuse axonal injury, GCS Glasgow coma score, HDL-C high-density lipoprotein cholesterol, LDL-C low density lipoprotein cholesterol



that the area under the curve (AUC) was 0.818 (95% CI 0.755–0.881;  $p < 0.001$ ).

#### Relationship Between Initial Serum HDL-C Quartiles and the Staging or Type of DAI

To further confirm that HDL-C quartiles were associated with the severity of DAI, we examined the associations of HDL-C quartiles with the staging of DAI (Table 3). Regarding the stage of DAI, 106 patients (60.2%) had stage 0 (no DAI), 24 patients (13.6%) had stage 1, 16 patients (9.1%) had stage 2, and 30 patients (17.0%) had stage 3. The proportion of patients in Q1 was higher in DAI stage 3 ( $p < 0.001$ ), stage 2 ( $p < 0.001$ ), or stage 1 ( $p < 0.001$ ) compared to DAI stage 0. However, no significant differences between the three grades of DAI (stage 1, 2, or 3) with respect to initial HDL-C.

Among the patients presenting with DAI, 33 of 70 patients (47.1%) showed coexistence of hemorrhagic and non-hemorrhagic DAI lesions. Hemorrhagic DAI lesions alone were found in 10 patients (14.3%), whereas non-hemorrhagic DAI lesions alone were noted in 27 (38.6%). No statistically significant difference was noted between different DAI lesion types with respect to HDL-C quartiles.

**Table 3 Analysis of HDL cholesterol quartiles by DAI in MRI**

Variable	Q1 %	Q2 %	Q3 %	Q4 %	p value
DAI type					
Hemorrhagic DAI	6/10 (60%)	3/10 (30%)	1/10 (10%)	0/10 (0%)	Reference
Non-hemorrhagic DAI	15/27 (55.6%)	5/27 (18.5%)	6/27 (22.2%)	1/27 (3.7%)	0.702
Coexistence	19/33 (57.6%)	6/33 (18.2%)	5/33 (15.2%)	3/33 (9.1%)	0.665
DAI stage					
0	6/106 (5.7%)	28/106 (26.4%)	35/106 (33.0%)	37/106 (34.9%)	Reference
1	13/24 (54.2%)	6/24 (25%)	4/24 (16.7%)	1/24 (4.2%)	<b>p &lt; 0.001</b>
2	9/16 (56.3%)	2/16 (12.5%)	4/16 (25%)	1/16 (6.3%)	<b>p &lt; 0.001</b>
3	18/30 (60%)	6/30 (20%)	4/30 (13.3%)	2/30 (6.7%)	<b>p &lt; 0.001</b>

Bold values indicate statistical significance ( $p < 0.05$ )

The HDL-C quartile boundaries were: the lowest quartile (Q1) corresponds to HDL-C  $\leq 0.77$  mmol/L,  $0.77$  mmol/L < quartile 2 (Q2)  $\leq 0.97$  mmol/L,  $0.97$  mmol/L < quartile 3 (Q3)  $\leq 1.22$  mmol/L, and the highest quartile (Q4)  $> 1.22$  mmol/L

DAI diffuse axonal injury, HDL high-density lipoprotein

**Table 4 Logistic regression analysis of HDL cholesterol quartiles to predict recovery of consciousness**

Variable	Adjusted odd ratio	95% CI	p value
HDL quartiles			
Q3	1.982	0.616–6.381	0.251
Q2	1.029	0.344–3.081	0.959
Q1	0.318	0.108–0.939	<b>0.038</b>

Bold value indicates statistical significance ( $p < 0.05$ )

The HDL-C quartile boundaries were: the lowest quartile (Q1) corresponds to HDL-C  $\leq 0.77$  mmol/L,  $0.77$  mmol/L < quartile 2 (Q2)  $\leq 0.97$  mmol/L,  $0.97$  mmol/L < quartile 3 (Q3)  $\leq 1.22$  mmol/L

