Value of PCT content for adjuvant diagnosis of neonatal septicemia and assessment of its severity

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ARTICLE INFO

Article history:
Received 12 Jun 2018
Received in revised form 19 Jun 2018
Accepted 28 Jun 2018
Available online 14 Jul 2018

Keywords:
Septicemia
Procalcitonin
Inflammatory response
Immune response

ABSTRACT

Objective: To evaluate the value of procalcitonin (PCT) content for adjuvant diagnosis of neonatal septicemia and assessment of its severity. Methods: A total of 100 newborns who were diagnosed with septicemia in the First People’s Hospital of Xianyang City between January 2017 and January 2018 were selected as the septicemia group of the research, and the newborns who were born in the First People’s Hospital of Xianyang City during the same period, with the general data matched with those of septicemia group and without neonatal diseases after born were selected as the control group. Serum was collected to determine the contents of PCT, inflammatory cytokines and immune cytokines, and peripheral blood was collected to determine the expression intensity of inflammatory signaling molecules and immune transcription factors. Results: Serum PCT, TNF-\(\alpha\), HMGB1, ICAM-1, IL-10 and IL-17 contents as well as peripheral blood TLR4, NF-\(\kappa\)B, MPO, ROR\(\gamma\)t and FOXP3 expression intensity of septicemia group were significantly higher than those of control group whereas peripheral blood HLA-DR expression intensity was lower than that of control group; peripheral blood TLR4, NF-\(\kappa\) B, MPO, ROR\(\gamma\)t and FOXP3 expression intensity as well as serum TNF-\(\alpha\), HMGB1, ICAM-1, IL-10 and IL-17 contents of septicemia group of newborns with high PCT were significantly higher than those of newborns with low PCT whereas peripheral blood HLA-DR expression intensity was lower than that of newborns with low PCT. Conclusion: The increase of PCT in serum of newborns with septicemia is related to the change of inflammatory response and immune response, and its content detection is valuable for assessing the severity of the disease.

1. Introduction

Septicemia is a common infectious disease of the newborn, which specifically refers to the systemic infection caused by bacteria or fungi that invade the blood circulation from infection site, multiply and produce toxins\(^1\). The excessive activation of inflammation and the disorder of immune responses are the important pathological features in the course of neonatal septicemia. The immune response disorder is not conducive to the removal of pathogens, and the continuously reproduced pathogens can cause inflammation cascade amplification and increase the occurrence risk of systemic inflammatory response syndrome and multiple organ dysfunction\(^2,3\). In clinical practice, early diagnosis of septicemia through the detection of serum indexes can improve the prognosis of the disease. Procalcitonin (PCT) is an infection index risen in recent years, which has strong sensitivity and specificity for the diagnosis of bacterial infection, and helps to early detect bacterial infection\(^4\). It has been reported that the detection of serum PCT can provide a basis for the diagnosis of neonatal septicemia\(^5\), but the value of this indicator for illness assessment is not yet clear. In the following studies, we specifically analyzed the value of PCT content for evaluating the inflammatory response and immune response in the course of neonatal septicemia.

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Fund Project: Shaanxi Provincial Achievements of Science and Technology No: 9612017Y0897.
2. Neonatal information and research methods

2.1 Neonatal inclusion and information

A total of 100 newborns who were diagnosed with septicemia in the First People’s Hospital of Xianyang City between January 2017 and January 2018 were chosen as the septicemia group of the study, and all newborns were diagnosed with septicemia by clinical symptoms, signs and blood culture bacteria positive. They included 52 males and 48 females, with 36-40 weeks of gestational age, 28 cases of which were premature infants and 72 cases were term infants. Moreover, 28 premature infants and 72 term infants who were born in the First People's Hospital of Xianyang City during the same period and without neonatal diseases after born were chosen as the control group. They included 54 males and 46 females, with 35-40 weeks of gestational age. There was no significant difference in general information between the two groups (P>0.05).

2.2 Laboratory detection methods

2.2.1 Serum index detection

About 2-3 mL of peripheral blood was collected and centrifuged to separate the upper serum, and the instructions of Elisa kit were followed to determine the contents of PCT, TNF-α, HMGB1, ICAM-1, IL-10 and IL-17.

2.2.2 Peripheral blood index detection

About 0.5-1.0 mL of peripheral blood was collected, centrifuged, joined by EDTA anticoagulant and then blended, 0.1 mL was taken and added in the reaction tube. The TLR4, NF-κB, MPO, HLA-DR, RORγt and FOXP3 fluorescent antibody were incubated respectively, and then their expression intensity was measured in flow cytometer.

2.3 Statistical analysis

Software SPSS 23.0 was adopted to input data, the differences in data between groups were analyzed by t test and P<0.05 showed that the difference was statistically significant.

3. Results

3.1 Comparison of serum PCT content

Serum PCT content of septicemia group was 16.52 ng/mL and serum PCT content of control group was (0.86±0.11) ng/mL. The t test analysis of the difference in serum PCT contents between the two groups of newborns showed that serum PCT content of septicemia group was significantly higher than that of control group, and the difference in serum PCT contents was statistically significant between the two groups of newborns (P<0.05).

