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ABSTRACT
of the dissertation for the degree of Doctor of Philosophy

BİOCHEMİCAL FEATURES OF NEONATAL SEPSİS, MODERN CLİNİCAL, LABORATORY AND PROGNOSTİC CRİTERİA

Speciality**:** **Biochemistry**

Field of science: **Biology**

Applicant: **Nazila Talat Karimova**

Baku – 2025

The research was conducted at the Scientific Research Institute of Pediatrics named K.Y. Farajova and within the 7th Maternity Hospital in Baku.

Scientific supervisor: Doctor of biological sciences, professor **Arif Mustafa Afandiyev**

Official opponents: Corresponding Member of the ANAS, Doctor of Biological Sciences, professor **Ilham Ayyub Shahmuradov**

Doctor of biological sciences, professor **Shafiga Anvarovna Topchiyeva**

PhD in Biological Sciences **Tarana Farahim Movsumova**

Dissertation Council FD 1.08 of Supreme Attestation Commission under the President of the Republic of Azerbaijan operating at the Institute of Physiology named after Academician Abdulla Garayev of the Ministry of Science and Education of Azerbaijan Republic.

Chairman of the Dissertation council: Doctor of Biological Sciences, Professor **Ulduz Fayizi Hashimova**

Scientific secretary of the Dissertation council: Doctor of Philosophy in Biology, Associate Professor **Yegana Ogtay Bayramova**

Chairman of the scientific seminar: Doctor of Biological Sciences, Professor **Adalat Nurulla Farajov**

GENERAL DESCRIPTION OF WORK

Actuality of the theme and the degree of research. Neonatal sepsis (NS) is a common purulent-septic polyethological inflammatory process, characterized by the presence of a primary focus and an infection circulating in the blood. initial focus and circulating infection in the blood. During NS, which manifests as infectious toxicosis, neonatal mortality can reach 30-40%[1].

Among the etiological factors of NS, the main place is occupied by gram-positive microorganisms (staphylococci, hemolytic streptococcus of group A), gram-negative flora (Escherichia coli, Pseudomonas aeruginosa, Klebsiella) and mixed flora (associations of staphylococcus, Candida in the gastrointestinal tract, urinary tract, skin and mucous membranes) [2] ,[3]. The risk of sepsis is significantly higher in at-risk neonates, including those with preterm birth, intrauterine hypoxia, intracranial birth trauma, surgical interventions, and other conditions. Risk factors on the part of the mother's organism (chorioamnionitis, vaginosis, urinary tract infections, multiple abortions, etc.) greatly increase the likelihood of infection in the newborn. In case of NS, as a result of an inadequate systemic inflammatory response in a child to common pathogenic, hospital-acquired, bacterial and viral infections, there is a risk of impaired hemostasis, microcirculation disorders, endothelial damage, the development of disseminated intravascular coagulation (DIC) syndrome and multi-organ deficiency. which, if left untreated, inevitably leads to a high risk of mortality[4].

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[1] Сурков Д.Н., Суркова А.Д., Иванов Д.О. Эпидемиология неонатального сепсиса: анализ работы отделения интенсивной терапии для новорожденных. Вестник современной клинической медицины. 2014 Том 7, вып. 6, с. 56-61

[2] Гаврилов А.В. Сепсис. Учебное пособие. Благовещенск, 2015, 63 с. https://www.amursma.ru/upload/iblock/141/Sepsis.pdf.

[3] Birju A Shah & James F Padbury.Neonatal sepsis, Virulence. 2014, 5:1, 170-178, DOI: 10.4161/viru.26906

[4] Singh M, Alsaleem M, Gray CP. Neonatal Sepsis. 2022 Sep 29. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan–. PMID: 30285373

The symptoms observed in NS are associated with dysfunction of the immune system, especially its phagocytic component, characterized by cytokine imbalance and a transient immunodeficiency state. At this time, the regulatory mechanisms of secretion into the blood of other pro- and anti-inflammatory mediators, especially antimicrobial peptides, are disrupted[5],[6].

Considering the above, identifying the subtleties of the pathogenetic mechanisms of sepsis in children and planning early diagnosis and treatment of sepsis in accordance with them remains one of the urgent tasks.

The object and subject of the research. The object of the study consisted of 100 neonates diagnosed with neonatal sepsis (65 preterm and 35 term neonates). The subject of the research was the clinical-diagnostic and prognostic significance of antimicrobial peptides (AMPs) and cytokines identified in the patients' blood serum.

The aim of the study. The aim of the study was to investigate the pathogenic mechanisms underlying changes in the immune system during sepsis in full-term and preterm neonates by determining the informational value of cytokines, antimicrobial peptides, and biochemical parameters.

The study objectives:

1. Study of dynamic changes in antimicrobial peptides and cytokines during the course of the disease in children with neonatal sepsis.

2. To determine the diagnostic and prognostic value of cytokine changes (IL-6 and IL-8) in term and preterm newborns with sepsis.

3. Studying changes in antimicrobial peptides (α-defensin, endotoxin) in various forms of the disease in term and preterm

[5] Raymond S.L., Stortz J.A., Mira J.C. et al. Immunological defects in neonatal sepsis and potential therapeutic approaches. Frontiers in Pediatrics. 2017; 5: 14

[6] Schuerholz et al. Antimicrobial peptides and their potential application in infl ammation and sepsis. Critical Care 2012, 16:207 http://ccforum.com/content/16/2/207

newborns with sepsis and determining their impact on the prognosis of sepsis.

