DOI 10.26724/2079-8334-2024-2-88-82-85
UDC 616.94-053.31-074

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ANTIMICROBIAL AND IMMUNE FACTORS IN THE DIAGNOSIS AND PROGNOSIS OF NEONATAL SEPSIS

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The purpose of the study was to determine the clinical significance of antimicrobial peptides, pro- and anti-inflammatory cytokines in sepsis in newborns. In the course of this study, the role of endotoxin and cytokines in early diagnosis of sepsis was also studied. The determination was carried out using the standard method of solid-phase ("sandwich" version) enzyme-linked immunosorbent assay on an automatic analyzer "Elaysis Uno" (Germany). The study included 72 newborns diagnosed with sepsis, early neonatal sepsis (n=15), late neonatal sepsis (n=50) and control group (n=7). The changes of the levels of endotoxin, pro-inflammatory and anti-inflammatory cytokines in the blood serum depends on the dynamics of septic process were revealed Thus, the determination of endotoxin and cytokines makes it possible to identify the development of sepsis and predict its severity and course in premature infants of various gestational ages.

Key words: sepsis, preterm and term infants, endotoxin, cytokines.

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АНТИМІКРОБНІ ТА ІМУННІ ФАКТОРИ У ДІАГНОСТИЦІ І ПРОГНОЗУВАННІ НЕОНАТАЛЬНОГО СЕПСИСУ

Мета дослідження полягала у визначенні клінічного значення антимікробних пептидів, про- та протизапальних цитокінів при сепсисі у новонароджених дітей. У ході цього дослідження було вивчено роль ендотоксину та цитокінів у ранній діагностиці сепсису. Визначення показників було проведено стандартним методом твердофазового («сендвіч» варіант) імуноферментного аналізу на автоматичному аналізаторі «Elaysis Uno» виробництва Німеччини. У дослідження було включено 72 новонароджених, з них 65 новонароджених з діагнозом сепсис: ранній неонатальний сепсис (n=15), пізній неонатальний сепсис (n=50), та контрольна група, здорові (n=7). Виявлено залежність між показниками антимікробних пептидів, про- та протизапальних цитокінів у сироватці крові та динамікою септичного процесу. Таким чином, визначення ендотоксину та цитокінів дозволяє простежити розвиток сепсису, прогнозувати його тяжкість та перебіг у недоношених дітей різного гестаційного віку.

Ключові слова: сепсис, недоношені та доношені діти, ендотоксин, цитокіни.

Sepsis is a pathological process based on the body's reaction in the form of generalized (systemic) inflammation to an infection of various nature (bacterial, viral, fungal), leading to acute organ dysfunction. Neonatal sepsis remains one of the leading causes of morbidity and mortality among full-term and preterm infants [11].

Generalized infectious disease with acyclic course caused by conditionally pathogenic bacterial microflora, which is based on dysfunction of the body's immune system with the development of a focus of purulent inflammation or bacteremia, systemic inflammatory reaction and multiple organ failure in children of the first month of life [4, 7].

Endotoxins are bacterial toxic substances that are structural components of certain bacteria and are released only during lysis (disintegration) of the bacterial cell [10].

Endotoxin circulates in the bloodstream and promotes the activation of monocytes and macrophages. As a result, mediators, including cytokines, are released and favorable conditions are created for systemic inflammation caused by infection. Endotoxin is a trigger for the release of cytokines and mediators. The presence of endotoxins in the blood is called endotoxemia. If the immune response is strong, endotoxemia can lead to septic shock. Septic shock is the most severe variant of the course of sepsis, characterized by severe circulatory, cellular, and metabolic disorders that cause an increased risk of death. It is believed that targeting endotoxin and its rapid elimination from the body are the most important tasks in the treatment of sepsis. The outcome of the reaction of lipopolysaccharide-binding protein (LBP) with the cells of the macroorganism depends on its concentration. Moderate activation of cells and systems at low doses of endotoxin with an increase in dose turns into hyperactivation, which is accompanied by increased production of inflammatory cytokines, increased activation of the complement system and blood clotting factors, which can result in the development of such formidable complications as disseminated intravascular coagulation (DIC), endotoxin shock and acute multiple organ failure. With excessive intake of endotoxin into the systemic circulation in conditions of relative insufficiency of lipopolysaccharides-binding (LPS-binding) factors, as well as with insufficiency of LPS-secreting systems (primarily kidneys), endotoxin can exhibit its numerous pathogenic properties [4, 5].

