Effect of continuous blood purification on inflammatory state, immune response and erythrocyte glycometabolism in patients with multiple injury and sepsis

Zhao-Hui Gan

Intensive Care Unit, Third Affiliated Hospital of Guangzhou Medical University, Guangzhou City, Guangdong Province, 510150, China

ARTICLE INFO

Objective: To analysis the effect of continuous blood purification on inflammatory state, immune response and erythrocyte glycometabolism in patients with multiple injury and sepsis.

Methods: A total of 78 patients with multiple injury and sepsis were randomly divided into observation group (n=39) and control group (n=39), control group received routine therapy, observation group received continuous blood purification treatment, and then the differences in inflammatory state, immune response, erythrocyte glycometabolism and other indexes were compared between the two groups after treatment.

Results: Inflammatory factor hs-CRP, TNF-α, PCT, sTREM-1 and HBP content in serum of observation group after treatment were significantly lower than those of control group; Th1 cytokines IL-2 and IFN-γ content in serum were lower than those of control group while Th2 cytokines IL-4 and IL-10 content were higher than those of control group; PFK and EGSH content in erythrocyte solution were higher than those of control group while G-6PD, AR and ELPO content were lower than those of control group; fluorescent intensity of CD11a, CD54, CD106 and CD49d in peripheral blood were significantly lower than those of control group.

Conclusions: Continuous blood purification can significantly reduce the systemic inflammatory response in patients with multiple injury and sepsis, and promote the immune function and erythrocyte metabolism to return to normal.

1. Introduction

Sepsis is the common complication after the occurrence of multiple injury, and without active treatment, inflammatory cascade can occur in patients and lead to multiple organ dysfunction[1,2]. Accumulation of inflammation and oxidative stress products in the body is the key link leading to internal environment disturbance and sepsis development, so early removing the massively produced inflammatory mediators, toxic immune cells and oxidation products in patients by blood purification is a reliable way to reverse the disease and optimize the treatment outcome[3,4]. Continuous blood purification (CBP) is a more respected way to eliminate the toxins in blood at present, and it has been successfully applied in patients with organophosphorus poisoning, etc. In the study, CBP was used in the treatment of patients with multiple injury and sepsis in our hospital, and the effect of the method on patients’ inflammatory state, immune response and erythrocyte glycometabolism was mainly elaborated.

2. Materials and methods

2.1. General information

A total of 78 patients with multiple injury and sepsis treated in our hospital between September 2013 and September 2015 were included in the study and randomly divided into observation group
(n=39) and control group (n=39). Observation group included 21 male cases and 18 female cases that were 28-67 years old and (45.37±6.09) years old in average; control group included 20 male cases and 19 female cases that were 29-69 years old and (46.59±6.14) years old in average. The two groups of patients showed no statistically significant difference in the distribution of gender and age (P>0.05) and could be subsequently compared.

2.2. Treatment methods

Control group received life support treatment, including oxygen uptake, volume resuscitation and bleeding control, accepted ventilator support or renal replacement therapy if necessary, and meanwhile, took broad-spectrum antibiotics to prevent infection. Based on support therapy, observation group received continuous blood purification treatment, specifically as follows: indwelling needle was incubated through the right femoral vein, extracorporeal circulation was established, Diapact CRRT machine was used, HV-CVVH mode treatment was conducted, and the line was one-time. The displacement liquid compositions included Na⁺ 135 mmol/L, K⁺ 2 mmol/L, Cl⁻ 108 mmol/L, Ca²⁺ 1.88 mmol/L, Mg²⁺ 0.75 mmol/L, glucose 1.5 g/L and lactate 33.75 mmol/L. Osmotic pressure of displacement fluid was 290 mmol/L, it was input in pre-dilution mode, flow rate was 3 500 mL/h and the blood flow was 250 mL/min. The ultrafiltration volume was set according to patients’ all-day curative dose and physiological requirement. Initial dose of low molecular heparin sodium (for anticoagulation) was 2 500 U, additional dose was 500 U/h, and the line and filter were flushed with normal saline (200 mL) every 30 min.

