Original Article

Peripheral inflammatory markers in patients with prolonged disorder of consciousness after severe traumatic brain injury

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Background: Inflammatory processes are known to be the key secondary effects of severe traumatic brain injury (sTBI). The aim of the present study was to assess the value of peripheral inflammatory markers in the chronic unconscious phase after sTBI.

Methods: This was a prospective cohort study. A total of 101 patients with prolonged disorder of consciousness (DoC) and 22 healthy controls (HC) were enrolled in the study. Serum levels of interleukin (IL)-1β, -4, -6, -10, -13, and tumor necrosis factor-α (TNF-α) were investigated in patients with prolonged DoC after sTBI. In addition, the Coma Recovery Scale-revised (CRS-R) was used to quantify the consciousness level, and clinical outcomes at 12 months were determined using the Glasgow Outcome Scale (GOS). Predictive logistic model was built based on the demographic characteristics and cytokine levels.

Results: At baseline, IL-6, -10, -13, and TNF-α levels were significantly higher in patients with prolonged DoC compared with controls, while no differences in cytokine levels were observed between patients in a vegetative state (VS) and those in a minimally conscious state (MCS). IL-13 and TNF-α were found to be correlated with behavioral scores in patients with prolonged DoC, and were associated with recovery 12 months later.

Conclusions: The results of the study provide information about long-term inflammatory responses in the chronic unconscious phase after brain trauma. Further larger studies are required to validate the value of these inflammatory markers.

Keywords: Severe traumatic brain injury (sTBI); prolonged disorder of consciousness (prolonged DoC); inflammation; recovery

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Introduction

Prolonged disorder of consciousness (DoC), also known as chronic DoC, is most frequently caused by severe traumatic brain injury (sTBI) and remained unconscious for more than 1 month, mainly includes a vegetative state (VS)/unresponsive wakefulness syndrome (UWS) and a minimally conscious state (MCS) (1,2). Previous studies have reported that patients in a traumatic VS for more than 1 year might remain in an unconscious state permanently (3). As for now, the Coma Recovery Scale-revised (CRS-R) is still considered to be the best behavioral scale for differential diagnosis of DoC, even though the misdiagnosis rate has reached 30–40%. In the last few years, new techniques have been developed to improve the diagnostic and prognostic value. Behavioral assessments, multimodal neuroimaging methods, and fluid biomarkers (cerebrospinal fluid, serum and plasma) have been reported as the potential predictors

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of recovery in DoC patients.

In addition to the primary local impact injury that occurs immediately upon trauma, secondary brain injury becomes progressively exacerbated during the post-injury period (4). Recently, there has been significant insight into the pathobiology of secondary injury, revealing that such injury involves metabolic disturbances, excitotoxicity, oxidative stress, inflammation, and apoptosis (5,6). Previous studies from our laboratory have shown that the inflammatory response plays a vital role in the chronic unconscious phase after sTBI (7). However, evidence of a relationship between the inflammatory response and DoC has been inconclusive.

Investigators have recently reported the dual roles of central and peripheral inflammation in TBI (8,9). Cytokines, a group of inflammatory mediators, have multiple functions and can induce, exacerbate, mediate, and inhibit subsequent pathophysiological processes in the injured brain (10,11). More specifically, high levels of inflammatory cytokines, such as interleukin (IL)-1β, -4, -6, -10, and -13, as well as tumor necrosis factor-α (TNF-α) have been reported in the peripheral blood stream after sTBI (12-14). IL-1β, -6, and TNF-α are pro-inflammatory cytokines, whereas IL-4, -10, and -13 perform anti-inflammatory functions. A number of researchers have evaluated the effects of inflammatory cytokines during the acute phase (15,16), the inflammatory cytokines not only induce secondary injury and neurodegeneration but also promote neural repair and functional recovery, however, uncertainty still exists about the roles of inflammatory cytokines during the chronic phase after sTBI.

The present study was designed to examine whether serum inflammatory markers are associated with clinical performance and long-term outcomes in patients with DoC after sTBI. The levels of inflammatory cytokines IL-1β, -4, -6, IL-10, -13, and TNF-α were compared between patients with VS/UWS and patients with MCS. We hypothesized that peripheral inflammatory markers offer clinically valuable information on the chronic unconscious phase after sTBI. We present the following article in accordance with the MDAR reporting checklist (available at https://dx.doi.org/10.21037/apm-21-1852).