4. To identify interactions between antimicrobial peptides, cytokines, and clinical-biochemical parameters (TB, DB, IDB, AsAT, AlAT) in newborns with a diagnosis of sepsis.

Research methods. The methods used included the collection of anamnestic data, clinical examination, laboratory testing (including general and biochemical blood analysis), and analysis of serum AMP levels (α-defensin, endotoxin), cytokines (IL-6, IL-8), bilirubin levels (total, direct, indirect), and AlAT, AsAT activities.

The main provisions for the defense:

– Antimicrobial peptides and cytokines play an important role in the pathogenesis of the NS and act as highly informative biomarkers in term and preterm neonates.

– Changes in cytokine status in term and preterm newborns with sepsis determine the course and prognosis of the disease.

– Determination of antimicrobial peptides (α-defensin and endotoxin) in newborns with various forms of neonatal sepsis is important for improving early diagnosis and choosing treatment tactics.

– The interaction of antimicrobial peptides and cytokines with clinical and biochemical parameters in newborns with sepsis, which determine the different course of the disease and the risk of complications, was noted.

Scientific novelty of the study:

– The role of antimicrobial peptides and cytokines in the pathogenesis of various forms of the disease in term and pretedrm newborns with sepsis has been studied.

– Based on changes in cytokines (IL-6 and IL-8) and antimicrobial peptides (defensin, endotoxin), the course of the disease in term and preterm newborns with sepsis was determined.

– Special criteria for early diagnosis have been developed, based on identifying the interaction of antimicrobial peptides and cytokines with other clinical and laboratory indicators in newborns with sepsis.

Theoretical and practical significance of the study. Studying the role of antimicrobial peptides and cytokines in various forms of the disease in newborns with sepsis will open up opportunities to determine the effectiveness of tactics for the prevention and treatment of the disease, as well as the effectiveness of the course of treatment in later stages of the newborn.

Understanding the effects of antimicrobial peptides on cellular mechanisms and cytokine status allows for the prediction of sepsis severity in term and preterm newborns.

Approbation and application of research results. The results of the research were presented at multiple international and national conferences, including: "Topical Issues in Medicine" dedicated to the 100th anniversary of Professor T.Ə.Əliyev (Baku, Azerbaijan, October 6–8, 2021). The 270th Anniversary of Shusha conference (Baku, Azerbaijan, 2022), "XIX Congress of Pediatricians of Russia" (Moscow, March 5–7, 2022). "1st International Azerbaijan Laboratory Medicine Congress" (Baku, Azerbaijan, May 3–5, 2023), Scientific conferences "Təbabətin aktual problemləri-2023" dedicated to the 100th anniversary of Heydər Əliyev (Baku, Azerbaijan, May 3–6, 2023), scientific-practical conference dedicated to the 80th anniversary of Professor A.M. Əfəndiyev (Baku, Azerbaijan, 2023).

Name of the organization where the dissertation is performed. The research was conducted at the Scientific Research Institute of Pediatrics named K.Y. Farajova and within the 7th Maternity Hospital in Baku.

Application of the obtained results. Research results were applied in the scientific research laboratory of the Scientific-Research Institute of Pediatrics named K.Y. Farajova, in the departments of Maternity Hospital No. 7, and in the educational process of the Biochemistry Department at Azerbaijan Medical University.

The results of the conducted research are reflected in 18 published scientific papers, 8 of which are articles published in journals recommended by the Higher Attestation Commission for dissertation work.

Structure and volume of the dissertation. The volume of the dissertation consists of 174 pages (217621 signs), introduction (7337 signs), literature review (43427 signs), materials and examination methods (11032 signs), 3 chapters reflecting personal research (112561 signs), conclusion (40956 signs), conclusions and practical recommendations (2308 signs), and a list of used literature.

The dissertation is illustrated with 8 tables and 27 pictures. The list of literature consists of 195 sources.

MATERIALS AND METHODS OF THE RESEARCH

The research was carried out at the Scientific-Research Institute of Pediatrics named after K.Farajova and Maternity Hospital No. 7 in Baku. The examinations were carried out in the Departments of Pathology of Newborns, Department of Anesthesiology and Intensive care of Newborns of the Scientific-Research Institute of Pediatrics and in the scientific research laboratory.

The study involved 130 newborn children. Of these, 100 people are inpatient children with a diagnosis of sepsis and 30 healthy newborns. The children who participated in the examination were divided into 3 groups:

Group I – newborn with sepsis, gestational age 38-41 weeks (n=35);

Group II - newborn with sepsis, gestational age 27-37 weeks (n=65);

Group III - control group: healthy newborn children (n=30).

In the process of the study, the collection of anamnestic data was carried out. In this case, there is a obstetrician history, the course of childbirth, etc.defined. The results obtained are reflected in special examination cards. These cards include all the necessary information about each patient: age, gender, notes characterizing the course of the disease, the results of clinical, laboratory tests and etc.