In recent years, the simultaneous measurement of interleukin-6 (IL-6) and lipopolysaccharide-binding protein has attracted the attention of many researchers. The interest is due to the fact that the active synthesis of IL-6 begins immediately after exposure to the cells of the immune system of bacteria, viruses, mitogens, and various mediators. The rapid and pronounced reaction to this diverse group of endogenous and exogenous substances indicates that this cytokine belongs to the category of early mediators. The half-life of IL-6 is 45 minutes, therefore, by measuring its serum content in dynamics, it is possible to control the development of an acute inflammatory response to surgical aggression, injury or infection. Depending on whether this reaction develops quickly or slowly, it is possible to predict the degree of risk of septic complication and its prognosis, including the likelihood of septic shock. It has been shown that an increase in the concentration of IL-6 with an increase in body temperature can help predict the infectious nature of the complication before the results of a microbiological examination of the culture are obtained and warn the doctor that the patient has a high risk of developing sepsis before other markers and symptoms manifest themselves. LBP is a well-studied compound that is one of the mediators of the immune system [1, 2]. LBP synthesis is induced within a few hours after contact with gram-positive and gram-negative bacteria, as well as during infection with fungi. An increase in the concentration of LBP above the normal level can warn the clinician about the onset of acute local inflammation of an infectious nature, systemic bacterial or fungal infection or septicemia. Moreover, it was shown that the highest concentrations of LBP were detected in severe sepsis and septic shock, the development of which was facilitated by the suppression of monocyte activity in response to LPS. Monitoring of LBP can also help in determining the etiology of the inflammatory process, since its concentration differs in bacterial or fungal infections and in inflammatory reactions caused by other pathogens or pathological processes, for example, viruses, parasites or those developed as a result of trauma and pancreatitis [10]. The main pathogenic factor of gram-negative bacteria is LPS, gram-positive bacteria contain a number of immunogenic components of the cell wall, which belong to exotoxins.

Thus, the body's response to gram-negative flora is overly pronounced, which leads to an imbalance of pro- and anti-inflammatory reactions and more often causes septic shock and multiple organ failure, DIC syndrome.

The main cause of death in gram-negative sepsis is endotoxin shock. Endotoxins have a global effect on the body, both at the humoral and cellular levels. LPS activates the complement cascade and modulates various pathways of the coagulation system, causing damage to the vascular endothelium and disseminated intravascular coagulation syndrome. At the same time, LPS stimulate myeloid cells to synthesize and secrete biologically active molecules, under the action of which lymphocytes are activated, mast cells and basophils produce chemotaxis factors, platelets secrete growth and coagulation factors, macrophages, monocytes. Free cytokines activate cells of various tissues and organs, leading to necrosis, and induce apoptosis [6, 8].

Of these, IL-6 and interleukin-8 (IL-8) are considered more informative. The amount of IL 6 is higher in early sepsis. IL-8 is an anti-inflammatory cytokine that accelerates neutrophil activation and chemotaxis, is not only a marker of sepsis, but also an indicator of the severity of infection.

The purpose of the study was to evaluate the changes in endotoxin and cytokines in newborns with sepsis.

Materials and methods. The research was conducted in the scientific research laboratory of the Scientific Research Institute of Pediatrics named after K. Farajeva in Baku. The work was performed in the departments of neonatal pathology, anesthesiology and intensive care units. The concentration of endotoxin and cytokines were determined by the method of enzyme immunoassay (ELISA) according to the “sandwich” principle – on an enzyme immunoassay analyzer “ElisysUno” manufactured in Germany. The blood of 72 newborns was examined, who were divided into 3 groups:

Group I – early neonatal sepsis (n=15),

Group II – late neonatal sepsis (n=50).

The control group consisted of 7 healthy newborn children from maternity hospital № 7 in Baku.

Statistical data processing was conducted by using descriptive statistical methods (mean, standard deviation, frequency, percentage). For evaluating the numeral data and comparison of results between groups the Wilcoxon (Mann-Whitney) U-test was performed.

Results of the study and their discussion. Newborns who have had sepsis have a high incidence of diseases. So, in group I, enterocolitis was in 23.5 %, ventriculitis – in 23.5 %, osteomyelitis – in 5.9 %, pneumonia – in 64.7 %, DIC syndrome – in 17.6 %. In group II, ventriculitis was – in 21.2 %, osteomyelitis – in 12.1 %, pneumonia – in 60.7 %, DIC syndrome – in 6.1 %. Mortality rate in group I was 52.9 %, in group II was 54.8 %. According to the presence of diseases of the organ systems, children of the main I

and II comparison groups do not significantly differ from each other, but the number of diseases, on average, in group I and II is 2 times higher than in the control group. Among infected newborns with a gestation period of 38–41 weeks the average age was 37.8±0.2 weeks, with a gestation period of 27–37 was 32.8±0.5 weeks. Among term newborn the average weigh was 3181±107.9 g, among preterm was 1983.6±91.5 g (Table 1).