3.2 Comparison of inflammatory mediators in peripheral blood and serum

Comparison of inflammatory mediators TLR4, NF-κB, MPO, TNF-α (ng/L), HMGB1 (ng/L) and ICAM-1 (μg/L) in peripheral blood and serum between the two groups of newborns as follows: peripheral blood TLR4, NF-κB and MPO expression intensity as well as serum TNF-α, HMGB1 and ICAM-1 contents of septicemia group were significantly higher than those of control group (P<0.05). Comparison of inflammatory mediators TLR4, NF-κB, MPO, TNF-α, HMGB1 and ICAM-1 in peripheral blood and serum between septicemia group of newborns with different PCT contents was as follows: peripheral blood TLR4, NF-κB and MPO expression intensity as well as serum TNF-α, HMGB1 and ICAM-1 contents of septicemia group of newborns with high PCT were significantly higher than those of newborns with low PCT (P<0.05).

Table 1.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>TLR4</th>
<th>NF-κB</th>
<th>MPO</th>
<th>TNF-α</th>
<th>HMGB1</th>
<th>ICAM-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Septicemia group</td>
<td>100</td>
<td>2.58±0.36</td>
<td>2.19±0.32</td>
<td>1.88±0.25</td>
<td>6.48±0.93</td>
<td>42.8±6.2</td>
<td>248.5±33.5</td>
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<tr>
<td>Control group</td>
<td>100</td>
<td>1.03±0.15</td>
<td>1.01±0.16</td>
<td>1.04±0.17</td>
<td>2.41±0.35</td>
<td>15.2±1.9</td>
<td>64.9±9.3</td>
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<tr>
<td>t</td>
<td>&lt;0.05</td>
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<td>P</td>
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</table>

Table 2.

<table>
<thead>
<tr>
<th>PCT content</th>
<th>n</th>
<th>TLR4</th>
<th>NF-κB</th>
<th>MPO</th>
<th>TNF-α</th>
<th>HMGB1</th>
<th>ICAM-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>High content</td>
<td>50</td>
<td>3.39±0.52</td>
<td>2.94±0.45</td>
<td>2.42±0.35</td>
<td>9.11±1.21</td>
<td>46.4±7.8</td>
<td>351.3±45.1</td>
</tr>
<tr>
<td>Low content</td>
<td>50</td>
<td>1.81±0.25</td>
<td>1.84±0.19</td>
<td>1.39±0.18</td>
<td>3.98±0.52</td>
<td>35.3±4.2</td>
<td>140.4±20.3</td>
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<tr>
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<td>P</td>
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</tbody>
</table>
Differences in immune indexes in peripheral blood and serum between septicemia group of newborns with different PCT contents.

Table 4.

Differences in immune indexes in peripheral blood and serum between the two groups of newborns.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>HLA-DR</th>
<th>ROR γ t</th>
<th>FOXP3</th>
<th>IL-10</th>
<th>IL-17</th>
</tr>
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<tbody>
<tr>
<td>Septicemia group</td>
<td>100</td>
<td>0.41±0.07</td>
<td>2.33±0.36</td>
<td>2.01±0.32</td>
<td>40.6±6.2</td>
<td>66.1±8.5</td>
</tr>
<tr>
<td>Control group</td>
<td>100</td>
<td>1.04±0.17</td>
<td>1.00±0.12</td>
<td>1.03±0.14</td>
<td>21.7±3.3</td>
<td>24.2±3.6</td>
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<tr>
<td>t</td>
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<td>&lt;0.05</td>
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<td>&lt;0.05</td>
</tr>
<tr>
<td>P</td>
<td></td>
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<td>&lt;0.05</td>
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<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

3.3 Comparison of immune indexes in peripheral blood and serum

Comparison of immune indexes HLA-DR, ROR γ t, FOXP3, IL-10 (ng/L) and IL-17 (ng/L) in peripheral blood and serum between the two groups of newborns was as follows: peripheral blood HLA-DR expression intensity of septicemia group was lower than that of control group whereas peripheral blood ROR γ t and FOXP3 expression intensity as well as serum IL-10 and IL-17 contents were significantly higher than those of control group (P<0.05). Comparison of immune indexes HLA-DR, ROR γ t, FOXP3, IL-10 and IL-17 in peripheral blood and serum between septicemia group of newborns with different PCT contents was as follows: peripheral blood HLA-DR expression intensity of septicemia group of newborns with high PCT was lower than that of newborns with low PCT whereas peripheral blood ROR γ t and FOXP3 expression intensity as well as serum IL-10 and IL-17 contents were significantly higher than those of newborns with low PCT newborns (P<0.05).