In addition to general clinical examinations, laboratory tests are carried out for all the children involved in the study. Hematological indicators of blood (Hb, erythrocytes, hematocrit number, MCV, MCH, leukocytes, platelets) were determined using the 3–part

hematological analyzer ”Mythic–18” (Switzerland) based on the impedance method, and the determination of ECS was based on the rate of blood sedimentation in 1 hour by the Panchenkov method. Determination of bilirubin (total (TB), direct (DB) and indirect (IDB)) in the blood, determination of the amount of characteristic enzymes (alaninaminotransferase (AlAT), aspartataminotransferase (AsAT)) in the blood was carried out in order to assess the functional state of the liver. The level of antimicrobial peptides and cytokines in the blood of children was carried out by the method of immunoferment analysis (ELISA) in the German-made immune enzyme analyzer "ElisysUno". The determination of endotoxins was analyzed using the Endotoxin (ET) ELISA Kit ("Abbexa"), and the determination of interleukins (IL-6, IL-8) was analyzed using the Russian-made "ВЕКТОР БЕСТ" kits. Statistical analysis was carried out in IBM Statistics SPSS-26 programs with the application of variational, variational, discriminant, correlation and ROC-analysis methods.

RESEARCH RESULTS AND THEIR DISCUSSION

We evaluated the indicators of the general analysis of blood in patients with neonatal sepsis. Among the identified indicators, parameters that have an important influence on the course of sepsis and future prognosis were analyzed in comparison in newborn with early (ENS) and late neonatal sepsis (LNS). It was established that the results differed slightly among the subgroups on most indicators. It has been determined that the results for most parameters (Hb, leukocytes, ESR) significantly differ from those of the control group. Between subgroups, during ENS and LNS, similar changes in the leukocyte formula were observed in response to inflammation. We conducted a correlation analysis to determine the likely interactions between these indicators and clinical signs that play a certain role in the course of sepsis. The correlation between Hb and age has been as negative as expected in preterm newborns with sepsis. This relationship significantly reflects the normal physiological tendency (change in the direction from the moment the child is born to the

decrease in Hb) (r=-0,456; p<0,001). A correlation relationship of the same nature was also traced between age and the amount of erythrocyten count (r=-0,538;p<0,001). By analogy with Hb, this parameter of the blood tends to decrease after birth, which is a process accompanied by the breakdown of fetal hemoglobin and the associated physiological decrease in both indicators. Also, in our study, significant differences in the number of monocytes were also not found during ENS and LNS. The correlation between ESR and age was significantly positive (r=+0.459;p=0.009).

However, a more pronounced dependence was established between the course and manifestations of neonatal sepsis and laboratory indicators. Thus, in preterm newborns with sepsis, with neonatal mortality, both Hb and erythrocytes showed a close correlation, which was significant in nature with both parameters (p=0,028 and p<0,001, respectively). This may be due to the results confirming a significant correlation of these blood parameters with the risk of neonatal mortality in patients with sepsis.

In the newborn with neonatal sepsis we examined, the following pathologies were observed: enterocolitis - 14%, ventriculitis - 15%, osteomyelitis - 8%, pneumonia - 56%, NEC III B - 14%, sclerema - 10%. During the course of the disease, 12% of patients developed septic shock, and 4% developed acute renal failure. Intestinal obstruction was noted in 1 patient.

Analyzing the correlation coefficients of these manifestations and general blood parameters, we obtained statistically significant results only for some of them. It should be noted that they mainly manifested themselves in preterm newborn with sepsis. So, with ventriculitis, both Hb and erythocytes demonstrated significant dependence. For example, significant negative correlations were found between ventriculitis and both Hb (r = -0.248, p = 0.046) and erythrocytes (r = -0.305, p = 0.015). Similar results were recorded between these parameters and osteomyelitis. This pathology was characterized by significant negative dependence on both Hb and erythocytes (respectively, r=-0,300;p=0,015; r=-0,328;p=0,009). A statistically significant relationship has been observed between necrotic enterocolitis and eosinophils (r=-0,443;p=0,012).

Interestingly, in segmented and band leukocyte species, the relationship with enterocolitis was weak. With severe septic shock, however, it demonstrated a close but negative relationship with segmented leukocytes (r=-0,359; p=0,047). However, a close correlation was also observed between septic shock and lymphocytes (r=+0.383; p=0.031). However, in contrast to the segmented form, the relationship with this species has been positive. It can be assumed that such interactions make it possible to take into account the significant prognostic role of this factor in the development of septic shock in newborns with neonatal sepsis.

Determining a number of biochemical indicators that play an important role in the course of sepsis and act as manifestations of polysystem dysfunction is one of the absolute conditions for treatment planning. With this in mind, we also studied what is considered important in our study (which characterizes liver, kidney function, etc.).) we have analyzed the parameters (TB, DB and IDB, as well as AsAT and AlAT, etc.).

Thus, when assessing the above indicators in children with ENS in general, we found that the TB level was 132.3±49.7 mkmol/L, DB was 20.0±6.3 mkmol/L, IDB bilirubin was 112.3±44.7 mkmol/l. Among those with LNS, TB was 146.6 ± 25.5 μmol/L, DB was 17.6 ± 3.0 μmol/L, and IDB was 121.9 ± 22.2 μmol/L. A general analysis of the enzyme AsAT activity showed that in ENS, this parameter was 95.3 ± 57.3 IU/l, and the AlAT activity was 90.8 ± 43.9 IU/l. In LNS, these parameters were AsAT = 90.6 ± 28.3 IU/L and AlAT = 70.6 ± 20.4 IU/L.