Methods

Patients

This prospective cohort study enrolled 101 patients with prolonged DoC admitted to the rehabilitation units of Hangzhou Wujing Hospital (Hangzhou, Zhejiang, China) from January 2015 to January 2018. All patients had suffered severe brain trauma and remained unconscious for more than 1 month. Patients with acute infectious diseases or liver dysfunction were excluded. The location of the region showing structural damage and any clinical complications following the trauma were carefully recorded. Twenty-two age- and sex-matched individuals participated in the study as healthy controls (HC).

All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethical Committee of the First Affiliated Hospital, Zhejiang University School of Medicine (No. 2015_310). Written informed consent was obtained from the patients' legal guardians. The trial was registered on ClinicalTrials.gov (ID No. NCT03908541).

Blood collection

Venous blood samples (5 mL) were collected from each patient using standardized phlebotomy procedures. Blood samples were collected in a Vacutainer tube without anticoagulant, centrifuged at 2,000 ×g for 5 min at 4 ℃, and stored at –80 ℃ until further analysis.

Measurement of inflammatory markers

The serum concentrations of IL-1β, -4, -6, -10, -13, and TNF-α were assessed using the MILLIPLEX MAP Kit (Millipore, Billerica, MA, USA). The data are expressed as picograms per milliliter. Cytokine sample processing and data analysis were performed according to the manufacturer's instructions. All measurements were performed at the same time on the same machine by one board-certified laboratory technician who was blinded to the patients' clinical characteristics.

Behavioral assessment

The CRS-R was used to separate the patients into the VS/UWS and MCS groups (17). The CRS-R consists of 23 hierarchically arranged items that constitute 6 subscales addressing auditory, visual, motor, oromotor/verbal, communication, and arousal processes. The clinical outcome was determined using the Glasgow Outcome Scale (GOS) at 12 months' follow up and was

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Table 1 Baseline characteristic of the included individuals

Covariates	DoC (n=101)			HC (n=22)	P value
	MCS (n=43)	UWS (n=58)	P value		
Age (years), mean ± SD	48.72±16.19	50.50±15.25	0.54	44.23±6.39	0.11
Sex, male (%)	30 (69.77)	40 (68.97)	0.93	16 (72.72)	0.75
Time points (day), mean ± SD	100.63±46.75	90.59±43.55	0.27	–	–
Course of lesion, n (%)			0.10	–	–
Traffic accident	25 (58.14)	46 (79.31)			
Fall	17 (39.53)	9 (15.52)			
Other	1 (2.3)	3 (5.17)			
Lesion region, n (%)			0.16	–	–
Left	10 (23.26)	11 (18.97)			
Right	15 (34.88)	12 (20.69)			
Bilateral	18 (41.86)	35 (60.34)			
CRS-R, mean ± SD	13.53±4.47	4.41±1.85	0.00	–	–

DoC, disorder of consciousness; MCS, minimally conscious state; UWS, unresponsive wakefulness syndrome; HC, healthy controls; SD, standard deviation; CRS-R, Coma Recovery Scale-revised.

dichotomized as favorable (GOS 3–5) or unfavorable (GOS 1–2), where GOS 1= death, GOS 2= VS, GOS 3= severe disability, GOS 4= mild-to-moderate disability, and GOS 5= good recovery (18).

Statistical analysis

One-way analysis of variance was applied to compare cytokine levels between the patient and control groups. Correlations between cytokine levels and CRS-R scores were evaluated using the Spearman correlation coefficient. A binary logistic regression analysis was performed to determine associations between the variables and clinical recovery at 12 months. All P values presented are two-tailed, and P<0.05 was considered statistically significant.

Results

Of the 300 patients with DoC admitted to Wujing Hospital between January 2015 and January 2018, 101 met the inclusion criteria and were included in the present. *Table 1* shows the demographic and clinical characteristics of the 101 patients and 22 controls. Patients with DoC had a mean age of 50 years and were predominantly male (69.3%). At the baseline assessment, 43 DoC cases were classified as MCS (30 males, 48.72±16.19 years) and 58 as VS/UWS (40 males, 50.50±15.25 years). The HC (16 males and 6 females) had a mean age of 44 years. There were no significant differences in age, sex, disease duration, disease cause, or lesion region among the MCS and VS/UWS groups.

