Bacteriological investigation and method of antigen connected lymphocytes (ACL) in defining etiological structure of sepsis in children in the early age

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Bacteriological investigation allowed identifying the etiological factor of sepsis in the blood of children at the early age only in 33.3% of cases; in other clinical material - in 48.3% of cases. Staphylococcus was the dominated microflora in the development of sepsis. As a leading etiological factor in other clinical material, Staphylococcus competed with gram-negative flora. Given limitations of bacteriological method in defining the etiologic factor in infant patients with sepsis, especially in its septicemic form, there is a need to find the most informative, less time-consuming diagnostic methods. We have used the indirect diagnostic method by considering the presence in the blood of antigen-connected lymphocytes (ACL) which react with the bacteria antigens - causative agents of the disease. The ACL method showed that gram-negative bacteria perform as a dominated microflora.

Key words: Hemoculture, antigen-connected lymphocytes, bacteriological investigation, sepsis.

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Introduction

Determination of the etiological factor in sepsis takes a significant place in the determination of the etiotropic therapy. Bacteriological investigation of the blood and other clinical material has the high importance in the diagnosis of sepsis and treatment of purulent-septic and purulent-inflammatory diseases.

Methods of the classic microbiology, oriented for the identification of a clear culture of microorganisms, have significant shortcomings: they are more difficult to perform, have long-term duration in the receiving results (7-10 days), and they are not always acceptable. Besides, preliminary therapy with use of antibiotics could sharply decrease the amount of isolated microorganisms in the blood culture and, at the same time, it could decrease the probability of its identification in the hemoculture.

Taking into account it, for the determination of etiological factor in development of sepsis in children at the early age we have also used the method of immunoscopy. This approach was implemented through identifying in the patient’s blood antigen-connected lymphocytes (ACL) which can react with the causative agents of the disease. The study considers the effectiveness of immunoscopic method in determining causative agent through ACL-method results with the results of bacteriological method of investigation samples of the clinical material in children at the early age who have sepsis.

Materials and methods

91 children in the early age were examined using bacteriological method and ACL-method. Given the most common point of view of the paramount significance of bacteriemia in the development of sepsis, the blood sterility test was made in all hospitalized patients in the first day. Microorganisms in blood were indicated in 33.3% of observed cases. Thus, microorganisms were not determined in more than 65% of patients, though the clinical and laboratory signs testified about the generalized character of the inflammation, about the sepsis. The low disclosure level of the causative agent from the
blood of the investigated patients was seemingly explained by the fact that the most children had been hospitalized in the clinic from the other hospitals. In previous hospitals they unsuccessfully received a few courses of therapy with use of antibiotics during the long-term period.

Particular difficulty in the identifying the etiology of the sepsis created the group of patients who had not a clear purulent focuses of the infection; absence of a growing hemoculture in such patients made also the difficulties to prescribe the rational therapy by use of the antibiotics.

Results and discussion

As is known, intensive reproduction and accumulation of microorganisms happens in intestines, nasopharynx, in the focuses of purulent infection and in the wound surfaces. Considering this, we performed bacteriological investigation of the specimens from fauces, intestines contents and urine. In the group of patients with septicemic type of sepsis, when it was not the clear purulent focus, the positive result of the bacteriological investigation was determined only in 44 (48.3%) children. This result was received from the smears of the pharynx, examinations of urine and stool.

The Table 1 shows that microorganisms most often were identified from intestine - 60.7%, in 26.5% of cases they were found in the urine, in 39.8% of patients they were established through examination of smears of the pharynx.

Within the whole revealed by the examination results, share of staphylococcus made 45.4% of all cases, presence of proteus was determined in 34.1% of cases, presence of blue pus and colon bacillus was noted in 2.2% and 6.8% of cases respectively, and Candida fungus was determined in 13.6%. In 3.3% of patients a microbe association was identified.

Thus, applying the bacteriological investigation allowed us to identify the etiological factor from the blood in children at the early age in 33.3% of cases; while this method in analysis the other clinical material provided finding microorganisms in 48.3% of cases. According to the data from hemoculture, the staphylococcus appeared as the dominated microflora in the development of the sepsis. Analysis of the culture of microorganisms from the other clinical material has found that staphylococcus was competing with gram-negative flora to be a dominated etiological factor. In this case, staphylococcus was identified in 43.1% of all cases and gram-negative flora was found in 45.4% of cases. Limitations of bacteriological method in detecting the etiological factor in children at the early age, especially in septicemic form of the disease, makes necessary to find out more informative, less difficult to use methods of identification the causative agent of the disease.