Table 1

Indices of endotoxin, IL-6, IL-8in newborns with sepsis

Parameters	Control group (n=7)	Early neonatal sepsis (n=15)		Late neonatal sepsis (n=50)		P	P1
		Before treatment	After treatment	Before treatment	After treatment		
Endotoxin, EU/ml	0.337±0.019 (0.25–0.39)	1.487±0.034 (1.28–1.73)	0.987±0.110 (0.46–1.61)	1.526±0.015 (1.27–1.69)	1.318±0.037 (0.45–1.61)	<0.000	<0.000
IL-6 (pq/ml)	3.1±0.3 (2.1–4.5)	44.5±6.5 (11.6–76.9)	24.2±2.49 (10.2–35.2)	50.8±2.7 (16.8–74.3)	27.03±1.09 (13.4–39.1)	<0.000	<0.000
IL-8 (pq/ml)	12.27±0.45 (10.8–14.5)	306.6±14.1 (173.2–378.7)	193.3±10.7 (119.4–233.9)	307.22±8.17 (134-396.1)	188.5±9.4 (53.8–378.3)	<0.000	<0.000

Note: statistical significance of differences: p – with the indices of the control group; p1 – between the indices of the main groups.

Endotoxin can cause or accelerate immune inflammation through multiple mechanisms that are quite informative markers of the severity of the inflammatory process.

The amount of endotoxin in group I, before treatment was 4.4 times more than in control group, but after treatment decreased and was 2.9 times more than in healthy newborn.

In group II the situation was similar: before treatment the level of endotoxin was 4.45 times than control, after treatment decreased but did not become at the same level than in healthy newborn.

Bacterial endotoxin is a permanent structural component of the outer cell wall of gram-negative bacteria, it is released when they are destroyed.

To assess the activity of the inflammatory process in premature and full-term infants diagnosed with sepsis, we also studied the content of IL-6 and IL-8.

The amount of IL-6 was 16.3 times more compared the control before treatment and decreased after treatment becoming 7.8 times more than healthy newborns. In the group II the same indicator was significantly higher compared healthy newborns and in the dynamics of septic process decreased to 8.8 times more than control.

The level of IL-8 in group of early sepsis at the first measurement was 24.9 times higher than in control, bur after treatment became 15.7 times more than healthy newborn by decreasing in several times. In newborn with late onset sepsis the amount of IL-8 in serum was 25 higher compared control group and in dynamics of septic process became 15.3 times more than healthy children.

Thus, changes in the indicators of the immune status and endotoxin content in full-term and premature septic children allow them to be used as additional criteria for the characterization of the inflammatory process in sepsis, prognosis of outcomes and assessment of the degree of immunodeficiency. When assessing the prognostic and diagnostic significance of various cytokines and mediators of the immune response, it should be taken into account that the development of sepsis and systemic inflammatory response syndrome (SIRS) is associated with the activation of the production of both pro-inflammatory and anti-inflammatory mediators, which are mainly synthesized in tissues. When assessing the significance of prognostic immunological markers, it should be taken into account that the immune status of patients who fully meet the criteria for sepsis is influenced by both the nature of the pathological process itself and many key parameters characteristic of a severe infection. IL-6 and IL-8 are frequently used in some countries and are considered sensitive “biomarkers of early anxiety” [1]. The advantage of using interleukins is an early peak in the manifestation of the infectious process, which can be convenient for early diagnosis. Endotoxin, IL-6, IL-8 can help in assessing the immune reactivity of patients, since they signal danger earlier than other clinical symptoms of the development of an infectious complication become apparent.

Eichberger J, et al reported that analyzing the combination of IL-6 and C-reactive protein, they revealed the cut-off values ranged from 36 to 100 pg/ml and from 10 to 60 mg/l, respectively, with a sensitivity of 75–100 %, and specificity varies among 37–74 %. The level of IL-6 in umbilical cord blood has been proposed to be used as an early diagnostic marker of sepsis in premature infants [6]. Our results supported the independently high reaction of IL-6 on dynamics of septic process and treatment, that confirms and emphasizes this concept of importance in the diagnosis of early sepsis.

It should be noted that compared to traditional antibiotics, the action of most AMPs is extremely rapid, and the bactericidal effect is observed at concentrations very close to the minimum inhibitory concentrations. In addition, some AMPs have an unusually broad spectrum of action and are capable of destroying multidrug-resistant pathogens [3].

However, some authors note that, despite the is of great importance of high concentrations of IL-6 and IL-8 in the diagnosis of neonatal sepsis, combined tests including early (IL-6, IL-8 or procalcitonin) and late (C-reactive protein) markers are still relevant [1, 4, 7].