Bilirubin metabolism abnormalities were evident, with TB, IDB, and DB levels in septic neonates being significantly different from the control group. ENS showed TB at 123.8 ± 8.0 μmol/L, significantly lower than LNS (156.3 ± 9.8 μmol/L). Similar trends were observed for IDB levels (102.9 ± 8.3 μmol/L vs. 139.5 ± 9.1 μmol/L, respectively).

Comparative evaluation of liver enzymes showed parallel changes in AsAT and AlAT. For ENS, AsAT was 55.3 ± 17.5 IU/L, slightly lower than in LNS (60.4 ± 9.7 IU/L). AlAT followed a similar trend (48.3 ± 16.4 IU/L in ENS vs. 53.8 ± 9.0 IU/L in LNS).

These findings highlight the need for more sensitive and informative markers for the early diagnosis of sepsis in neonates. Based on the study objectives, dynamic levels of antimicrobial peptides (α-defensin, endotoxin) and cytokines (IL-6, IL-8) were evaluated on days 2 and 5 of sepsis.

The results revealed significant deviations in AMP and cytokine levels in septic neonates compared to healthy controls. In full-term neonates with sepsis, α-defensin levels were 771.8 ± 37.6 ng/mL in the early stages, significantly differing from the control group ($p < 0.001$). These levels decreased over time to 380.0 ± 21.1 ng/mL, reflecting the resolution of the inflammatory process (table). The changes during ENS and GNS exhibited a similar pattern. The alterations in AMP levels observed in the 2nd and 5th days in the ENS and LNS groups are presented in the Table.

Table. Changes in α-defensin and endotoxin levels in full-term neonates with sepsis compared to healthy neonates

Indicators	Group	N	M	±m	95% CI		Min	Max
					LB	UB		
α-defensin (1), ng/mL	Control	23	48.4	1.3	45.6	51.1	31.2	58.4
	Early sepsis	11	760.1	74.3	594.6	925.7	323.9	1098
	Late sepsis	24	777.1	44.2	685.8	868.5	397.2	1045
α-defensin (2), ng/mL	Control	23	48.4	1.3	45.6	51.1	31.2	58.4
	Early sepsis	11	370.7	47.1	265.6	475.7	113.4	625.7
	Late sepsis	24	384.3	22.7	337.2	431.3	212.4	566.3
Endotoxin (1), EU/ml	Control	23	0.410	0.018	0.374	0.447	0.27	0.57
	Early sepsis	11	1.449	0.048	1.343	1.555	1.24	1.69
	Late sepsis	24	1.523	0.027	1.467	1.579	1.27	1.77
Endotoxin (2), EU/ml	Control	23	0.410	0.018	0.374	0.447	0.27	0.57
	Early sepsis	11	1.027	0.142	0.711	1.343	0.37	1.57
	Late sepsis	24	1.208	0.084	1.034	1.381	0.37	1.68

Note: 1 – 2nd-day assessment, 2 – 5th-day assessment; 95% CI – 95% confidence interval; LB – lower bound, UB – upper bound.

Similar trends were observed in endotoxin levels, which were significantly elevated in the sepsis group (1.500 ± 0.024 EU/ml) compared to the control group (0.410 ± 0.018 EU/ml, $p < 0.001$).

Over time, the endotoxin levels decreased to 1.151 ± 0.073 EU/ml. A similar pattern and dynamics were observed in preterm neonates, with significant differences in α-defensin (727.9 ± 27.2 ng/ml and 363.2 ± 14.5 ng/ml in early and follow-up assessments, respectively, compared to 41.9 ± 4.2 ng/ml in the control group, $p < 0.001$) and endotoxin levels (1.517 ± 0.014 EU/ml and 1.242 ± 0.042 EU/ml, respectively, compared to 0.337 ± 0.019 EU/ml in the control group, $p < 0.001$). These findings characterized the general trends observed during ES and LS of neonates.

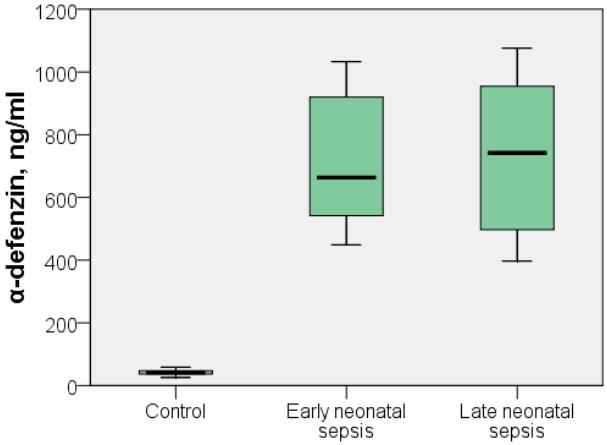
In term neonates with NS, IL-6 levels were significantly elevated during the early stages (51.3 ± 3.7 pg/ml) compared to the control group ($p < 0.001$) but decreased over time to 26.6 ± 1.4 pg/ml. This reduction likely reflects the attenuation of inflammation due to treatment, as IL-6, a sensitive inflammatory cytokine, demonstrated decreased levels. Similarly, IL-8 levels showed a comparable decline (325.1 ± 8.0 pg/ml initially, reducing to 203.0 ± 12.1 pg/ml) and were significantly different from the control group ($p < 0.001$). Comparable changes were noted in preterm neonates with sepsis: IL-6 levels decreased from 49.3 ± 2.6 pg/ml to 26.4 ± 1.0 pg/ml ($p < 0.001$), while IL-8 levels declined from 307.1 ± 7.0 pg/ml to 189.6 ± 7.6 pg/ml ($p<0.001$).