2.3. Serum indexes

Two weeks after treatment, 3 mL of fasting peripheral venous blood was extracted from two groups of patients and cryopreserved in -70 °C refrigerator for test, and the specific detected indexes were as follows: (1) inflammatory factors: enzyme-linked immunosorbent assay was used to determine hypersensitive C-reactive protein (hs-CRP), tumor necrosis factor-α (TNF-α), procalcitonin (PCT), soluble triggering receptor expressed on myeloid cells-1 (sTREM-1) and heparin-binding protein (HBp); (2) Th1/Th2 cytokines: ELISA was used to determine the content of Th1/Th2 cytokines, including Th1 cytokines interleukin-2 (IL-2) and interferon-γ (IFN-γ) as well as Th2 cytokines interleukin-4 (IL-4) and interleukin-10 (IL-10).

2.4. Immune cells

Two weeks after treatment, 3 mL of fasting peripheral venous blood was extracted from two groups of patients, mononuclear cells were purified, then the cell density was adjusted to 1×10⁷/mL, fluorescent antibodies of CD11a, CD54, CD106 and CD49d were incubated for 20 min, and after PBS washing for three times, flow cytometer was used to determine the mean fluorescence intensity of peripheral blood lymphocyte CD11a, CD54, CD106 and CD49d.

2.5. Erythrocyte glycometabolism indexes

Two weeks after treatment, 3 mL of fasting peripheral venous blood was extracted from patients to collect erythrocyte solution, and then the phosphofructokinase (PFK), glucose-6-phosphate dehydrogenase (G-6PD), aldose reductase (AR), erythrocyte lipid peroxides (ELPO) and erythrocyte glutathione (EGSH) content were determined.

2.6. Statistical methods

Data in the study was input in software SPSS23.0, measurement data was analyzed by t test and P<0.05 indicated statistical significant differences.

3. Results

3.1. Inflammatory factor content

<table>
<thead>
<tr>
<th>Groups</th>
<th>Case No.</th>
<th>hs-CRP (mg/L)</th>
<th>TNF-α (pg/mL)</th>
<th>PCT (ng/mL)</th>
<th>sTREM-1 (pg/mL)</th>
<th>HBp (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observation</td>
<td>39</td>
<td>9.26±0.95</td>
<td>37.29±4.15</td>
<td>9.23±0.95</td>
<td>43.27±5.19</td>
<td>13.82±1.76</td>
</tr>
<tr>
<td>Control</td>
<td>39</td>
<td>28.45±3.12</td>
<td>72.35±8.39</td>
<td>27.15±3.09</td>
<td>92.64±10.12</td>
<td>20.76±2.49</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Groups</th>
<th>Case No.</th>
<th>IL-2</th>
<th>IFN-γ</th>
<th>IL-4</th>
<th>IL-10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observation</td>
<td>39</td>
<td>124.37±15.93</td>
<td>18.32±2.04</td>
<td>45.38±5.92</td>
<td>17.23±1.94</td>
</tr>
<tr>
<td>Control</td>
<td>39</td>
<td>159.15±16.34</td>
<td>31.47±3.98</td>
<td>31.67±4.09</td>
<td>11.52±1.76</td>
</tr>
<tr>
<td>t</td>
<td></td>
<td>8.495</td>
<td>6.125</td>
<td>7.283</td>
<td>6.372</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Table 1

Comparison of inflammatory factor content in serum between two groups after treatment.

Table 2

Comparison of Th1/Th2 cytokine content in serum between two groups after treatment (pg/mL).
Serum hs-CRP, TNF-α, PCT, sTREM-1 and HBP content of observation group were significantly lower than those of control group (P<0.05), shown in Table 1.

