

Original Article

Plasma cytokine changes and its clinical significance in intracranial infection secondary to traumatic brain injury

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Abstract: Objective: This study aimed to investigate the plasma cytokine changes and its clinical significance in intracranial infection secondary to traumatic brain injury. Methods: A total of 60 cases with intracranial infection secondary to traumatic brain injury admitted to our hospital from January 2017 to December 2019 were selected as the research objects, of whom, 24 cases with mild infection, 20 with moderate infection, and 16 with severe infection. Another 60 cases without intracranial infection secondary to traumatic brain injury during the same period were selected as the uninfected group. A comparison of infected and uninfected groups on changes of plasma cytokines (IL-1 β , IL-2, IL-6, IL-8, TNF- α , IFN- γ) and high mobility group-1 protein (HMGB1) were conducted to analyze the correlation between plasma cytokines and disease severity. Results: The data of IL-1 β , IL-2, IL-6, IL-8, TNF- α , IFN- γ and HMGB1 levels in both groups on day 1, day 3 and day 5 after the surgery were obtained. The results indicated that for infected group, the differences were significant among these 3 days ($P < 0.05$), and the data on day 5 were all higher than that on day 1 and day 3 ($P < 0.05$). While for uninfected group, there was no significant difference among those 3 days ($P > 0.05$). The differences in different severity of infection on day 5 showed statistically significance ($P < 0.05$), and it was positively correlated with the severity ($P < 0.05$). Conclusion: The cytokines content in intracranial infection secondary to traumatic brain injury increased significantly, which was closely related to the severity of the infection. These factors can be used as monitoring indicators for diagnosis of intracranial infection and assessment of the severity.

Keywords: Intracranial infection secondary to traumatic brain injury, interleukin, TNF- α , IFN- γ , HMGB1

Introduction

With the increasing popularity of vehicles, the incidence of traffic accidents is increasing, and so is the incidence of traumatic brain injury (TBI). Clinically, surgical treatment is mostly used for TBI. However, the risk of postoperative intracranial infection is relatively high. If it is not diagnosed and treated in time, it will seriously affect the prognosis and even endanger life [1]. It is reported that by 2020, TBI will surpass many other diseases to become the main cause of death and disability [2]. TBI induces stress response, which can lead to metabolic and functional disorders. Some studies have shown that patients with TBI will produce a large number of inflammatory mediators under long-term pathological stress, leading to further aggravation of the disease, which will

worsen the prognosis [3]. Currently, whether the patient has infection is usually diagnosed according to the clinical manifestations and results of cerebrospinal fluid (CSF) bacterial culture. However, there are problems such as difficulty in obtaining specimen and long-time consumption, which are not conducive to early infection detection. Various plasma cytokines (such as interleukin, tumor necrosis factor, interferon) are closely related to inflammatory responses [4-6], high mobility group-1 protein (HMGB-1) is a cytokine with pro-inflammatory effect that has received extensive attention in recent years [7]. Studies have found that the level of HMGB-1 in infected patients significantly increased [8, 9]. In order to investigate the application value of plasma cytokines and HMGB-1 levels in the evaluation and diagnosis of patients with intracranial infection secondary

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to traumatic brain injury, this study selected the patients with intracranial infection secondary to traumatic brain injury for analysis.

Data and methods

Data collection

A total of 60 cases with postoperative intracranial infection secondary to TBI admitted to our hospital during January 2017-December 2019 were selected as infected group. TBI patients were all treated within 72 hours of injury; clinically manifested as intracranial hypertension and meningeal irritation, such as fever, headache, vomiting, disturbance of consciousness; intracranial infection was confirmed by head CT and CSF bacterial culture were included. While patients with diabetes, hypertension, malignant tumor, with complication of other inflammation, infection or autoimmune diseases were excluded from the current study. Another 60 cases of traumatic brain injury patients without intracranial infection admitted in the same period were taken as the uninfected group. This study was approved by the hospital ethics committee.

Criteria for disease severity

The diagnosis of intracranial infection is based on the turbidity of CSF and white blood cell (WBC) count in CSF. Mild infection: mild turbidity of CSF, WBC count in CSF specimen is $<5 \times 10^6/L$. Moderate infection: moderate turbidity of CSF, and the WBC count is $5 \times 10^6/L-6 \times 10^6/L$. Severe infection: severe turbidity of CSF, WBC count is $>6 \times 10^6/L$.