The serum levels of peripheral inflammatory markers in the MCS, VS/UWS, and control groups are presented in *Figure 1*. Compared with controls, patients with MCS had significantly higher levels of IL-10 (10.68±6.79 *vs.* 2.61±1.38 pg/mL, P<0.001), IL-13 (3.42±2.48 *vs.* 1.41±0.53 pg/mL, P<0.001), IL-1β (1.19±0.64 *vs.* 0.90±0.21 pg/mL, P=0.04), IL-6 (4.29±3.72 *vs.* 0.97±0.68 pg/mL, P=0.001), and TNF-α (33.25±11.77 *vs.* 18.00±6.49 pg/mL, P<0.001). Patients with VS/UWS had significantly higher levels of IL-10 (8.91±5.20 *vs.* 2.61±1.38 pg/mL, P<0.001), IL-13 (2.64±2.35 *vs.* 1.41±0.53 pg/mL, P=0.04), IL-6 (4.61±3.90 *vs.* 0.97±0.68 pg/mL, P<0.001), and TNF-α (30.96±14.42 *vs.* 18.00±6.49 pg/mL, P<0.001) compared with the controls. However, there were no significant differences in the inflammatory markers between MCS and VS/UWS patients.

The correlation coefficients between CRS-R scores and cytokine levels are shown in *Figure 2*. IL-13 and TNF-α concentrations were significantly correlated with CRS-R

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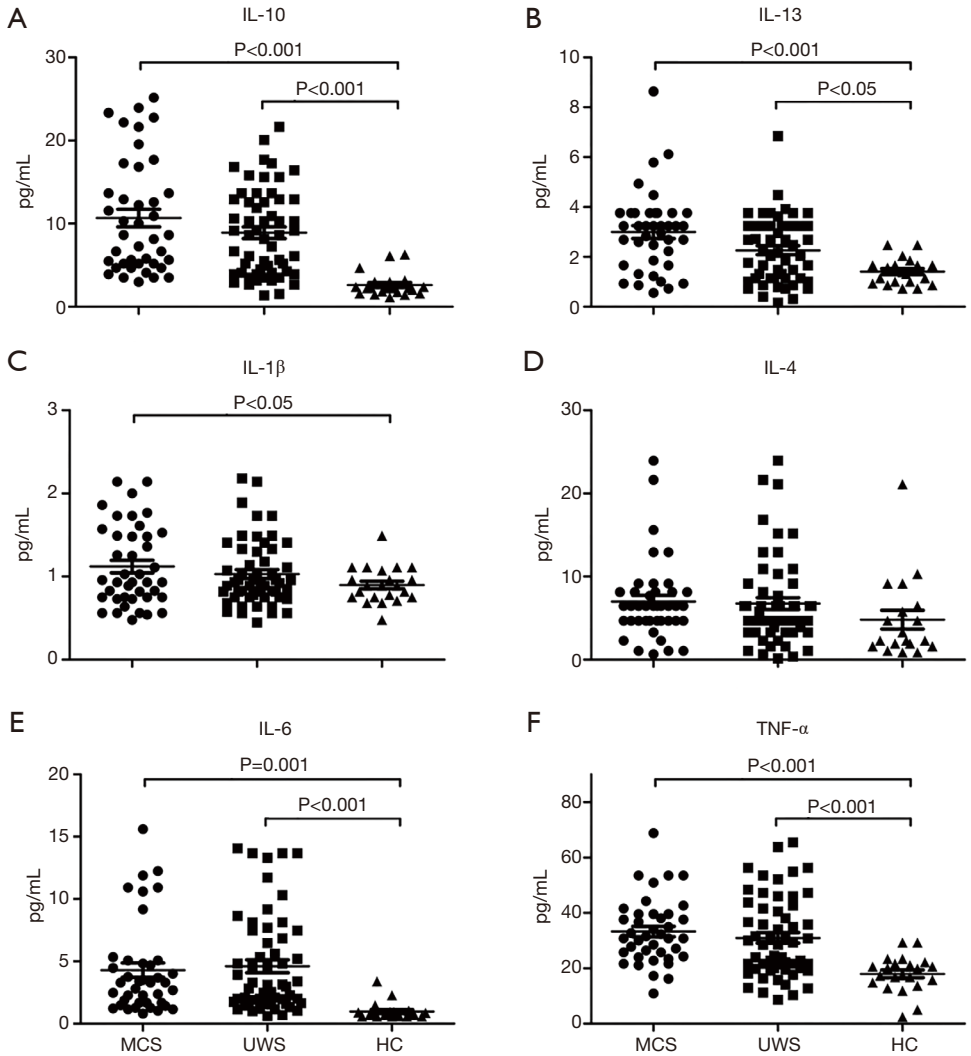