4. Discussion

Bacterial infection is a common neonatal infectious disease. As the immune system development of the newborns is not mature, and the ability to defense and remove pathogens is weak, they are relatively vulnerable to bacteria invasion and infection[6]. When the bacteria in the infection site enter into the blood circulation and are massively reproduced, the disease will progress into neonatal septicemia, and the bacterial metabolites within the blood circulation will further activate the systemic inflammatory response syndrome. Neonatal septicemia is difficult to treat and with high mortality, and early diagnosis and treatment are the key measures to improve prognosis[7,8]. The detection of serum infection indexes is a common auxiliary means for clinical diagnosis of septicemia, and PCT is a serum index with high sensitivity and specificity for the diagnosis of bacterial infection. PCT is the precursor of calcitonin, which is mainly synthesized and secreted by thyroid C cells in physiological conditions[9]; in the pathologic condition of bacterial infection, pro-inflammatory cytokines can act on the cells in multiple solid organs in the body to significantly increase the expression and secretion of PCT. It has been reported that PCT has high sensitivity and specificity for the diagnosis of neonatal septicemia[10,11]. In the above studies, analysis of the changes of serum PCT contents in neonates with septicemia showed that serum PCT content of septicemia group was significantly higher than that of control group.

This means that in the occurrence and development of neonatal septicemia, the synthesis and secretion of PCT increase significantly, and the detection of serum content can provide the basis for the diagnosis and evaluation of the disease.

The excessive activation of inflammatory response is an important pathological change after bacterial infection, and the mass release of a variety of pro-inflammatory factors during the action of bacterial infection can directly promote the synthesis and secretion of PCT. The activation of inflammatory response after bacterial infection depends on the combination of multiple pattern recognition receptors with bacteria and the transduction of corresponding signaling pathways. TLR4 is the most important pattern recognition receptor in the body. It identifies and binds to bacteria to transmit inflammation signals into the cells and make NF-κB dissociate with its inhibitory molecule IκB through MyD88-dependent or non-dependent pathway, and the free NF-κB is able to enter the nucleus and start the expression of inflammatory factors such as TNF-α, HMGB1 and ICAM-1[12,13]. TNF-α and HMGB1 are cytokines with pro-inflammatory activity, which can mediate the inflammatory response cascade activation[14,15]; ICAM-1 is a cytokine with intercellular adhesion activity, which can promote the adhesion and infiltration of inflammatory cells in the lesion[16,17]. At the same time, neutrophils are massively activated during the activation of inflammatory reaction, and the activated neutrophils highly express MPO and can increase the generation of reactive oxygen species through the activity of MPO, causing tissue damage by oxidative stress reaction[18]. Analysis of the changes in serum inflammatory mediators in neonates with septicemia showed that peripheral blood TLR4, NF-κB and MPO expression intensity as well as serum TNF-α, HMGB1 and ICAM-1 contents of septicemia group were significantly higher than those of control group. This means that the inflammatory response tends to be overactive in neonates with septicemia. Further analysis of the relationship between PCT content and inflammatory reaction activation in neonates with septicemia showed that peripheral blood TLR4, NF-κB and MPO expression intensity as well as serum TNF-α, HMGB1 and ICAM-1 contents of septicemia group of newborns with high PCT increased significantly. It means that the mass PCT secretion in newborns with septicemia is closely related to the excessive activation of inflammatory response, and the determination of PCT content can assess the abnormal activation of inflammatory response in the course of neonatal septicemia.

Immature immune system is the important reason for neonatal bacterial infection. HLA-DR is an important molecule involved in the immune response regulation, the molecule is also an important part of MHC-II compound, which can present the antigen to T cells, regulate the differentiation and maturation of regulatory T cells,
and then remove bacteria through the biological activities of T cell subsets; when HLA-DR expression reduces, the antigen presenting process is disturbed and it directly affects the differentiation of T cells, resulting in the disorder of immune response[19,20]. CD4+T cells are important immune cells involved in the removal of pathogens, and Th17 and Treg subsets play different roles. Th17 cells differentiate and mature under the action of ROR γt, and secrete IL-17 to exert pro-inflammatory effect, which is conducive to the activation of systemic inflammatory reaction after bacterial infection[21,22]; Treg cells differentiate and mature under the action of FOXP3, secrete IL-10 to exert immunosuppressive activity, and can restrain the differentiation of a variety of immune cells and the release of immune active molecules, which is bad for the body to remove the pathogen[23,24]. Analysis of the changes of above serum immune indexes in neonates with septicemia showed that peripheral blood HLA-DR expression intensity of septicemia group was lower than that of control group whereas peripheral blood ROR γt and FOXP3 expression intensity as well as serum IL-10 and IL-17 contents were significantly higher than those of control group. This indicates that the disorder of immune response is closely related to the occurrence of neonatal septicemia. Further analysis of the relationship between PCT content and immune response disorder in neonates with septicemia showed that peripheral blood HLA-DR expression intensity of septicemia group of newborns with high PCT significantly decreased whereas peripheral blood ROR γt and FOXP3 expression intensity as well as serum IL-10 and IL-17 contents significantly increased. It means that the mass PCT secretion in newborns with septicemia is closely related the immune response disorder, and the determination of PCT content can evaluate the degree of immune response disorder in the course of neonatal septicemia.

The above results show that the serum PCT content significantly increases in neonates with septicemia, and is closely related to the activation of inflammatory response and the disorder of immune response; therefore, the detection of PCT in serum can assess the severity of septicemia.

References