Test on cytokine level

5 ml peripheral venous blood was sampling from all patients under fasting, centrifuged at 3000 r/min for 5 min, and then supernatant was collected. Enzyme-Linked Immunosorbent Assay (ELISA) was adopted to detect the level of IL-1 β , IL-2, IL-6, IL-8, TNF- α and IFN- γ . The operation steps were strictly carried out in accordance with the instructions of the kit (GENZYME).

HMGB-1 test

The HMGB-1 was detected by ELISA with the kit made by Shino-Test, Japan. The experimental steps were performed in strict accordance with the kit instructions.

Th1, Th2 cell test

Reagent: The flow antibody CD3 (PerCP), CD8 (APC), IL-4 (PE), IFN- γ (FITC), IgG2a (PE), IgG1 (FITC), FACSLysing hemolysates and cell stimulator leukemia activation were purchased from Becton Dickison. FIX & PERM Kit was purchased from Life Technologies. FACS Calibur flow cytometer was purchased from Becton Dickison.

Fasting hemospasia was performed in all patients to collect 5 ml peripheral venous blood, with 1 μ l cell stimulator added to the bottom of the control tube and experimental tube respectively. 50 μ l of whole blood was added to each tube, shaken and mixed evenly, and placed in a 37°C 5 mg/dl CO₂ incubator for 4.5 h (shaken and mixed evenly every 2 h). Next, antibody CD3 (PerCP) 5 μ l and CD8 (APC) 5 μ l were added in both tubes and mixed evenly, and incubated at room temperature in dark for 15 min. 50 μ l of fixative A was added to the two tubes and incubated in dark for 15 min, and washed with normal saline to remove supernatant. 50 μ l of FIX & PERM B was added to the two tubes respectively. 5 μ l of IL-4 (PE) and 5 μ l of IFN- γ (FITC) labeled antibody were added to the experimental tube, and the corresponding same type of control antibody was added to the control tube, and incubated at room temperature in dark for 30 min. 360 μ l cell suspension was obtained after washing with normal saline, and the proportion of Th1 and Th2 positive cells were detected.

Statistical analysis

SPSS 23.0 statistical software was used for data analysis. The counting data was represented by number and rate [n (%)]. The chi-square test was used for inter-group comparison. Measurement data was represented by mean \pm standard deviation ($\bar{x} \pm s$). Variance analysis was used for multi-group comparison. R3.6.1 software was used for drawing graphics. Pearson correlation analysis was used for correlation analysis. $P < 0.05$ was considered statistically significant.

Results

General information

There are 24 cases of mild infection, 20 cases of moderate infection and 16 cases of severe infection. By comparison, the difference in

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Table 1. Comparison of general information of patients

General data	Infected group n = 60	Uninfected group n = 60	t/ χ^2	P
Age	54.11 ± 7.37	52.62 ± 7.25	1.116	0.266
Gender				
Male	37	35	0.139	0.709
Female	23	25		

terms of general data between the infected group and uninfected group was not statistically significant ($P > 0.05$, **Table 1**).

Comparison of cytokine level in two groups

For infected group, the differences of levels of IL-1 β , IL-2, IL-6, IL-8, TNF- α and IFN- γ on day 1, day 3 and day 5 after the surgery were statistically significant ($P < 0.05$), and the data on day 5 were all higher than that on day 1 and day 3 ($P < 0.05$). While for uninfected group, there was no significant difference in levels of IL-1 β , IL-2, IL-6, IL-8, TNF- α , IFN- γ on day 1, day 3 and day 5 ($P > 0.05$, **Table 2**).

HMGB-1, Th1, Th2 level in two groups

For infected group, the differences of HMGB-1, Th1, Th2 level on day 1, day 3 and day 5 after the surgery were statistically significant ($P < 0.05$), and the data on day 5 were all higher than that on day 1 and day 3 ($P < 0.05$). While for uninfected group, there was no significant difference in HMGB-1, Th1, Th2 level on day 1, day 3 and day 5 ($P > 0.05$, **Table 3**).

Cytokine level in patients with different severity of infection

Cytokines (IL-1 β , IL-2, IL-6, IL-8, TNF- α , IFN- γ) level of patients with different severity of infection on day 5 after the surgery was statistically significant ($P < 0.05$) (**Table 4**).