#### Associations of Serum HDL-C with Recovery of Consciousness in Patients Post-DAI

We then investigated whether serum HDL-C levels were associated with recovery of consciousness in patients with DAI lesions to assess their potential predictive value to recovery of unconsciousness following DAI. Of 70 TBI patients with DAI, consciousness was regained in 38 patients. Among these patients, the duration of recovery to consciousness after TBI was <1 month for 17 patients, 1–3 months for 11 patients, 3–6 months for seven patients, and more than 6 months for three patients. HDL-C level in Q4 was only found in four patients with DAI which all of them (100%) had regained consciousness. The multivariate analyses are summarized in Table 4. After adjustments for other factors were made, including age, sex, GCS score, Rotterdam CT score, and cause of accident, patients with HDL-C levels in Q1 were less likely to regain consciousness at 6 months post-DAI than those with HDL-C levels in all other quartiles (OR 0.318, 95% CI 0.108–0.939,  $p = 0.038$ ).

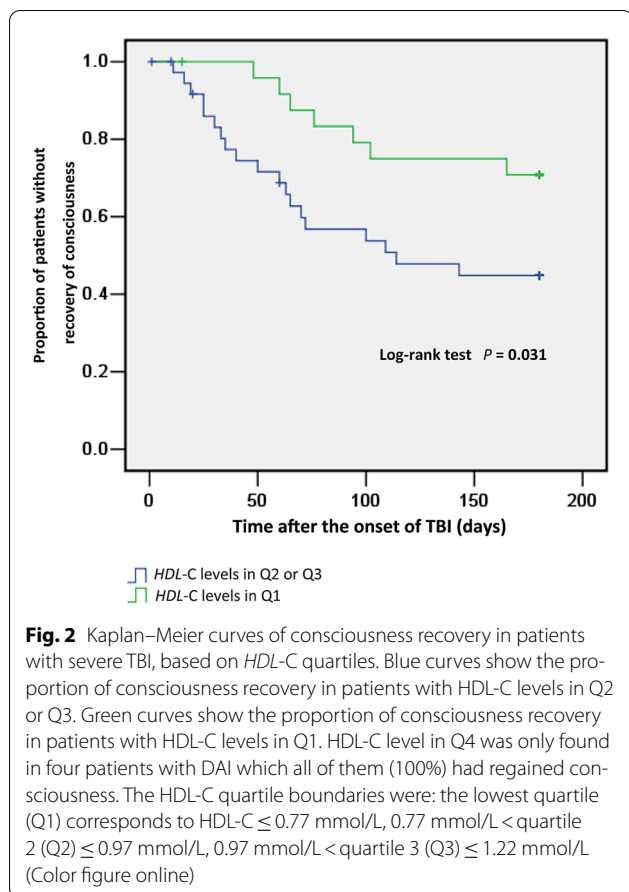
Furthermore, the times to recovery from unconsciousness for DAI patients with HDL levels in Q1, versus all other quartiles were compared by Kaplan–Meier survival curves, which showed the proportion of DAI patients remaining in an unconscious state for 6 months following TBI (Fig. 2). The analysis showed that HDL levels in Q1 significantly increased the risk of patients with DAI lesions on MRI remaining in an unconscious state within 6 months after injury (log-rank test,  $p = 0.031$ ).

#### Discussion

In this retrospective study, we have reported results indicating that initial serum HDL-C levels was independently associated with DAI visible on subsequent MRIs of TBI patients. Of the HDL-C quartiles, serum HDL-C levels falling in our Q1 were specifically correlated with the presence of DAI. In addition, we demonstrated that the lower HDL-C level was found to be, the higher the probability of that DAI patient remaining in an unconscious state at 6 months post-TBI. To our knowledge, this is the first study to clearly establish the relationship between serum lipid profile variables and DAI lesions detected on subsequent MRIs in patients with TBI.