In preterm neonates as with other inflammatory processes, sepsis exhibited specific characteristics distinct from term neonates. These differences can be attributed to gestational age-related immaturity and inadequate immune reactivity in this group. Among the AMPs analyzed, α-defensin levels in septic neonates demonstrated a significant increase compared to the control group during the early stages. This statistically significant elevation was observed in both early-onset and late-onset sepsis (716.7 ± 53.3 ng/ml and 731.3 ± 31.8 ng/ml, respectively). These high levels reflect the pronounced manifestation of inflammation from the initial days in both forms of sepsis (Picture 1).

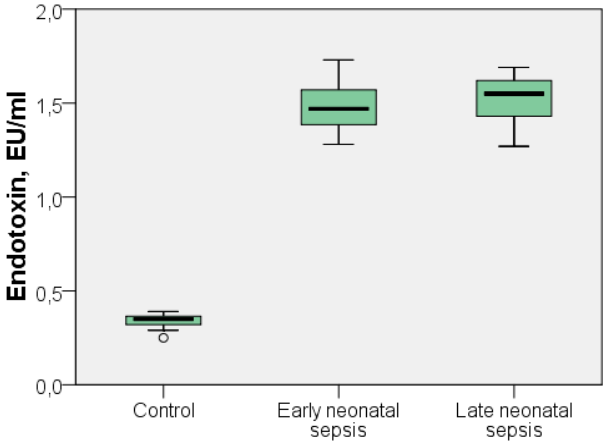
A similar trend was observed with endotoxin levels. During the initial assessment, this AMP was found at nearly identical levels in preterm neonates with ENS and LNS (1.487 ± 0.034 EU/ml and 1.526 ± 0.015 EU/ml, respectively). No significant difference was

observed between these subgroups (p>0.05). However, the results were statistically significantly higher compared to the control group (p<0.001) (Picture 2).

The endotoxin levels decreased in the 8th day more significantly in neonates with ENS, and this reduction was statistically significant compared to previous values (1.487 ± 0.034 EU/ml to 0.987 ± 0.110 EU/ml; p < 0.001).



Picture 1. Changes of α-defensin levels in term newborns with sepsis compared to healthy newborn



Picture 2. Changes of endotoxin levels in preterm newborns with sepsis compared to healthy newborn

In preterm neonates with LNS, endotoxin levels slightly decreased compared to initial values but remained higher than those of the control group (1.526 ± 0.015 EU/ml to 1.318 ± 0.037 EU/ml, respectively). Considering that bacterial endotoxin is a constant structural component of the outer cell wall of Gram-negative bacteria, its release during bacterial lysis contributes to increased endotoxin levels in the bloodstream. It can be hypothesized that this process occurs more rapidly and prominently in ENS, with a faster tendency toward reduction.

By analyzing the levels and dynamic changes of cytokines, it was observed that IL-6 levels in preterm with NS were significantly elevated in early forms (44.5 ± 6.5 pg/ml) compared to the control group ($p < 0.001$). This parameter was also elevated in LNS (50.8 ± 2.7 pg/ml). In dynamic monitoring, compared to preterm neonates without sepsis, the levels remained significantly different, showing a reduction in both early-onset and late neonatal sepsis compared to previous results. Over time, despite a reduction compared to initial values, IL-6 levels in both ENS and LNS remained higher than those of the control group (24.15 ± 2.49 pg/ml in ENS and 27.03 ± 1.09 pg/ml in LNS). This reduction can be attributed to the attenuation of inflammation during treatment, as reflected in the decreased levels of IL-6, a sensitive inflammatory cytokine. IL-8 exhibited a similar trend. In the initial assessment, IL-8 levels in ENS were 306.59 ± 14.09 pg/ml and were comparable in LNS (307.22 ± 8.17 pg/ml), both significantly higher than the control group ($p < 0.001$). In the follow-up assessment, IL-8 levels showed a tendency to decrease (respectively, 193.3 ± 10.7 pg/ml in ENS and 188.5 ± 9.4 pg/ml in LNS), yet these values remained significantly higher than the control group.

These findings suggest that both early and late neonatal sepsis in preterm neonates are characterized by statistically significant elevations in AMPs and cytokines. We hypothesized that analyzing

correlation relationships could provide more precise data in such cases. Neonates with sepsis demonstrated strong and statistically significant correlations between AMPs and cytokines.