HMGB-1, Th1 and Th2 level in patients with different severity of infection

There were significant differences in HMGB-1, Th1 and Th2 level between patients with different severity of infection on day 5 after the surgery ($P < 0.05$) (**Table 5**; **Figures 1** and **2**).

The correlation between cytokine level and infection severity in infected patients

The IL-1 β , IL-2, IL-6, IL-8, TNF- α , IFN- γ and HMGB-1 level were positively correlated with the severity of infection ($P < 0.05$) (**Figures 3-9**).

Discussion

Previous studies have shown that the main causes of intracranial infection secondary to TBI are low resistance, severe injury, open brain injury, post-operative CSF leakage, and long operation time or multiple operations, etc. [10]. At present, the early diagnosis of intracranial infection is mainly assisted with the clinical indicators including the patient's body temperature, CSF lab test, peripheral blood (WBC level, CRP level) and other indicators. However, these indicators are non-specific, the intracranial infection are susceptible to many factors [11]. The golden standard for diagnosis of intracranial infection is positive bacteriology in CSF. However, due to the limitation of sampling process and bacterial culture methods, the sensitivity and specificity of routine and biochemical test of CSF are not high. Therefore, it is very important to explore a better way to detect early intracranial infection. In this study, the changes of plasma cytokines in patients were analyzed. It is expected to provide guidance for early diagnosis of intracranial infection secondary to TBI.

The levels of plasma cytokines (IL-1 β , IL-2, IL-6, IL-8, TNF- α , IFN- γ), HMGB-1, Th1 and Th2 on day 1, day 3 and day 5 after the surgery in infected group were examined and compared. The results indicated that for infection group, the differences of these data on those 3 days were statistically significant ($P < 0.05$), and the data on day 5 were all higher than that on day 3, the data on day 3 were all higher than that on day 1 ($P < 0.05$). While for uninfected group, there was no significant difference of these data on those 3 days ($P > 0.05$). It indicates that the plasma cytokines, HMGB-1, Th1 and Th2 level in infected group were significantly changed in the early stage. The correlation between plasma cytokines, HMGB-1, Th1, Th2 level and the severity of infection in infected group showed that the IL-1 β , IL-2, IL-6, IL-8, TNF- α , IFN- γ , HMGB-1 level were positively correlated with severity of infection ($P < 0.05$), which indicates that levels of IL-1 β , IL-2, IL-6, IL-8, TNF- α , IFN- γ , HMGB-1 can be used as monitoring indicators for diagnosis of intracranial infection and infection severity.

Th1 cells primarily secrete pro-inflammatory factors, such as IL-2 and IFN- γ to mediate cellular immune response. Th2 cells mainly secrete anti-inflammatory factors, such as IL-4,

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Table 2. The comparison of cytokine level in two groups (ng/mL $\bar{x} \pm s$)

Indicators	Infected group n = 60			F	P	Uninfected group n = 60			F	P
	Day 1	Day 3	Day 5			Day 1	Day 3	Day 5		
IL-1 β	18.97 \pm 3.28	25.61 \pm 4.16 ^A	32.81 \pm 4.71 ^{A,B}	23.125	0.001	15.17 \pm 2.69	15.48 \pm 3.11	15.24 \pm 3.16*	22.636	0.002
IL-2	39.11 \pm 6.12	68.26 \pm 8.23 ^A	91.06 \pm 11.57 ^{A,B}	21.361	0.011	33.15 \pm 5.67	34.18 \pm 6.13	34.27 \pm 6.52*	24.566	0.014
IL-6	57.54 \pm 8.25	76.88 \pm 10.63 ^A	102.36 \pm 19.31 ^{A,B}	20.365	0.002	49.28 \pm 8.17	49.66 \pm 7.95	50.37 \pm 8.64*	26.546	0.021
IL-8	78.47 \pm 11.79	105.24 \pm 14.97 ^A	141.36 \pm 16.44 ^{A,B}	24.658	0.012	66.31 \pm 9.67	67.64 \pm 10.65	67.32 \pm 10.32*	25.365	0.003
TNF- α	1.15 \pm 0.24	1.48 \pm 0.28 ^A	2.62 \pm 0.36 ^{A,B}	25.697	0.003	1.12 \pm 0.28	1.18 \pm 0.27	1.23 \pm 0.29*	29.565	0.001
IFN- γ	3.75 \pm 0.67	6.94 \pm 1.14 ^A	13.86 \pm 3.67 ^{A,B}	26.355	0.001	2.71 \pm 0.52	2.83 \pm 0.56	2.90 \pm 0.58*	27.566	0.002

Note: A. P<0.05, compared with day 1; B. P<0.05, compared with day 3; *, P<0.05, compared with day 5 in the infected group.

