Original Article
Correlations of prognosis of severe purulent meningitis in children after rehabilitation with serum inflammatory cytokines, humoral immunity and the expression of TLR4 before and after treatment

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Abstract: Objective: Our study aims to investigate the correlation of the prognosis of severe purulent meningitis in children with serum inflammatory cytokines, humoral immunity and the expressions of Toll-like receptor 4 (TLR4) before and after treatment. Methods: A total of 60 children with severe purulent meningitis, who met the inclusion criteria treated from January 2016 to October 2017 were enrolled. According to the Glasgow score, these patients were divided into a good prognosis group (n=38) and a poor prognosis group (n=22). Venous blood and cerebrospinal fluid (CSF) were collected before and after treatment, and the levels of interleukin-6 (IL-6), IL-17, CSF protein and TLR4 were measured. Pearson’s correlation analysis was adopted to explore the correlations of the difference value of changes in the above-mentioned indexes before and after treatment with the Glasgow score. Results: Before treatment, the levels of IL-6, IL-17, CSF protein and TLR4 in the good prognosis group were notably lower than those in the poor prognosis group. After treatment, the levels of these indexes in the good prognosis group were remarkably lower than those in the poor prognosis group (P<0.05). The difference in values of changes in the levels of IL-6, IL-17, CSF protein and TLR4 before and after treatment were positively associated with the Glasgow score. Conclusion: The reduced levels of IL-6, IL-17, CSF protein and TLR4 before and after treatment are correlated to the favorable prognosis of children with severe purulent meningitis, which provides basis for the diagnosis and evaluation of purulent meningitis.

Keywords: Severe purulent meningitis in children, inflammatory cytokine, humoral immunity, TLR4, correlation

Introduction

Purulent meningitis refers to pediatric nervous system infections commonly found in clinical practice, which clinically manifests as fever, meningeal irritation, increased intracranial pressure and cerebrospinal fluid (CSF) purulent changes [1]. Currently, it is considered that purulent meningitis is a major disease that causes pediatric death [2]; especially severe purulent meningitis in children, which is characterized by rapid onset, fast development, severe injury and difficulty treatment, thus resulting in poor prognosis as well as high mortality and morbidity rates in children. In some children, the efficacy of conventional antibiotic treatment is relatively poor, and the disease frequently relapses. Besides, there is still a large possibility of disability or death in patients after antibiotic treatment. Hence, the correlations of the prognosis of severe purulent meningitis in children with certain clear risk factors are of great significance to identify, in order to reduce the mortality and disability rates of severe purulent meningitis in children. This study aims to explore the correlations of the prognosis of severe purulent meningitis in children with serum inflammatory cytokines, humoral immunity and the expression of Toll-like receptor 4 (TLR4).

Patients and methods

General data

A total of 60 children with severe purulent meningitis who met the inclusion and exclusion cri-
teria admitted to our hospital from January 2017 to July 2017 were enrolled. Venous blood and CSF were collected before treatment, and these patients were followed after routine drug treatment. During the follow-up, venous blood and CSF were collected again, and the prognosis was evaluated via the Glasgow clinical outcome scoring. The above-mentioned 60 children were divided into a good prognosis group (n=38), including 20 males and 18 females with an average age of (1.28±0.78) years old, and a poor prognosis group (n=22), including 13 males and 9 females with an average age of (1.53±0.33) years old. The study was reviewed and approved by Ethics Committee of our hospital and all patients’ parents participating in the study signed the informed consent.

**Inclusion criteria**

Inclusion criteria: 1) children who had their first onset, 2) children who clinically manifested with fever, convulsions, increased intracranial pressure, altered consciousness, meningeal irritation, etc., 3) children whose laboratory examinations indicated severe bacterial infection, 4) children with pathogenic bacteria found in the CSF cultivation, 5) children manifested with moderate or severe disturbance of consciousness for more than 48 h, or complicated with other organ dysfunction or convulsions, fever and limb dysfunction for more than one week.

**Exclusion criteria**

Exclusion criteria: 1) children with primary immune deficiency, 2) children with craniocerebral trauma or undergoing surgery, 3) children whose CSF are positive in cultivation only, but other manifestations such as clinical symptoms and laboratory tests did not meet the standard, or 4) children who had been treated outside the hospital.