### 3.2. Th1/Th2 cytokine content

Th1 cytokines IL-2 and IFN-γ content in serum of observation group were significantly lower than those of control group while Th2 cytokines IL-4 and IL-10 content were significantly higher than those of control group (P<0.05), shown in Table 2.

### 3.3. Immune molecule expression

Fluorescence intensity of CD11a, CD54, CD106 and CD49d in peripheral blood mononuclear cells of observation group were significantly lower than those of control group (P<0.05), shown in Table 3.

#### Table 3

Comparison of fluorescence intensity of immune cells in peripheral blood between two groups after treatment.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Case No.</th>
<th>CD11a</th>
<th>CD54</th>
<th>CD106</th>
<th>CD49d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observation</td>
<td>39</td>
<td>15.48±1.93</td>
<td>22.37±2.93</td>
<td>30.12±3.42</td>
<td>19.37±2.12</td>
</tr>
<tr>
<td>Control</td>
<td>39</td>
<td>21.65±2.79</td>
<td>34.12±3.86</td>
<td>41.53±4.98</td>
<td>33.12±3.98</td>
</tr>
<tr>
<td>t</td>
<td></td>
<td>6.834</td>
<td>7.128</td>
<td>6.394</td>
<td>9.273</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

### 3.4. Erythrocyte glycometabolism indexes

PFK and EGSH content in erythrocyte solution of observation group were significantly higher than those of control group while G-6PD, AR and ELPO content were significantly lower than those of control group (P<0.05), shown in Table 4.

#### Table 4

Comparison of erythrocyte glycometabolism indexes between two groups after treatment.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Case No.</th>
<th>PFK (U/gHb)</th>
<th>G-6PD (U/gHb)</th>
<th>AR (U/gHb)</th>
<th>ELPO (μmol/gHb)</th>
<th>EGSH (μmol/gHb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observation</td>
<td>39</td>
<td>1.54±0.18</td>
<td>10.36±1.87</td>
<td>0.26±0.03</td>
<td>12.37±1.75</td>
<td>1352.84±150.67</td>
</tr>
<tr>
<td>Control</td>
<td>39</td>
<td>0.92±0.09</td>
<td>17.25±1.93</td>
<td>0.45±0.05</td>
<td>17.19±1.95</td>
<td>1175.93±120.64</td>
</tr>
<tr>
<td>t</td>
<td></td>
<td>5.384</td>
<td>7.823</td>
<td>5.475</td>
<td>6.934</td>
<td>12.345</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

### 4. Discussion

Most patients with multiple injuries are complicated with systemic inflammatory response syndrome, sepsis and so on, and severe cases may cause multiple organ dysfunction. Sepsis is a serious complication of patients with multiple injuries, and is one of the common leading causes of death in patients with multiple injuries. Many studies believe that massively accumulated inflammatory mediators, oxidizing media, acid-base disturbance and so on in patients with sepsis are all internal causes of sepsis development, so it is recommended that blood purification is introduced in treatment of such patients[5]. Via convection, dispersion, adsorption and other mechanisms of action, CBP exchanges and removes the plentiful toxic and side metabolites in the patients, thus reducing the negative effect on the body's normal functions[6]. The feasibility of CBP therapy in patients with multiple injury and sepsis has received the attention from many scholars, but the specific clinical curative effect of treatment is still not clear, and there is less clinical research in the field at present. In order to define the therapeutic effect of CBP for patients with multiple injury and sepsis, it was introduced in the treatment of patients in our hospital, and the effect of CBP therapy on inflammatory state, immune response and erythrocyte glycometabolism was mainly elaborated.