We have used method of indirect indication of the infection by the presence in the blood of the patients’ antigen-connected lymphocytes (ACL) which react with the antigens of the bacterial causative agents of the disease. According to the literature, sepsis data in children at the early age “problem microbes” are the representatives of the conditionally-pathogenic flora: staphylococcus, Klebsiella, proteus, blue pus bacillus, streptococcus and Candida fungus. In connection with that for the identification of ACL an antigens to the mentioned microbes were used as diagnosticums. The presence of ACL to the causative agents of conditionally-pathogenic flora in healthy children delivered evidence that this flora was not foreign for the organism.

ACL reaction was evaluated as positive when the amount of ACL in the blood was 3% and more. This level was considered as pathologically significant for the patients; it pointed on the evidence of the dominated role of “bacteria-leader” in the septic process.

ACL-method was implemented in examination of 91 sick children with septicemic type of sepsis. Such group was distinguished as a most difficult in the diagnosis of the etiological factor of the disease. The results received by ACL-method were compared with the data of the bacteriological investigation. ACL-method to the bacterial agents provided data on
registration frequency of the causative agents. In 136 positive results the presence of
staphylococcus was noted in 29% of cases (42 patients), Klebsiella was registered in 23%
of cases (33 patients), presence of colon bacillus - in 6.2% (9 patients), presence of
Candida fungus - in 27.2% (37 patients), presence of proteus - in 11.7% (16 cases),
presence of blue pus bacillus - in 3.4% (5 cases), and the presence of streptococcus was
determined in 2% (3 cases). Microbe association was determined in 54% of patients. From
all investigated patients positive result due to ACL was established in 84 patients, which
made up 92.3% of patients.

It is seen in the Table 1 that registration of the causative agents by use ACL-method
significantly increases determination frequency of microbes comparing to use of
bacteriological method. In 78.4% of patients, microbes registered by bacteriological
method coincided with the results of causative agents by use of ACL-method.

Generalization of the registration frequency has shown that during the bacteriological
investigation the sowing percentage of gram-positive and gram-negative flora was almost
the same, while during the analyses of the results after using ACL-method showed that
the dominated microflora was gram-negative bacteria. Yeast fungus were registered most
often.

### Table 1. The Results of Determination of Causative Agents of Sepsis by Use of ACL-Method and Bacteriological Method

<table>
<thead>
<tr>
<th>Causative agents</th>
<th>The amount of cases determined by use of bacteriology from hemoculture</th>
<th>Determination percentage of the bacteria from hemoculture</th>
<th>The amount of cases determined by use of ACL</th>
<th>Determination percentage of ACL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus</td>
<td>20</td>
<td>21.9</td>
<td>42</td>
<td>29</td>
</tr>
<tr>
<td>Klebsiella</td>
<td>–</td>
<td>0</td>
<td>33</td>
<td>23</td>
</tr>
<tr>
<td>Colon bacillus</td>
<td>3</td>
<td>3.2</td>
<td>9</td>
<td>6.2</td>
</tr>
<tr>
<td>Blue pus bacillus</td>
<td>1</td>
<td>1.09</td>
<td>5</td>
<td>3.4</td>
</tr>
<tr>
<td>Proteus</td>
<td>15</td>
<td>16.4</td>
<td>16</td>
<td>11</td>
</tr>
<tr>
<td>Candida fungus</td>
<td>6</td>
<td>6.6</td>
<td>37</td>
<td>25.5</td>
</tr>
<tr>
<td>Streptococcus</td>
<td>–</td>
<td>0</td>
<td>3</td>
<td>2.0</td>
</tr>
<tr>
<td>Total</td>
<td>45</td>
<td>49.2</td>
<td>145</td>
<td>99.9</td>
</tr>
<tr>
<td>Gram-positive flora</td>
<td>20</td>
<td>21.9</td>
<td>45</td>
<td>31</td>
</tr>
<tr>
<td>Gram-negative flora</td>
<td>19</td>
<td>20.7</td>
<td>54</td>
<td>46.3</td>
</tr>
</tbody>
</table>

### Conclusion

Thus, the ACL-method used for the etiological diagnostic of the sepsis has a big
informative importance then bacteriological method. Also, it has a good specificity: ACL-
results in the blood allow considering about significance of the antigen loading in the
organisms of children having sepsis. Accelerated implementation process is another
feature and advantage of this method. It is believed that use of the ACL-method in
diagnostic of the sepsis in children at the early age can help us to prescribe purposeful
therapy.

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