Figure 1 Serum inflammatory markers in patients with DoC after sTBI. Serum levels of (A) IL-10, (B) IL-13, (C) IL-1β, (D) IL-4, (E) IL-6, and (F) TNF-α in the MCS, VS/UWS, and control groups. Statistical analysis was performed using one-way analysis of variance. Data are expressed as means (pg/mL). DoC, disorder of consciousness; sTBI, severe traumatic brain injury; IL, interleukin; TNF-α, tumor necrosis factor-α; MCS, minimally conscious state; VS, vegetative state; UWS, unresponsive wakefulness syndrome.

score, with correlation coefficients of 0.27 (P=0.002) and 0.23 (P=0.023), respectively (*Figure 3*). However, no other cytokine level was correlated with CRS-R score. In addition, IL-10 level was significantly correlated with levels of IL-6 and TNF-α; IL-13 level was significantly correlated with levels of IL-4, -6, and TNF-α; IL-1β level was significantly correlated with levels of IL-4 and -6; and IL-6 level was significantly correlated with TNF-α level.

To examine the associations between the peripheral inflammatory markers and clinical recovery, logistic regression analysis was performed (*Table 2*). In this analysis, significant associations were found between IL-13 level and outcome [odds ratio (OR): 1.802, 95% confidence interval (CI): 1.099–2.954, P=0.02] and TNF-α level and outcome (OR: 0.952, 95% CI: 0.909–0.998, P=0.04). No statistically significant associations were found between age, sex, or time points.

Discussion

General pathophysiological cascades of secondary brain damage are initiated a few hours or even weeks after sTBI,

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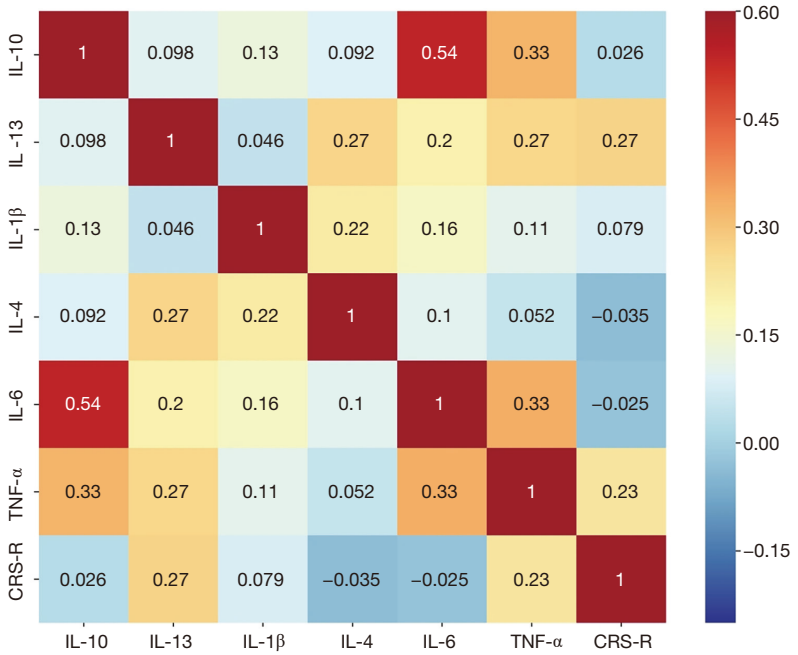

Figure 2 Correlation between the CRS-R score and cytokine levels. Correlations of serum levels of IL-1β, -4, -6, -10, -13, and TNF-α with the CRS-R score were examined. CRS-R, Coma Recovery Scale-revised; IL, interleukin; TNF-α, tumor necrosis factor-α.

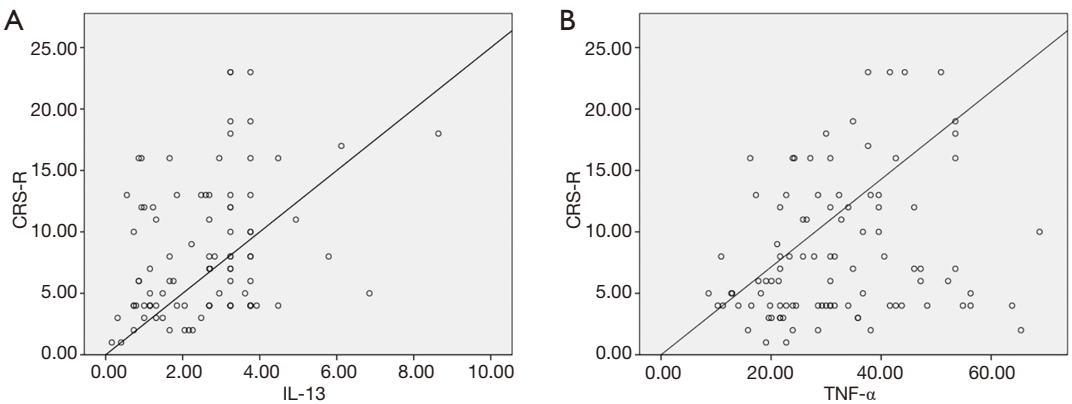

Figure 3 Association of (A) IL-13 and (B) TNF-α levels with the CRS-R score. IL, interleukin; TNF-α, tumor necrosis factor-α; CRS-R, Coma Recovery Scale-revised.