In previous works, it has been reported that post-traumatic brain lesions typically occur by two main mechanisms: direct injury (contact with the skull) and indirect injury (shearing strain) [23–25]. Gennarelli [26] reported that TBI can be classified into two categories: focal injuries and diffuse injuries. DAI was first described by Strich [27] and is usually caused by the shearing strain of angular or rotational acceleration–deceleration forces on the brain. Axonal breakage, caused by axonal retraction balls and changes to glia cells, has been reported to be involved in the pathological mechanism underlying DAI





[21, 28]. It is estimated that 25% of the total amount of cholesterol in the body is localized to the brain, most of it present in myelin. Since up to half of the brain's white matter may consist of myelin, it is unsurprising that the brain is the most cholesterol-rich organ in the human body. Cholesterol levels in the brain are often affected in neurodegenerating disorders, such as Alzheimer disease, and wherein the capacity for cholesterol transport seems to be of particular importance in development of the disease [29]. In the injured CNS, the need for cholesterol is much higher than that in the uninjured state [30].

DAI is associated with microglial/macrophage-mediated immune inflammatory response [18]. Under normal conditions, the BBB effectively prevents the uptake of lipoprotein cholesterol from the circulation. However, HDL-C, as the major component of lipoprotein, plays a key role in the transport of cholesterol and lipids in human, as well as being involved in the regulation of several neural functions in the CNS. HDL-C is thus able to traverse the BBB under both normal and pathological conditions. In sepsis, HDL can attenuate the chain of inflammation, once activated, by suppressing the production of proinflammatory cytokines from macrophages

[31]. The disruption of the BBB is also responsible for traumatic axonal injury [16]. Higher serum HDL-C is associated with lower degrees of BBB injury [12]. In this study, we found that lower serum HDL-C levels were significantly correlated with the presence of DAI in patients suffering from TBI. This implies that HDL-C levels in Q1 ( $<0.77$  mmol/L) could be used as a marker of warrant DAI workup. We speculated that low levels of serum HDL-C in the initial phases following TBI may impair the innate immune response against inflammation and heighten susceptibility to inflammatory damage and BBB disruption, then facilitating the onset of DAI. However, it is impossible to ascertain whether low levels of serum HDL-C are a cause or a consequence of DAI from this study. Further studies on the potential mechanism of HDL-C involvement in DAI will be of great benefit to the field.

In this study, we applied the widely used DAI grading system of Adams et al. [21] to modern MRI imaging. We found no significant differences between the three grades of DAI (stage 1, 2 or 3) with respect to initial HDL-C, which is consistent with the findings of some previous studies [5, 21]. In some previous works, DAI grading according to Adams et al.'s scale did not show any significant relationship to long-term patient outcome [21]. This most widely used classification for DAI has been reported to lack prognostic value in comatose patients [5]. Therefore, an alternative tool is clearly needed to offer more accurate outcome predictions for these patients.

It is well known that DAI can influence degree of consciousness [32]. Patients with DAI often present more prolonged post-traumatic comas, or even remain in persistent vegetative state [33]. Unconsciousness resulting from DAI is frustrating for clinicians and distressing for patients' families, particularly since the mechanisms behind the recovery from unconsciousness are largely unknown and accurate prognoses are especially challenging to provide. The ascending reticular activating system (ARAS) of the brain is primarily responsible for the regulation of consciousness [34]. It has been proposed that impaired consciousness level post-DAI may be due to widespread damage of axons, which are the key components of the ARAS particularly in such areas as the brainstem, or the disconnection of white matter between the thalamus and cerebral cortex [35, 36]. In this study, HDL-C was found to be independently associated with the recovery from unconsciousness. Notably, patients with HDL-C levels in Q1 were significantly associated with an increased risk of persistent unconsciousness. This indicated that measuring serum HDL-C may be used to predict the risk of a patient remaining an unconscious state at 6 months post-TBI. HDL has been implicated in several brain functions, including protection

against inflammatory response, pathogenic BBB injury, and oxidative stress [10–12]. To date, the mechanisms underlying DAI after TBI are very complicated, and inflammation and BBB disruption may have a prominent role, as well as oxidative stress [16, 37]. Myelin damage also contributes to axonal degeneration in the traumatic brain suggesting that promotion of myelin regeneration could provide important targets for the treatment of DAI [38]. HDL-C is needed for membrane lipid synthesis and axonal myelin repair [29], so a significant decline in HDL-C may correspond to severe axonal damage, implying a poor prognosis. Thus, the present observation that HDL-C was independently associated with the recovery from unconsciousness for patients with DAI, as visualized on subsequent MRIs, can be explained by our assumption that these protective functions of HDL-C are essential for preserving axon structural integrity and promoting the recovery from axonal damage.