**Research methods**

Venous blood and CSF were collected from children with severe purulent meningitis. Enzyme-linked immunosorbent assay (ELISA) was adopted to detect the levels of interleukin-17 (IL-17) and IL-6 in venous blood and the CSF protein (Invitrogen, Carlsbad, CA, USA). The follow-up was carried out after the treatment was completed, during which venous blood and CSF protein were collected again. ELISA was employed to detect the levels of IL-17 and IL-6 in venous blood and the CSF protein in CSF. The rate of TLR4 positive cells was measured via a flow cytometer, and the Glasgow comat score was applied for scoring so as to assess the prognosis.

**ELISA detection**

1) Sample addition: 100 μL standard references or serum to be tested was added to each well, and the reaction plate was placed at 37°C for 40 min for reaction after thorough mixing. 2) Plate washing: The reaction plate was fully washed with washing solution 4-6 times, and printed dry on the filter paper. 3) Each well was mixed with 50 μL distilled water and 50 μL first antibody working solution in the kit (except the blank group), and the reaction plate was placed at 37°C for 20 min of reaction after intensive mixing. 4) Plate washing: The reaction plate was fully washed with washing solution 4-6 times and printed dry on the filter paper. 5) Each well was mixed with 100 μL enzyme solution in the kit, and the reaction plate was placed at 37°C to react for 10 min. 6) Plate washing: The reaction plate was fully washed with washing solution 4-6 times and printed dry on the filter paper. 7) Each well was mixed with 100 μL substrate working solution in the kit and placed in a darkroom at 37°C for 15 min of reaction. 8) Each well was mixed with 100 μL stop buffer in the kit and mixed well. 9) A microplate reader was used to detect the optical density value at 450 nm.

**Detection of the rate of TLR4 positive cells via a flow cytometer**

1) The flow cytometer switch was turned on for preheating for 30 min. 2) A total of 200 μL venous blood was taken from each patient and divided into 2 tubes. 3) Then 100 μL venous blood and 20 μL TLR4 antibody were added to each centrifuge tube, followed by reaction for 10 min away from light after intensive mixing. One mL phosphate-buffered saline (PBS) was added for centrifugation at 1500 rpm for 5 min. Then the supernatant was discarded, followed by the addition of 0.5 mL PBS. 4) A flow cytometer was applied for detection.
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Table 1. Glasgow clinical outcome scoring criteria

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 points</td>
<td>Cured: Symptoms and signs disappeared, and the results of cerebrospinal fluid protein detection for two consecutive times are normal without complications and sequelae. Markedly improved: Symptoms and signs are markedly improved, and the results of cerebrospinal fluid protein detection are normal. Complications are improved with no sequelae.</td>
</tr>
<tr>
<td>4 points</td>
<td>Patients have central nervous system dysfunction and convulsions, and CSF protein and complications are not improved through detection.</td>
</tr>
<tr>
<td>3 points</td>
<td>Severe central nervous system dysfunction.</td>
</tr>
<tr>
<td>2 points</td>
<td>In a vegetative state.</td>
</tr>
<tr>
<td>1 point</td>
<td>Death.</td>
</tr>
</tbody>
</table>

Table 2. Comparisons of general data in children

<table>
<thead>
<tr>
<th>Group</th>
<th>Case (n)</th>
<th>Gender (n)</th>
<th>Age (years old)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Good prognosis group</td>
<td>38</td>
<td>Male (20)/female (18)</td>
<td>1.28±0.78</td>
</tr>
<tr>
<td>Poor prognosis group</td>
<td>22</td>
<td>Male (13)/female (9)</td>
<td>1.53±0.33</td>
</tr>
<tr>
<td>P value</td>
<td>0.6279</td>
<td>0.1589</td>
<td></td>
</tr>
</tbody>
</table>

Glasgow clinical outcome scoring

Details of Glasgow clinical outcome scores are shown in Table 1. Patients with Glasgow score of 1-4 points had poor prognosis and were included into the poor prognosis group, and those with Glasgow score of 5 points had good prognosis and were included into the good prognosis group.