Li Q., et al. showed that IL-6 levels increased significantly with increasing severity of sepsis ($p<0.001$). IL-6 levels were associated with recovery from sepsis and a negative correlation was found. According to the authors' results, the lower level of IL-6, was the sign of faster recovery of patients after treatment [9]. These data coincide with the results we obtained: the level of the studied indicator IL-6 in the blood over time was characterized by certain changes, which was manifested by a decrease in the level of IL-6 along with a decrease in inflammation during treatment.

Berka et al. [2] studied the dynamics of IL-6 levels within 24 hours after birth in premature newborns with the aim of using it as a predictor of early sepsis. The authors noted that measurement of IL-6 levels can be used to rule out early sepsis within the first 24 hours after birth. The potential use of IL-6 as a marker in the diagnosis of early neonatal sepsis in term and preterm infants was investigated. The range of sensitivity and specificity of IL-6 in the samples was 42.1–100 % and 43–100 %, respectively, diagnostic accuracy was higher in premature infants. In conclusion, it was emphasized that the earlier blood samples are collected when sepsis is suspected, the higher the sensitivity.

Conclusions

1. The level of endotoxin in term newborns with sepsis, before treatment was 4.4 times more than in control group, but after treatment decreased and was 2.9 times more than in healthy newborn. In preterm newborns before treatment the level of endotoxin was 4.45 times more than control, after treatment decreased but did not become at the same level than in healthy newborn.

2. IL-6 serum level was 16.3 times more at the beginning of sepsis compared the control and decreased after treatment becoming 7.8 times more than healthy newborns. In the group II the same indicator was significantly higher compared healthy newborns and in the dynamics of septic process decreased to 8.8 times more than control.

3. In newborn with early sepsis the level of IL-8 at the first measurement was 24.9 times higher than in control and 25 higher in group with late sepsis compared control group, but after treatment became 15.7 times and 15.3 times more than healthy newborn, respectively by decreasing in several times.

References

1. Ahmed AM, Mohammed AT, Bastawy S, Attalla HA, Yousef AA, Abdelrazek MS, et al. Serum Biomarkers for the Early Detection of the Early-Onset Neonatal Sepsis: A Single-Center Prospective Study. Adv. Neonatal Care. 2019;19: 26–32. doi: 10.1097/ANC.0000000000000631.

2. Berka I, Korček P, Straňák Z. Serial Measurement of Interleukin-6 Enhances Chance to Exclude Early-Onset Sepsis in Very Preterm Infants. Clin Pediatr (Phila) 2023; 62(4), 288–94. doi: 10.1177/00099228221124672.

3. Bhat BV. Fine-Tuning the Duration of Antibiotic Therapy for Neonatal Sepsis. Indian J. Pediatr. 2022; 89:323–324. doi: 10.1007/s12098-021-04063-2.

4. Birju ASh, Padbury JF. Neonatal sepsis, Virulence. 2014; 5(1): 170-178, DOI: 10.4161/viru.26906

5. Brady MT, Polin RA. Prevention and management of infants with suspected or proven neonatal sepsis. Pediatrics 2013; 132:166–8. doi: 10.1542/peds.2013-1310

6. Eichberger J, Resch B. Reliability of Interleukin-6 Alone and in Combination for Diagnosis of Early Onset Neonatal Sepsis: Systematic Review. Front Pediatr. 2022; 10, 840778. doi: 10.3389/fped.2022.840778.

7. Eichberger J, Resch E, Resch B. Diagnosis of Neonatal Sepsis: The Role of Inflammatory Markers. Front Pediatr. 2022; 10, 840288. doi: 10.3389/fped.2022.840288.

8. Escobar GJ, Puopolo KM, Wi S, Turk BJ, Kuzniewicz MW, Walsh EM, et al. Stratification of risk of early-onset sepsis in newborns ≥ 34 weeks' gestation. Pediatrics. 2014 Jan;133(1):30–6. doi: 10.1542/peds.2013-1689

9. Li Q, Yan W, Liu S, Li H. Study on the correlation and clinical significance of T-lymphocyte Subsets, IL-6 and PCT in the severity of patients with sepsis. Pak J Med Sci 2023; 39(1), 227–31. doi: 10.12669/pjms.39.1.5711

10. Sato M, Matsuyama R, Kadokura T, Mori R, Kumamoto T, Nojiri K. et al. Severity and prognostic assessment of the endotoxin activity assay in biliary tract infection. J. Hepatobiliary Pancreat. Sci. 2014; 21 (2): 120–127. doi: 10.1002/jhbp.10.

11. Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, et al. The third international consensus definitions for sepsis and septic shock (Sepsis-3). JAMA. 2016; 315: 801–10. doi: 10.1001/jama.2016.0287

Стаття надійшла 28.03.2023 р.