At the present study, presence of previous hyperlipidemia requiring statins was an exclusion criterion because that statins exert both potential salutary and negative effects in diseases of the CNS. Preclinical studies have shown that statins may exert significant benefit in TBI models by multiple mechanisms, such as their anti-inflammatory and anti-inflammatory properties. Thus, statins are considered as potential therapeutic drug for TBI [39]. However, statins also have been shown to have negative impact of statin on TBI. It has been reported that statins could hinder myelin formation by interfering with Ras and Rho signaling in mature oligodendrocytes [40]. As mentioned above, myelin damage is associated the axonal degeneration post-TBI and the repair of DAI. Hence, we excluded the patients received treatment of stains from this study in order to eliminate the interference effect of stains on the presence/outcome of DAI.

### Limitations

Although these findings are interesting, important limitations should be considered given our retrospective study design. First, inherent selection biases due to nonrandomized treatment assignments may affect the primary outcome of the patients. Second, this study lack of analysis of nutrition for patients within this cohort. Third, we did not investigate cholesterol and lipoprotein profiles in the cerebral spinal fluids. As HDL as a molecule can be considered a phase with the constituents come and go with distinct half-lives, one of cholesterol particles that act as an acute phase reactant and can change in the setting of severe inflammation and specifically severe TBI, thus the level of HDL within a week of TBI might not be the accurate baseline level for this particular patient, yet having a low level might reflect a lower baseline and might indicate the lack of the available protective

molecules against DAI. Fourth, this study was performed in single center and lack of ethnic diversity. Last but not the least, there was only a single evaluation of lipids; long-term follow-up was not performed.

### Conclusions

We conclude that initial serum HDL-C levels were independently associated with DAI lesions visible on subsequent MRIs of TBI patients. HDL-C levels in Q1 in patients presenting with DAI were significantly associated with an increased risk of a persistent unconscious state (>6 months). However, it remains unclear whether low levels of serum HDL-C are a cause or a consequence of the DAI presence. Our study would be further strengthened if we had also obtained cholesterol and lipoprotein profiles in the cerebral spinal fluids. Further experimental studies are needed to support the data of this study, and these investigations may hopefully provide deeper insights into the roles HDL plays in DAI.

### Author Contributions

ZYH contributed to the study design, data collection, data analysis and interpretation, and critical writing of the manuscript. BZ contributed to data analysis and interpretation, and critical writing of the manuscript. RHH contributed to the data collection and critical appraisal of the manuscript. ZZ contributed to the data collection and critical appraisal of the manuscript. SQZ contributed to the data analysis and interpretation. YW contributed to the data collection and interpretation. JZF contributed to critical appraisal of the manuscript.

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### Conflict of interest

The authors have no conflict of interest to declare.

### Ethical Approval

Since all data were extracted from routine hospital records and fully anonymized before analysis, the need for formal review and consent was waived. This study was carried out in accordance with the recommendations of Nanfang Hospital, Southern Medical University. The protocol was approved by our Institutional Review Board.

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### References

1. Maas AI, Stocchetti N, Bullock R. Moderate and severe traumatic brain injury in adults. *Lancet Neurol*. 2008;7(8):728–41.
2. Jiang JY, Gao GY, Feng JF, Mao Q, Chen LG, Yang XF, et al. Traumatic brain injury in China. *Lancet Neurol*. 2019;18(3):286–95.
3. Tang C, Bao Y, Qi M, Zhou L, Liu F, Mao J, et al. Mild induced hypothermia for patients with severe traumatic brain injury after decompressive craniectomy. *J Crit Care*. 2017;39:267–70.