Statistical methods

Statistical Product and Service Solutions (SPSS) 20.0 software was adopted for statistical analysis in this study. Data were expressed as mean ± standard deviation. Data in line with the normal distribution and the homogeneity of variance were detected by the t-test, those in line with normal distribution and heterogeneity of variance were examined via the corrected t-test, and those not in line with the normal distribution and the homogeneity of variance were tested using the non-parametric tests. The rank-sum test was conducted for ranked data, and the chi-square test was applied for enumeration data. Correlation analysis was carried out via Person’s correlation analysis. P<0.05 represented that the difference was statistically significant.

Results

No differences in age and gender between the good and poor prognosis groups

In the good prognosis group, there were 20 males and 18 females aged (1.28±0.78) years old. In the poor prognosis group, there were 13 males and 9 females aged (1.53±0.33) years old. There were no differences in age and gender between the two groups of patients before treatment, and these data were comparable (Table 2).

Levels of serum inflammatory cytokines, IL-17 and IL-6 in the good prognosis group were lower than that in the poor prognosis group

Before treatment, the levels of IL-17 and IL-6 in the good prognosis group were significantly lower than those in the poor prognosis group (P<0.05). After treatment, the levels of IL-17 and IL-6 in the good prognosis group were clearly lower than those in the poor prognosis group (P<0.05) (Table 3).

Level of CSF protein and the rate of TLR4 positive cells in the good prognosis group were lower than that in the poor prognosis group

Before treatment, the level of CSF protein and the rate of TLR4 positive cells in the good prognosis group were notably lower than those in the poor prognosis group (P<0.05). After treatment, the CSF protein level and the rate of TLR4 positive cells in the good prognosis group were markedly lower than those in the poor prognosis group (P<0.05) (Table 4).

The levels of IL-17, IL-6, CSF protein and TLR4 before and after treatment in the good prognosis group were changed significantly over those in the poor prognosis group

The change ranges of the levels of IL-17, IL-6, CSF protein and TLR4 before and after treatment in the good prognosis group were significantly greater than those in the poor prognosis group (P<0.05) (Table 5).
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IL-17, IL-6, CSF, TLR4 showed significant correlation with the prognosis of severe purulent meningitis

According to calculation, the correlation analysis of the difference value of changes in the survivors with pediatric purulent meningitis still have sequelae according to epidemiological statistics [3]. It has been indicated that the main sequelae of pediatric purulent meningitis include visual impairment, hearing impairment, changes in state of consciousness and mental

Table 3. Levels of IL-17 and IL-6 in serum before and after treatment (x±s, pg/mL)

<table>
<thead>
<tr>
<th>Group</th>
<th>Before treatment</th>
<th>After treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IL-17</td>
<td>IL-6</td>
</tr>
<tr>
<td>Good prognosis</td>
<td>378.21±12.33 *</td>
<td>265.84±15.67 *</td>
</tr>
<tr>
<td>Poor prognosis</td>
<td>463.58±17.86</td>
<td>421.36±14.33</td>
</tr>
<tr>
<td>P value</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Note: *P<0.05 vs. poor prognosis group.

Table 4. Levels of CSF protein and the rate of TRL4 positive cells before and after treatment (x±s)

<table>
<thead>
<tr>
<th>Group</th>
<th>Before treatment</th>
<th>After treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CSF protein (mg/L)</td>
<td>TLR4 positive cell rate (%)</td>
</tr>
<tr>
<td>Good prognosis</td>
<td>838.67±27.68 *</td>
<td>15.87±6.46 *</td>
</tr>
<tr>
<td>Poor prognosis</td>
<td>1162.87±35.23</td>
<td>28.69±9.21</td>
</tr>
<tr>
<td>P value</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Note: *P<0.05 vs. poor prognosis group.

Table 5. Difference values of changes in the levels of inflammatory cytokines, CSF protein and TLR4 (x±s)

<table>
<thead>
<tr>
<th>Group</th>
<th>IL-17 (pg/mL)</th>
<th>IL-6 (pg/mL)</th>
<th>CSF protein (mg/L)</th>
<th>TLR4 positive cell rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Good prognosis</td>
<td>288.54±6.65 *</td>
<td>250.17±9.38 *</td>
<td>315.51±15.44 *</td>
<td>4.51±2.22 *</td>
</tr>
<tr>
<td>Poor prognosis</td>
<td>152.31±14.31</td>
<td>183.78±16.21</td>
<td>207.03±19.23</td>
<td>3.21±1.12</td>
</tr>
<tr>
<td>P value</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.0132</td>
</tr>
</tbody>
</table>

Note: *P<0.05 vs. poor prognosis group.