For instance, α-defensin showed a positive correlation with IL-6 in both assessments in preterm neonates. This relationship was highly significant during the initial measurement ($r = +0.715$; $p < 0.001$) and remained significant in the follow-up assessment ($r = +0.625$; $p < 0.001$). A similar correlation was noted in term children with sepsis ($r=+0.687$; $p<0.001$ and $r=+0.673$; $p<0.001$, respectively). This correlation indicates the parallel dynamics of pro-inflammatory markers.

In preterm neonates with sepsis α-defensin also exhibited a strong positive correlation with IL-8 in both assessments ($r = +0.427$; $p < 0.001$ during the first measurement and $r = +0.395$; $p = 0.001$ during the follow-up assessment).

Correlations between AMPs (α-defensin and endotoxin) were observed in both assessments, albeit without statistical significance ($r = +0.056$; $p = 0.659$ and $r = +0.195$; $p = 0.120$, respectively). Although endotoxin demonstrated correlations with inflammatory markers, most of these relationships were not statistically significant. Only one significant correlation was observed between endotoxin and IL-6 during the follow-up assessment ($r = +0.282$; $p = 0.023$).

Cytokine interactions were more pronounced during the follow-up assessment. A significant positive correlation was identified between IL-6 and IL-8 ($r = 0.300$; $p = 0.015$). This correlation emerged during the follow-up period, suggesting a closer relationship between these cytokines during the progression of sepsis.

Thus, AMPs and cytokines are active participants in every inflammatory process and serve as critical indicators of significant changes during the course of generalized inflammatory pathologies such as sepsis.

One of the key findings of our study was the identification of diagnostic and prognostic markers among various laboratory parameters in neonates with NS)that exhibit high informativity, are clinically useful, and easy to implement in practice. To achieve this,

we analyzed the interrelationships between sepsis risk factors and clinical manifestations based on the parameters discussed in previous sections.

During the analysis of term neonates with sepsis, certain patterns were observed. Of particular interest is the statistically significant correlation between neonatal mortality and AMPs. Specifically, α-defensin exhibited a negative correlation with neonatal mortality in both the initial and follow-up measurements ($r = -0.341$; $p = 0.045$ and $r = -0.402$; $p = 0.017$, respectively). A strong negative correlation was also noted between endotoxin levels and neonatal mortality in both measurements ($r = -0.424$; $p = 0.011$ and $r = -0.515$; $p = 0.002$). Furthermore, an increase in correlation strength was observed dynamically. However, cytokine correlations with neonatal mortality were weaker and statistically insignificant.

The correlation of enterocolitis with both AMPs was logical, though their differing nature between α-defensin and endotoxin is noteworthy. The correlation with α-defensin was positive during both the initial and dynamic assessments; however, the initial measurement was statistically significant, while the dynamic correlation weakened and lost statistical significance ($r = +0.421$; $p = 0.012$ and $r = +0.256$; $p = 0.138$, respectively). This can likely be explained by the decrease in α-defensin levels due to treatment. Conversely, endotoxin showed a negative correlation during both measurements, with the initial correlation being non-significant ($r = -0.187$; $p = 0.135$) but becoming statistically significant in the dynamic assessment ($r = -0.295$; $p = 0.017$). This suggests that endotoxin may serve as a more sensitive indicator during the course of enterocolitis in sick neonates.

For ventriculitis in term neonates with sepsis, α-defensin levels demonstrated a strong positive correlation in both measurements ($r = +0.338$; $p = 0.047$ and $r = +0.347$; $p = 0.041$). Similarly, endotoxin levels were positively correlated ($r =+ 0.378$; $p = 0.025$ and $r = +0.418$; $p = 0.012$). The correlations with cytokines, particularly IL-6, were weaker and statistically insignificant, whereas IL-8 exhibited stronger correlations during both assessments, with the follow-up

measurement achieving statistical significance ($r = +0.329$; $p = 0.054$ and $r = +0.347$; $p = 0.041$).

Regarding pneumonia, a common manifestation, correlations were observed with only a few of the analyzed parameters. Among AMPs, α-defensin showed weak correlations (initially negative and later positive) in both assessments, though neither was statistically significant. Endotoxin demonstrated a positive correlation in the initial measurement but weakened dynamically ($r = +0.179$; $p = 0.303$ and $r = +0.142$; $p = 0.417$). IL-8, however, emerged as a sensitive indicator, showing a significant positive correlation with pneumonia in the initial measurement ($r = 0.534$; $p = 0.001$), though this weakened and lost significance dynamically ($r = 0.315$; $p = 0.066$).

NEC showed significant correlations only with IL-6 during both measurements, with statistical significance observed in the follow-up assessment ($r = +0.344$; $p = 0.043$). This suggests that IL-6 could be an important factor in dynamic monitoring of NEC in neonates.

Among biochemical indicators, TB exhibited statistically significant negative correlations with α-defensin during both measurements ($r = -0.465$; $p = 0.015$ and $r = -0.384$; $p = 0.048$, respectively). Similar significant negative correlations were found between DB and α-defensin ($r = -0.498$; $p = 0.008$ and $r = -0.421$; $p = 0.029$, respectively). However, in the follow-up assessment, the significance was lost, and the correlation became weaker. This could potentially be explained by the restoration of bilirubin metabolism during treatment.

In our opinion, this finding confirms that IDB, as a sensitive parameter of metabolism, is characterized by stronger associations with certain inflammatory markers. This relationship is also reflected in the correlation observed with IL-6.