4. Abu Hamdeh S, Marklund N, Lewén A, Howells T, Raininko R, Wikström J, et al. Intracranial pressure elevations in diffuse axonal injury: association with nonhemorrhagic MR lesions in central. *J Neurosurg.* 2018;14:1–8.
5. Abu Hamdeh S, Marklund N, Lannsjö M, Howells T, Raininko R, Wikström J, et al. Extended anatomical grading in diffuse axonal injury using MRI—hemorrhagic lesions in the substantia nigra and mesencephalic tegmentum indicate poor long-term outcome. *J Neurotrauma.* 2017;34(2):341–52.
6. Ohno N, Ikenaka K. Axonal and neuronal degeneration in myelin diseases. *Neurosci Res.* 2019;139:48–57.
7. Balazs Z, Panzenboeck U, Hammer A, Sovic A, Quehenberger O, Malle E, et al. Uptake and transport of high-density lipoprotein (HDL) and HDL-associated alpha-tocopherol by an in vitro blood–brain barrier model. *J Neurochem.* 2004;89(4):939–50.
8. Roheim PS, Carey M, Forte T, Vega GL. Apolipoproteins in human cerebrospinal fluid. *Proc Natl Acad Sci USA.* 1979;76(9):4646–9.
9. Borghini I, Barja F, Pometta D, James RW. Characterization of subpopulations of lipoprotein particles isolated from human cerebrospinal fluid. *Biochim Biophys Acta.* 1995;1255(2):192–200.
10. Rosenson RS. Beyond low-density lipoprotein cholesterol. A perspective on low high-density lipoprotein disorders and Lp(a) lipoprotein excess. *Arch Intern Med.* 1996;156(12):1278–84.
11. Burger D, Dayer JM. High-density lipoprotein-associated apolipoprotein A-I: the missing link between infection and chronic inflammation? *Autoimmun Rev.* 2002;1(1–2):111–7.
12. Fellows K, Uher T, Browne RW, Weinstock-Guttman B, Horakova D, Posova H, et al. Protective associations of HDL with blood–brain barrier injury in multiple sclerosis patients. *J Lipid Res.* 2015;56(10):2010–8.
13. Yuan Q, Wu X, Cheng H, Yang C, Wang Y, Wang E, et al. Is intracranial pressure monitoring of patients with diffuse traumatic brain injury valuable? An observational multicenter study. *Neurosurgery.* 2016;78(3):361–9.
14. Licastro F, Hrelia S, Porcellini E, Malaguti M, Di Stefano C, Angeloni C, et al. Peripheral inflammatory markers and antioxidant response during the post-acute and chronic phase after severe traumatic brain injury. *Front Neurol.* 2016;7:189.
15. Yoo RE, Choi SH, Oh BM, Do Shin S, Lee EJ, Shin DJ, et al. Quantitative dynamic contrast-enhanced MR imaging shows widespread blood–brain barrier disruption in mild traumatic brain injury patients with post-concussion syndrome. *Eur Radiol.* 2019;29(3):1308–17.
16. Johnson VE, Weber MT, Xiao R, Cullen DK, Meaney DF, Stewart W, et al. Mechanical disruption of the blood–brain barrier following experimental concussion. *Acta Neuropathol.* 2018;135(5):711–26.
17. Hottman DA, Chernick D, Cheng S, Wang Z, Li L. HDL and cognition in neurodegenerative disorders. *Neurobiol Dis.* 2014;72:22–36.
18. Kelley BJ, Lifshitz J, Povlishock JT. Neuroinflammatory responses after experimental diffuse traumatic brain injury. *J Neuropathol Exp Neurol.* 2007;66(11):989–1001.
19. Ezaki Y, Tsutsumi K, Morikawa M, Nagata I. Role of diffusion-weighted magnetic resonance imaging in diffuse axonal injury. *Acta Radiol.* 2006;47(7):733–40.
20. Tong KA, Ashwal S, Holshouser BA, Nickerson JP, Wall CJ, Shutter LA, et al. Diffuse axonal injury in children: clinical correlation with hemorrhagic lesions. *Ann Neurol.* 2004;56(1):36–50.