Table 3. The comparison of HMGB-1, Th1, Th2 level between two groups ($\bar{x} \pm s$)

Indicators	Infected group n = 60			F	P	Uninfected group n = 60			F	P
	Day 1	Day 3	Day 5			Day 1	Day 3	Day 5		
HMGB-1 (ng/mL)	121.25 \pm 16.67	155.29 \pm 17.23 ^A	206.34 \pm 17.75 ^{A,B}	27.648	0.021	82.24 \pm 15.21	83.37 \pm 16.13	84.86 \pm 16.34*	23.568	0.011
Th1 (%)	17.65 \pm 1.39	15.37 \pm 1.32 ^A	14.16 \pm 1.25 ^{A,B}	26.369	0.016	22.54 \pm 1.83	23.21 \pm 1.89	23.18 \pm 1.72*	21.695	0.021
Th2 (%)	3.42 \pm 0.67	4.29 \pm 0.81 ^A	5.72 \pm 0.83 ^{A,B}	21.697	0.014	2.79 \pm 0.47	2.94 \pm 0.52	2.95 \pm 0.56*	22.689	0.036

Note: A. P<0.05, compared with day 1; B. P<0.05, compared with day 3; *, P<0.05, compared with day 5 in the infected group.

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Table 4. Cytokine level in patients with different severity of infection (ng mL, $\bar{x} \pm s$)

Indicators	Infected group			F	P
	Mild n = 24	Moderate n = 20	Severe n = 16		
IL-1 β	29.26 \pm 2.78	33.41 \pm 4.12	37.38 \pm 2.85	29.462	0.001
IL-2	81.13 \pm 7.26	93.23 \pm 5.32	103.24 \pm 9.04	46.291	0.011
IL-6	86.32 \pm 7.88	101.38 \pm 9.12	127.64 \pm 17.63	61.021	0.002
IL-8	128.13 \pm 8.02	140.05 \pm 10.18	162.84 \pm 9.56	68.692	0.001
TNF- α	2.31 \pm 0.13	2.61 \pm 0.15	3.09 \pm 0.25	94.947	0.021
IFN- γ	10.21 \pm 1.66	13.67 \pm 1.94	18.07 \pm 1.56	98.836	0.032

Table 5. HMGB-1, Th1 and Th2 level in patients with different severity of infection ($\bar{x} \pm s$)

Indicators	Infected group			F	P
	Mild n = 24	Moderate n = 20	Severe n = 16		
HMGB-1 (ng/mL)	188.64 \pm 10.61	205.24 \pm 12.01	222.85 \pm 11.63	43.948	0.001
Th1 (%)	16.25 \pm 0.82	14.81 \pm 1.13	10.21 \pm 0.81	208.698	0.002
Th2 (%)	4.54 \pm 0.41	5.46 \pm 0.76	7.92 \pm 0.42	182.824	0.001

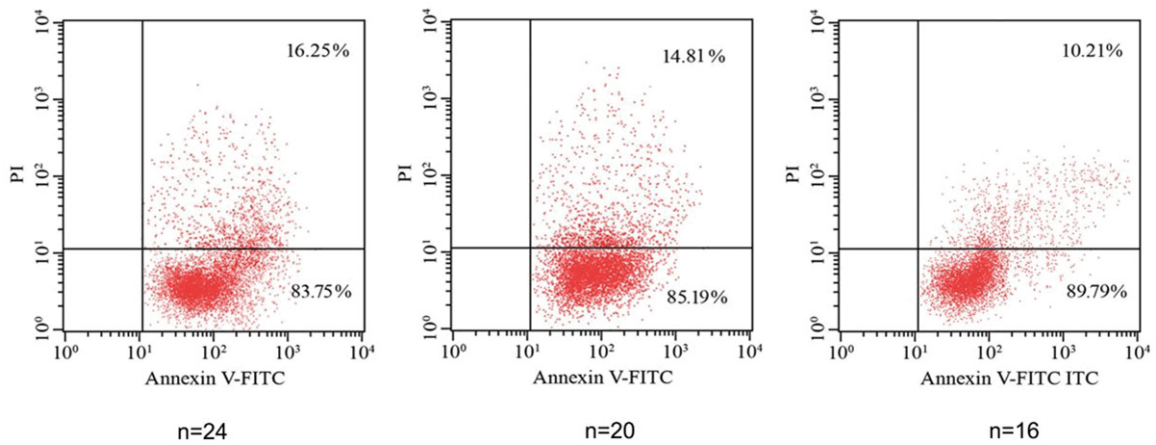


Figure 1. Flow cytometer of Th1.