Dynamic monitoring revealed significant correlations between AsAT activity and IL-6 levels ($r = +0.568$; $p = 0.014$) and between AlAT activity and IL-6 in both measurements ($r = +0.518$; $p = 0.028$ and $r = 0.607$; $p = 0.008$).

The findings suggest that AMPs and cytokines are associated with specific clinical and laboratory parameters in term neonates during sepsis. These associations play a significant role during sepsis and can be utilized for early diagnosis and prognosis.

In preterm neonates with confirmed sepsis, correlation relationships were identified among the studied parameters, and these were analyzed during the research.

In preterm neonates with sepsis, it was observed that the correlation between enterocolitis and α-defensin was positive during both the initial and follow-up measurements, but only the initial correlation was statistically significant. Conversely, the correlation with endotoxin was negative in both assessments; however, while it was non-significant during the first measurement ($r = -0.187$; $p = 0.135$), it became statistically significant in the follow-up assessment ($r = -0.295$; $p = 0.017$). This suggests that endotoxin might serve as a more sensitive marker during the progression of enterocolitis in preterm neonate with sepsis. Correlations with cytokines were generally weak and positive.

In preterm neonates with sepsis, ventriculitis exhibited statistically significant correlations with α-defensin during both measurements ($r = +0.326$; $p = 0.008$ and $r = +0.332$; $p = 0.007$, respectively). Similarly, IL-6 showed positive correlations in both measurements, with significance only observed during the follow-up ($r = +0.270$; $p = 0.030$).

For pneumonia, significant correlations were noted only with endotoxin in both assessments ($r = +0.264$; $p = 0.034$ and $r = +0.387$; $p = 0.001$). Additionally, IL-8 demonstrated significant correlations during the initial measurement ($r =+ 0.308$; $p = 0.012$), which weakened and lost significance in the follow-up assessment ($r = +0.042$; $p = 0.740$). These findings indicate that IL-8 may serve as an important marker in the dynamic monitoring of preterm neonates with pneumonia.

It is noteworthy that NEC demonstrated a statistically significant correlation with several analyzed parameters. Among AMP, α-defensin exhibited a statistically significant positive correlation during the initial days, followed by a positive but

statistically non-significant correlation later (respectively, r=+0.265; p=0.033 and r=+0.223; p=0.074). Endotoxin, on the other hand, showed a statistically significant inverse correlation in both evaluations, with a weaker correlation over time (respectively, r=-0.364; p=0.003 and r=-0.298; p=0.016). Similarly, in term neonates, this finding suggests that endotoxin could serve as a sensitive early marker in the context of NEC development during sepsis. IL-6 can also be noted as a sensitive marker for this pathology, as its correlation with NEC was statistically significant in both measurements (respectively, r=+0.414; p=0.001 and r=+0.248; p=0.046). This indicates that IL-6 might act as an early marker as well.

For other pathologies, a statistically significant correlation was identified between IL-6 and sclerema. This relationship was statistically significant in the initial measurement but lost its significance over time (respectively, r=+0.317; p=0.010 and r=+0.153; p=0.222).

The study of correlations between biochemical parameters, antimicrobial peptides, and cytokines revealed no statistically significant correlations in preterm neonates. By assessing total, direct, and indirect bilirubin, it was determined that inflammatory markers in both assessments exhibited weak correlations with these bilirubin levels. Similarly, enzymes such as AsAT and AlAT showed no significant correlation with inflammatory markers.

In conclusion, multidirectional correlation analysis revealed that antimicrobial peptides and cytokines interact with clinical and laboratory manifestations to varying degrees. These interactions exhibit unique features in the context of sepsis in preterm and term NS. Based on our findings, it is important to consider that the immunological status of septic patients is influenced by the pathological process itself as well as numerous parameters characteristic of severe infections. The early onset of sepsis in term and preterm neonates is associated with elevated levels of AMP and cytokines, particularly IL-6. This could be attributed to disruptions in maternal homeostasis caused by prolonged persistence of infectious

agents (viral, bacterial, fungal, or intestinal dysbiosis), leading to pre-activation of fetal monocytes/macrophages.

In our study, the practical informativeness of the analyzed parameters was assessed using ROC analysis. This statistical approach allowed for a more precise evaluation of the diagnostic value of markers such as IL-6 and IL-8 during various manifestations of sepsis (e.g., enterocolitis, pneumonia). Furthermore, our results provide insights into the prognostic importance of these markers in predicting severe complications such as neonatal mortality.

The ROC analysis revealed that during neonatal sepsis, α-defensin demonstrated the highest sensitivity for NEC (92.9%), pneumonia (85.7%), septic shock (91.7%), and neonatal mortality (92.3%). It also exhibited high specificity for ventriculitis (77.6%) and enterocolitis (74.4%). Endotoxin's predictive value was characterized by high sensitivity for enterocolitis (78.6%), NEC (100.0%), and neonatal mortality (71.8%) and high specificity for ventriculitis (71.8%).

Regarding interleukins, IL-6 demonstrated high sensitivity in diagnosing ventriculitis (100.0%), NEC (100.0%), pneumonia (80.4%), septic shock (83.3%), and neonatal mortality (79.5%) while exhibiting high specificity for enterocolitis (86.0%). The predictive evaluation of IL-8 revealed high sensitivity for NEC and neonatal mortality (respectively, 92.9% and 89.7%) and high specificity for ventriculitis and septic shock (respectively, 84.7% and 92.0%).