21. Adams JH, Doyle D, Ford I, Gennarelli TA, Graham DI, McLellan DR. Diffuse axonal injury in head injury: definition, diagnosis and grading. *Histopathology.* 1989;15(1):49–59.
22. Giacino JT, Kalmar K, Whyte J. The JFK Coma Recovery Scale-Revised: measurement characteristics and diagnostic utility. *Arch Phys Med Rehabil.* 2004;85(12):2020–9.
23. Li L, Tan HP, Liu CY, Yu LT, Wei DN, Zhang ZC, et al. Polydatin prevents the induction of secondary brain injury after traumatic brain injury by protecting neuronal mitochondria. *Neural Regen Res.* 2019;14(9):1573–82.
24. Meaney DF, Morrison B, Dale Bass C. The mechanics of traumatic brain injury: a review of what we know and what we need to know for reducing its societal burden. *J Biomech Eng.* 2014;136(2):021008.
25. Holbourn AHS. The mechanics of brain injuries. *Br Med Bull.* 1945;3:147–9.
26. Gennarelli TA. Mechanisms of brain injury. *J Emerg Med.* 1993;11(Suppl1):5–11.
27. Strich SJ. Diffuse degeneration of cerebral white matter in severe dementia following head injury. *J Neurol Neurosurg Psychiatry.* 1956;19(3):163–85.
28. Tang-Schomer MD, Johnson VE, Baas PW, Stewart W, Smith DH. Partial interruption of axonal transport due to microtubule breakage accounts for the formation of periodic varicosities after traumatic axonal injury. *Exp Neurol.* 2012;233(1):364–72.
29. Haley RW, Dietsch JM. Is there a connection between the concentration of cholesterol circulating in plasma and the rate of neuritic plaque formation in Alzheimer disease? *Arch Neurol.* 2000;57(10):1410–2.
30. Björkhem I, Meaney S. Brain cholesterol: long secret life behind a barrier. *Arterioscler Thromb Vasc Biol.* 2004;24(5):806–15.
31. Chien JY, Jerng JS, Yu CJ, Yang PC. Low serum level of high-density lipoprotein cholesterol is a poor prognostic factor for severe sepsis. *Crit Care Med.* 2005;33(8):1688–93.
32. Skandsen T, Kvistad KA, Solheim O, Strand IH, Folvik M, Vik A. Prevalence and impact of diffuse axonal injury in patients with moderate and severe head injury: a cohort study of early magnetic resonance imaging findings and 1-year outcome. *J Neurosurg.* 2010;113(3):556–63.
33. Gennarelli T, Thibault L, Adams J, Graham D, Thompson C, Marcincin R. Diffuse axonal injury and traumatic coma in the primate. *Ann Neurol.* 1982;12(6):564–74.
34. Jang SH, Kwon HG. The ascending reticular activating system from pontine reticular formation to the hypothalamus in the human brain: a diffusion tensor imaging study. *Neurosci Lett.* 2015;590:58–61.
35. Niemeier JP, Perrin PB, Holcomb MG, Rolston CD, Artman LK, Lu J, et al. Gender differences in awareness and outcomes during acute traumatic brain injury recovery. *J Womens Health (Larchmt).* 2014;23(7):573–80.
36. Zhong YH, Wu HY, He RH, Zheng BE, Fan JZ. Sex differences in sex hormone profiles and prediction of consciousness recovery after severe traumatic brain injury. *Front Endocrinol (Lausanne).* 2019;10:261.
37. Ma J, Zhang K, Wang Z, Chen G. Progress of research on diffuse axonal injury after traumatic brain injury. *Neural Plast.* 2016;2016:9746313.
38. Mu J, Li M, Wang T, Li X, Bai M, Zhang G, et al. Myelin damage in diffuse axonal injury. *Front Neurosci.* 2019;13:217.
39. Wible E, Laskowitz DT. Statins in traumatic brain injury. *Neurotherapeutics.* 2010;7(1):62–73.
40. Klopffleisch S, Merkler D, Schmitz M, Klöppner S, Schedensack M, Jeserich G, et al. Negative impact of statins on oligodendrocytes and myelin formation in vitro and in vivo. *J Neurosci.* 2008;28(50):13609–14.