and an inflammatory response is a prominent component of these processes (19,20). Inflammatory cytokines play central roles in the acute phase after sTBI (21); however, few studies have investigated long-term cytokine levels. Although TBI-related complications persist for years, little is known about the role of the inflammatory response in the chronic phase (22). To the best of our knowledge, the present study is the first to determine the effects of specific inflammatory markers in the chronic unconscious phase after sTBI.

A growing body of evidence suggests that cytokines play multiple roles in sTBI, depending on the time course and concentration (10,23). In the present study, IL-10, -13, -6, and TNF-α levels were significantly higher in patients with sTBI during the chronic unconscious phase than in controls, suggesting a persistent inflammatory response.

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Table 2 Binomial logistic regression analysis for the clinical recovery of patients with DoC according to demographic characteristics and cytokine levels

Covariates	OR	95% CI	P value
Age	0.994	0.955–1.034	0.748
Sex	1.357	0.425–4.329	0.606
Time points	0.993	0.979–1.007	0.346
IL-10	1.038	0.935–1.152	0.489
IL-13	1.802	1.099–2.954	0.020*
IL-1β	1.764	0.476–6.530	0.396
IL-4	0.948	0.849–1.058	0.340
IL-6	0.889	0.742–1.067	0.206
TNF-α	0.952	0.909–0.998	0.041*

*, statistical significance at P<0.05. DoC, disorder of consciousness; OR, odds ratio; CI, confidence interval; IL, interleukin; TNF-α, tumor necrosis factor-α.

However, no significant differences in these cytokine levels were found between the VS/UWS and MCS groups, suggesting a lack of specificity in differentiating the degree of consciousness.

All of these cytokines have been reported to contribute to the inflammatory response following sTBI (24). IL-6, which performs pro-inflammatory functions, is a key effector in the damaged brain. Similarly, the plasma level of the anti-inflammatory cytokine IL-10 was significantly higher in TBI patients and was also associated with the TBI outcome. Interestingly, an elevated IL-6/IL-10 ratio was previously found to be associated with outcome in TBI patients (9). The level of IL-4, a cytokine with both pro- and anti-inflammatory properties, has been reported to increase after trauma and has been found to be more prominent in patients with favorable recovery (25). However, in the present study, no significant difference in IL-4 level was found in the chronic stage after sTBI.

Many previously published studies have reported that IL-6, -1, -8, -10, and TNF-α levels are associated with worse outcomes (11,24). In contrast, IL-13, a protective and anti-inflammatory cytokine, has not been reported to be elevated in TBI studies. Although extensive studies have been carried out on inflammation after sTBI, the experimental data are unsatisfactory and controversial due to practical constraints. Inconsistent with the previous studies, our results indicated that specific inflammatory cytokines may be predictors of unfavorable outcomes in patients with DoC after sTBI.

Prolonged inflammation has been implicated in many chronic neurodegenerative diseases (26), whereas chronic inflammatory responses have not been well characterized following sTBI, especially in the chronic unconscious stage. It is important to understand the role of cytokines in the chronic phase after sTBI to improve the management of patients with DoC. Understanding the mechanisms underlying the disease course may offer novel intervention options, as well as therapeutic targets, for sTBI patients (27). One limitation of the present study was the small and homogeneous sample. Further studies involving a larger cohort of patients are needed to confirm our findings.

Conclusions

The findings of the present study indicated a peripheral inflammatory response during the late period after sTBI. Although chronic inflammatory activity has not been well characterized, several peripheral inflammatory markers could be used in long-term pathophysiological processes for sTBI. Future larger studies are required to further elucidate the value of these inflammatory markers.

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Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at https://dx.doi.org/10.21037/apm-21-1852

Data Sharing Statement: Available at https://dx.doi.org/10.21037/apm-21-1852

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://dx.doi.org/10.21037/apm-21-1852). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All procedures performed in this study involving human participants were

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in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethical Committee of the First Affiliated Hospital, Zhejiang University School of Medicine (No. 2015_310). Written informed consent was obtained from the patients' legal guardians.

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