After the occurrence of sepsis in patients with multiple injuries, plentiful proinflammatory factors are released, the body’s anti-inflammatory ability is suppressed and the inflammatory cascade continues to progress. hs-CRP is the most sensitive inflammatory acute phase protein that can be massively produced in the early inflammatory response and further promote the production of TNF-α and other proinflammatory factors[7]. PCT is a sensitivity index to judge the degree of inflammatory response, and is less affected by other humoral factors. Soluble triggering receptor expressed on myeloid cells-1 (sTREM-1) is widely expressed on the surface of mature monocytes and macrophages, it is closely related to the triggering and amplification of inflammation, and high level of sTREM-1 has been found in plasma of patients with infectious diseases, which is considered as the reliable index to reflect the degree of infection in the body[8,9]. HBP is a part of human neutrophil defense system, massive secretion of neutrophils can further prompt the release of HBP, and HBP affects the vascular permeability to exert chemotaxis effect on mononuclear macrophages[10]. In the study, the serum levels of the above five typical inflammatory markers were detected, and it was found that serum hs-CRP, TNF-α, PCT, sTREM-1 and HBP content of observation group were lower, indicating that the continuous blood purification has actively filtered the inflammatory factors, and helps to reduce the systemic inflammatory state in patients.

The general immune disorder in patients with sepsis is one of the important causes of continuous sepsis progression. The balance of Th1/Th2 cytokines also greatly determines the patients’ immune status, IL-2 and IFN-γ belong to the Th1 cytokines, and the IL-4 and IL-10 belong to the Th2 cytokines. Under physiological state, the levels of the two are in dynamic equilibrium state, and when the infectious diseases occur, Th1 cytokines are activated and massively secrete IL-2 and IFN-γ, and they also inhibit the activity of Th2 cytokines, which causes that IL-4 and IL-10 content decrease, Th1/Th2 balance tends to develop in the direction of Th1, the
functional T lymphocyte activation is disabled and the body shows immunosuppression[11,12]. At the same time, there are also specific immune function-related cells in the circulating blood of the body, and they also play important roles in the changes of patients' immune status. CD11a is called lymphocyte function-associated antigen-1, CD54 can mediate leukocyte activation and endothelial cell damage, and high levels of CD54 can promote leukocyte adhesion process and aggravate inflammatory injury. CD106 and CD49d is a pair of receptor and ligand, and they play an important role in mediating lymphocyte adhesion[13,14]. In the study, the immune fluorescence intensity of the above cells of two groups were tested after treatment, and it was found that the fluorescence intensity of immune cells CD11a, CD54, CD106 and CD49d in circulating blood of observation group were weaker after treatment, indicating that the continuous blood purification can filter the excessive toxic immune cells and reduce the occurrence of hazardous immune behavior.

Erythrocytes are with the richest content in the blood system, they have the important functions such as carrying oxygen and scavenging oxygen free radicals, and both inflammation and oxidative stress damage may lead to erythrocyte function impairment, specifically characterized by the blocked erythrocyte glycometabolism process. Erythrocytes mainly regulate the body's oxidative stress through glucose metabolism pathway, and the PFK, G-6PD and AR are the key enzymes of three glucose metabolism pathways of erythrocytes. PFK is a rate-limiting enzyme of glycolysis process, and when the body suffers from infection or stress and other injuries, the glycolysis process is restrained, PFK activity decreases, the other two pathways are strengthened, and the G-6PD and AR activity increase[15]. ELPO and EGSH content can represent the oxidative stress state of erythrocytes, ELPO belongs to oxidation product, EGSH represents the antioxidant capacity of erythrocytes, and the ELPO and EGSH equilibrium can decide the erythrocyte metabolism state in patients to a great extent[16]. It was found in the study that the PFK and EGSH content in erythrocyte solution of observation group were higher while G-6PD, AR and ELPO content were lower in observation group than the control group. This demonstrated that the continuous blood purification can optimize the health state in patients to a great extent.

To sum up, it is concluded as follows: continuous blood purification can significantly reduce the systemic inflammatory response in patients with multiple injuries complicated with sepsis, and prompt the immune function and erythrocyte metabolism to return to normal, and it is worth popularization and application in clinical practice in the future.

References