In literature focused on sepsis, information about the informativeness of AMP in various pathologies is often fragmented. Therefore, our applied approach – combining correlation analysis and ROC analysis– facilitates a more precise practical understanding of these parameters.

In conclusion, AMP, IL-6, and IL-8 are highly sensitive markers that change during the early phases of septic processes. Among these, IL-6 and IL-8 are considered particularly important as "early biomarkers." Consequently, determining AMP and cytokine levels in combination with other diagnostic methods may significantly enhance early diagnosis and the prognosis of infectious processes.

RESULTS

1. In term neonates with a confirmed diagnosis of sepsis, α-defensin levels were 15.9 times higher than those in the control group during the early days of the disease, with statistically significant differences ($p < 0.001$). Similarly, endotoxin levels were 3.7 times higher than those in the control group ($p < 0.001$). A comparable dynamic was observed in preterm neonates, with α-defensin levels being 17.4 times higher ($p < 0.001$) and endotoxin levels 4.5 times higher ($p < 0.001$) than those in the control group [7, 13, 16].
2. The level of IL-6 in premature infants with neonatal sepsis was 15.7 times higher than those in the control group during the early days ($p < 0.001$), and IL-8 levels demonstrated similar changes, being 28 times higher ($p < 0.001$). Comparable changes were observed in preterm neonates with sepsis: IL-6 levels were 15.9 times higher ($p < 0.001$) and IL-8 levels were 25 times higher ($p < 0.001$) than those in the control group [16, 17].
3. In neonatal sepsis, α-defensin has the highest sensitivity for NEC (92.9%), pneumonia (85.7%), septic shock (91.7%), and neonatal mortality (92.3%), as well as high specificity in relation to ventriculitis (77.6%) and enterocolitis (74.4%). The predictive value of endotoxin was characterized by high sensitivity to enterocolitis (78.6%), NEC (100.0%) and neonatal mortality (71.8%), and high specificity to ventriculitis (71.8%) [13, 17].
4. Informativeness of IL-6 in newborns with sepsis is characterizied by high sensitivity in relation to ventriculitis (100.0%), NEC (100.0%), pneumonia (80.4%), septic shock (83.3%), neonatal mortality (79. 5%) and high specificity (86.0%) for enterocolitis. The predictive value of IL-8 showed its high sensitivity for NEC and neonatal mortality (92.9% and 89.7%, respectively) and high specificity for ventriculitis and septic shock (84.7% and 92.0%, respectively) [17].
5. In full-term newborns with neonatal sepsis, TB with α-defensin ($r=-0.465$; $p=0.015$ and $r=-0.384$; $p=0.048$, respectively), IDB with α-defensin ($r=-0.498$; $p=0.008$ and $r=-0.421$; $p=0.029$, respectively), AlAT with IL-6 ($r=+0.518$; $p=0.028$ and $r=+0.607$; $p=0.008$, respec-

tively) showed a significant correlation in the dynamics of the disease [13, 17].

PRACTICAL RECOMMENDATIONS

1. The assessment of clinical and laboratory parameters in children with neonatal sepsis should be carried out in complex dynamics from the first days, so that complications that may arise during the course of the disease can be prevented.
2. In term and preterm newborns with sepsis, it is necessary to determine antimicrobial peptides (a-defensin and endotoxin) and cytokines (IL-6 and IL-8), which can serve as the basis for early diagnosis of various pathological manifestations.
3. Assessing biomarkers with high predictive value (α-defensin, endotoxin, IL-6 and IL-8) in neonates with sepsis may be important both to determine prognostic risks and to determine the need for intensive care.

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20.

LIST OF ABBREVIATIONS

ALP	–	Alkaline phosphatase
AlAT	–	Alanine aminotransferase
AMPs		Antimicrobial peptides
AsAT	–	Aspartate aminotransferase
IDB	–	Indirect bilirubin
DB	–	Direct bilirubin
ENS	–	Early neonatal sepsis
ESR	–	Erythrocyte sedimentation rate
GGT	–	Gamma-glutamyl transferase
IL	–	Interleukins
LNS	–	Late neonatal sepsis
NEC		Necrotizing enterocolitis
NS	–	Neonatal sepsis
TB	–	Total bilirubin

The defense of the dissertation will be held on «06» march 2025 at «11.00» at the meeting of the Dissertation Council FD 1.08 of Supreme Attestation Commission under the President of the Republic of Azerbaijan operating at Institute of Physiology named after academician Abdulla Garayev.

Address: Sharifzada Street 78, Baku. AZ 1100

The dissertation is available at the Library of the Institute of Physiology named after academician Abdulla Garayev of the Ministry of Science and Education of Azerbaijan Republic.

Electronic versions of the abstract are posted on the official website (www.physiology.az) of the Institute of Physiology named after academician Abdulla Garayev.

Abstract was sent to the required addresses on «31» january 2025.

Signed for print: 21.01.2025
Paper format: 60x84 1/16
Volume: 35.827 characters
Order: 209
Number of hard copies: 20
“Tabib” publishing house