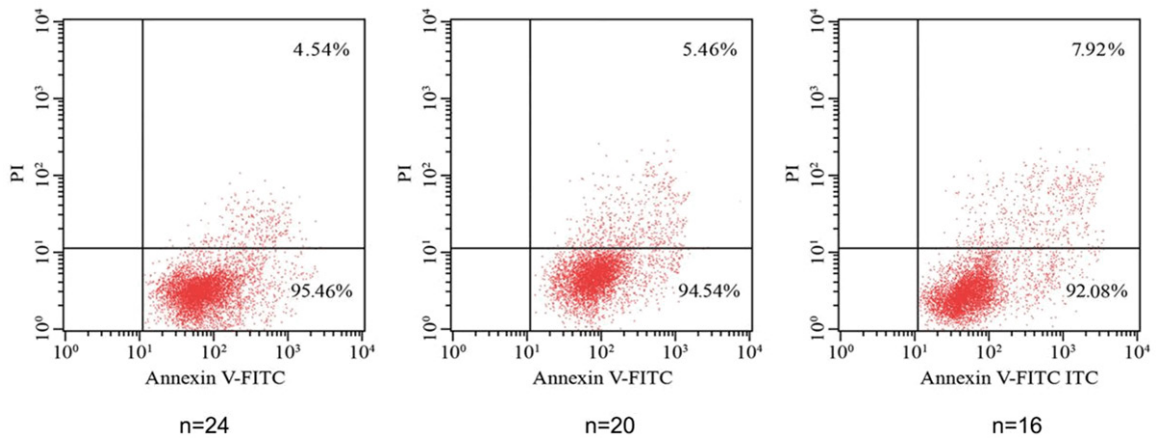


Figure 2. Flow cytometer of Th2.

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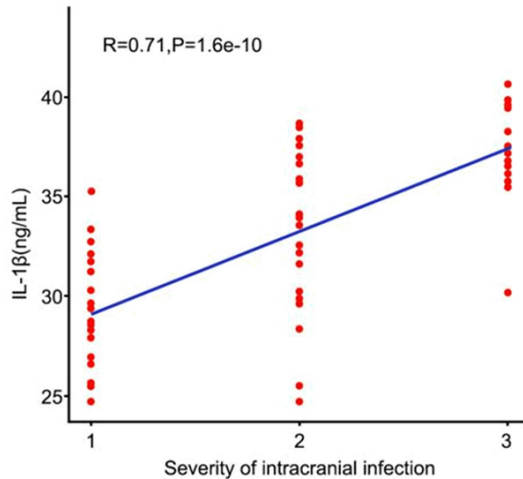


Figure 3. Correlation between the level of IL-1 β and the severity of infection in patients in the infected group. The level of IL-1 β was positively correlated with the severity of infection ($R = 0.71$, $P < 0.05$).

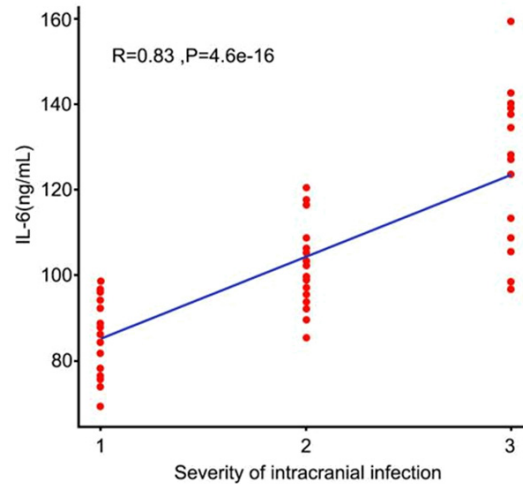


Figure 5. Correlation between the level of IL-6 and the severity of infection in patients in the infected group. The level of IL-6 was positively correlated with the severity of infection ($R = 0.83$, $P < 0.05$).