Discussion

Although the survival rate of children with purulent meningitis has notably increased owing to the development of antibiotics, nearly 16.4% of...
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state [4]. Statistics have revealed that about 41% of children with purulent meningitis suffer from dysfunction of varying degrees, including learning disabilities and behavior disorders [5]. In particular, severe purulent meningitis in children generally presents with rapid onset, a poor prognosis, likeliness to suffer from sequelae and a higher degree of overall risk. Hence, the identification of the risk factors for severe purulent meningitis in children and associated prognosis is of relatively high clinical significance.

Inflammation is one of the crucial pathological reactions of purulent meningitis, during which inflammatory cytokines, IL-6 and IL-17, play key roles. IL-17, as one of the important pro-inflammatory cytokines, can induce the secretion of inflammatory mediators by T cells and macrophages. It can be directly involved in the inflammatory reaction of purulent meningitis at the same time, so as to mediate immune injury and exert dual effects of stimulating inflammation and mediating the immune injury [6-9]. In particular, a large amount of IL-17 is secreted by the stimulation of pathogenic bacteria and inflammatory mediators, and it participates in inflammation-related cascades and immune responses to protect against the injury from pathogenic bacteria [10]. In addition, there is a close correlation between IL-17 and other cytokines. According to previous studies, IL-6 and transforming growth factor beta (TGF-β) exert a synergistic effect on stimulating the differentiation of T helper 17 (Th-17) cells to secrete IL-17 that interacts with other cytokines to regulate immune responses. As a multi-functional inflammatory factor, IL-6 exerts a vital effect on immune regulation of the body, and it is found to be abnormally expressed in many diseases [11-13]. When nervous system infections occur in the body, the important regulatory factor of the IL-6 response can activate the immune response, mediate inflammation, secrete a mass of cytokines and protect against injury in the early stage, but persistent immune responses and inflammation will give rise to secondary injury

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Figure 2. Correlation between change of IL-6 and Glasgow score.

Figure 3. Correlation between change of CSF protein and Glasgow score.
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It was revealed from this study that IL-6 and IL-17 were closely correlated with the prognosis of severe purulent meningitis in children. Children with severe purulent meningitis whose IL-6 and IL-17 levels were relatively low were basically found with good prognosis, indicating that the levels of IL-6 and IL-17 are closely associated with severe purulent meningitis in children and can be used as one of the potential indicators for prognosis evaluation and treatment guidance.

Normally, CSF protein mainly manifests as albumin whose level is less than 1% of plasma protein. However, the CSF protein level in patients with purulent meningitis is significantly raised by inflammation-induced release of a large number of cytokines that affect the blood-brain barrier. The protein level of CSF is intimately correlated with the degree of inflammation and indirectly reflects the severity of inflammation. CSF protein is a significant indicator for the diagnosis of intracranial infection, and its relationship with the prognosis of severe purulent meningitis in children is not yet clear. The results of this study revealed that children with severe purulent meningitis whose CSF protein level was low showed good prognosis over children with severe purulent meningitis whose CSF protein level was high, indicating a promising indicator for prognosis.

TLR4, as a type of pattern recognition receptor in the cell surface, is regarded as one of the effective indicators for early diagnosis of bacterial infection. Studies have demonstrated that TLR4 mainly recognizes Gram-negative bacteria, activates the body's immune response to bacteria and regulates the body's inflammatory response. The overexpression of TLR4 can result in nerve injury or neuronal necrosis. Besides, purulent meningitis is closely related to cell signaling pathways mediated by TLR4, especially inflammatory signaling pathways, and TLR4 can intensify immune and inflammatory responses, but the overexpression of TLR4 aggravates nervous tissue injury [18-20].

Conclusion

Our study demonstrate that the reduction of IL-17, IL-6, CSF, TLR4 was associated with a good prognosis in children with severe purulent meningitis, which provides basis for the evaluation of the disease progression as well as further targeted treatment.

Disclosure of conflict of interest

None.

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