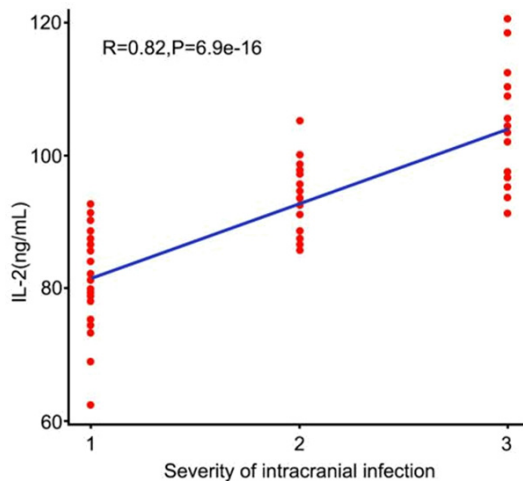


Figure 4. Correlation between the level of IL-2 and the severity of infection in patients in the infected group. The level of IL-2 was positively correlated with the severity of infection ($R = 0.82$, $P < 0.05$).

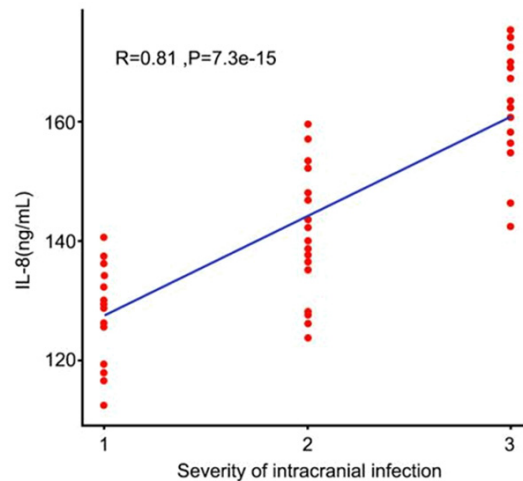


Figure 6. Correlation between the level of IL-8 and the severity of infection in patients in the infected group. The level of IL-8 was positively correlated with the severity of infection ($R = 0.81$, $P < 0.05$).

IL-6, TNF- α to mediate humoral immune response [12]. Normally, the proportion of Th1 and Th2 cells maintains a dynamic balance to ensure homeostasis. When the body presents with symptoms such as trauma or infection, tissue damage or bacterial endotoxin stimulates inflammatory cells, resulting in cascade release of inflammatory mediators. Then, the inflammatory response syndrome occurs in the body, promoting differentiation of Th1 and Th2 cells [13]. The results of this study are consistent

with previous studies, which have found that Th1 cell function decreases and Th2 cell function increases when the body is injured or infected. IL-1 β is a monocyte factor produced by mononuclear macrophages and mainly acts on lymphocytes. It has pro-inflammatory function and plays an important role in immune regulation [14]. Normally, only a small amount of IL-1 β and its receptor are expressed. However, under pathological conditions such as brain injury and craniocerebral injury caused by

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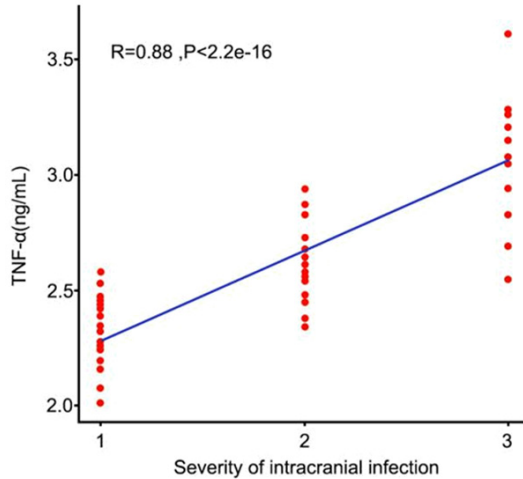


Figure 7. Correlation between the level of TNF- α and the severity of infection in patients in the infected group. The level of TNF- α was positively correlated with the severity of infection ($R = 0.88$, $P < 0.05$).

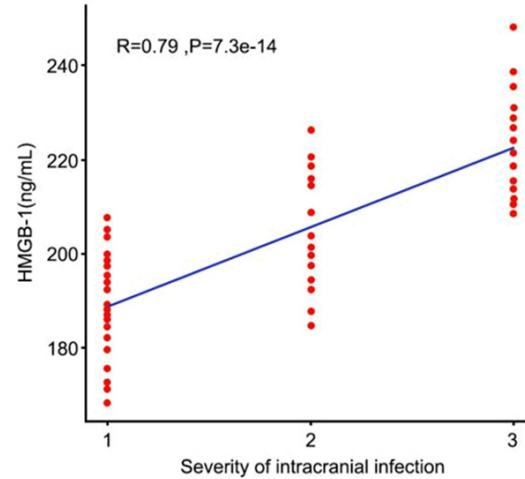


Figure 9. Correlation between the level of HMGB-1 and the severity of infection in patients in the infected group. The level of HMGB-1 was positively correlated with the severity of infection ($R = 0.86$, $P < 0.05$).

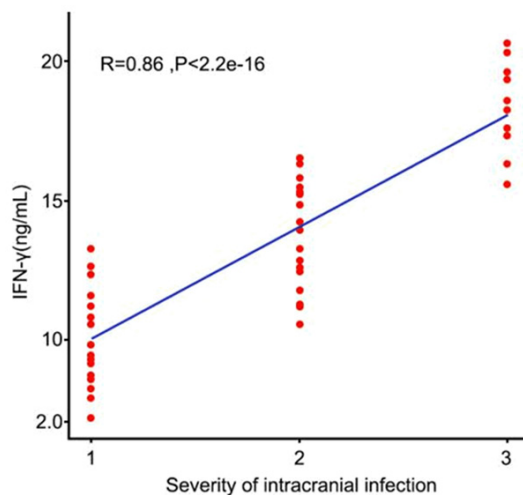


Figure 8. Correlation between the level of IFN- γ and the severity of infection in patients in the infected group. The level of IFN- γ was positively correlated with the severity of infection ($R = 0.86$, $P < 0.05$).

intracranial infection, the level of IL-1 β evidently increases [15], which is consistent with our finding. TNF- α is a cytokine mainly produced by the action of bacterial toxins or other inflammatory stimuli on macrophages and monocytes [16]. When bacteria invade the CSF and release endotoxin phosphatidic acid and other inflammatory components of the cell wall, it can stimulate macrophages and monocytes to produce TNF- α . IL-6 is multifunctional single-stranded glycoprotein cytokines widespread in the central nervous system, with a wide range of bio-

logical activities. Intracranial IL-6 is mainly expressed in neurons and microglia [17]. IL-6 is involved in the immune response and inflammatory response, and plays a role in the nervous system and the stress response. Its regulation disorder is related to a variety of inflammatory diseases [18]. IL-8 is a chemokine of neutrophils, T cells and basophils, under the stimulation of inflammatory factors and bacteria. IL-8 can be synthesized and expressed by various cells. IL-8 is an important pro-inflammatory factor [19]. HMGB-1 is a kind of pro-inflammatory factor, which normally only exists in the nucleus. When the body's pro-inflammatory factors level rises and binds to the corresponding receptors, it can promote the release of HMGB-1, induce apoptosis, stimulate cell growth, destroy blood-brain barrier and other reactions [20]. Studies have found that in infectious diseases, the HMGB-1 level increased significantly, aggravating inflammatory reaction and vascular injury, and inhibiting tissue repair [21]. Therefore, monitoring changes of IL-1 β , IL-2, IL-6, IL-8, TNF- α , IFN- γ , HMGB-1 levels will contribute to early diagnosis of intracranial infection secondary to TBI and evaluation of disease severity. Despite that IL-1 β , IL-2, IL-6, IL-8, TNF- α , IFN- γ and HMGB-1 levels were positively correlated with the severity of infection, the multivariate correlation analysis was not performed. In the future, the research will be conducted with multi-factor analysis of relevant data in order to provide a basis for clinical treatment.

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In summary, plasma cytokines (IL-1 β , IL-2, IL-6, IL-8, TNF- α , IFN- γ) and HMGB-1 level is closely correlated with the occurrence of intracranial infection secondary to TBI, and plasma cytokines (IL-1 β , IL-2, IL-6, IL-8, TNF- α , IFN- γ) and HMGB-1 level has an upward trend with the aggravation of infection, suggesting that these factors can be used as a monitoring indicators for diagnosis of intracranial infection and assessment of its severity.

Disclosure of conflict of interest

None.

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