JMS 2020;3(2):3-12
UDC:616.94-097-053.31

DIAGNOSTIC VALUES OF BIOCHEMICAL MARKERSIN ASPHYXED NEWBORNS WITH PROVEN SEPSIS

Sofijanova Aspazija**,** Naunova Timovska S, Mangjukova H, Shuperliska E,
Jordanova O, Bojadzieva S
PHI University Clinic for Children Diseases, Medical Faculty, University "Ss.Cyril and Methodius",
Skopje, Republic of North Macedonia

Abstract

Introduction**:** The aim of this study was to investigate the predictive values of biochemical parameters, including Procalcitonin (PCT), as an early diagnostic and prognostic marker for sepsis in asphyxed newborns with proven sepsis.

Materials and Methods: This study was designed as a prospective study, where we included 110 (M:F=67:43) newborns with proven sepsis hospitalized in the Intensive Care Unit at the PHI University Children's Hospital – Skopje.PCT and CRP,WBC one serum blood sample was obtained from each patient at the 24h at admission, as well asday 3 and day 7. Procalcitoninlevels were measured by using an immunoassay system Vidas, based on the Enzyme Linked Fluorescent Assay (ELFA) principles.

Results: The newborns with proven sepsis have been divided into two groups. The first group included 50 proven septicpreterm newborns with a positive blood culture and the second group included 50 proven sepsis full-term newborns.We isolated forty two that had two or three bacteria at the same time. The identified bacteria included *Staphylococcus aureus* (n=56) *mecA*,*Streptococcus* (n=6), *Acinetobacter baumannii* (n=18), *Serratia marcescens* (n=9) and *Entrobacteriaceae* (n=31), *Candida albicans*(n= 1), *Candida parapsilosis*(n=1).Statistical analysis confirmed significantly different values of PCT in the analyzed time period in preterm newborns with proven sepsis $p<0.001$.Statistical analysis confirmed significantly different values of PCT in the analyzed time period in newborns with proven sepsis with asphyxia $p<0.001$.

Conclusion: The levels of PCT have important clinical significance in predicting the prognosis of asphyxed newborns with sepsis, to prevent the development of severe sepsis and septic shock.

Keywords:Sepsis,C-reactive protein,procalcitonin PCT, asphyxed newborns

Introduction

Asphyxia is a lack of gas exchange or blood flow during, before or after childbirth. Lack of oxygen can lead to systemic and neurological sequelae.When compromised or completely stopped, placental (prenatal) or pulmonary (postnatal) gas exchange occurs in part (hypoxia) or complete (anoxia), resulting in lack of oxygen to vital organs [1] .

This will lead to progressive hypoxemia and hypercapnia. Severe asphyxia will lead to the development of anaerobic glycolysis and lactic acidosis, with severe neurological consequences.Prenatal asphyxia can occur in a haemodynamically compromised mother, uterine rupture, placental abruption, node or compromised umbilical cord and infection. Postnatal asphyxia occurs in the following conditions: severe forms of cyanogenic congenital heart disease, severe pulmonary disease, severe anemia sufficient to reduce the oxygen content of the blood, shock due to sepsis or massive blood loss, intracranial or adrenal hemorrhagia[2].

Asphyxia occurs for reasons that occur intrapartum, such as inadequate relaxation of the uterus due to excess oxytocin, prolonged delivery, and knotting of the umbilical cord around the neck of the infant.

Risk factors for asphyxia include: elderly or young mothers, multiple births, prolonged rupture of the membranes, low birth weight infants, meconium-stained fluid, malpresentation, antepartum hemorrhage, lack of antenatal care, severe eclampsia and pre-eclampsia, antepartum and intrapartum

anemia, and augmentation of labour with oxytocin[3].The most common symptoms and signs of asphyxia before childbirth are: an abnormal heart rate or rhythm, an increased acid level in the baby's blood. At birth, the most common symptoms and signs are: bluish or pale skin color, weak muscle tone and reflexes gasping or weak breathing, low heart rate, weak cry and meconium – the first stool passed by the baby –in the amniotic fluid, which can block small airways and interfere with breathing [4].
Diagnostic criteria for hypoxic ischemic encephalopathy in neonates includes: metabolic acidosis in gas analyzes-pH <7.0, presence of multiple organ-system failures, clinical evidence of encephalopathy: hypotonia, weak or absent sucking reflex, abnormal oculomotor or pupillary movements, hyperpnea, apnea or clinical seizures, neurological outbreaks that cannot be attributed to another disease.
Treatment may include: giving the mother extra oxygen before delivery, emergency delivery or caesarean section, assisted ventilation and medications to support the baby's breathing and blood pressure, extracorporeal membrane oxygenation (ECMO).

Neonatal sepsis is the systemic response to infection by microbial organisms and it is a major cause of mortality and morbidity in newborns, with the highest incidence occurring among infants of very low birthweight and gestation [5,6]. Without timely treatment, sepsiscan rapidly lead to tissue damage, organ failure, and death in newborns. Preterm newborns are at higher risk for sepsis than term newborns, as they tend to require more invasive procedures than term newborns[7,8].Biochemical monitoring is of great importance in the risk assessment for developing sepsis, severe sepsis or septic shock. Unfortunately, the leading factor in morbidity and mortality in the postnatal period is still bacterial inflammation[9]. Newborns with clear signs of sepsis present with features of infection and clinical manifestations of inflammation.Severe sepsis is the development of hypoperfusion with organic dysfunction in a septic patient.Septic shock is the hypoperfusion with multiorgan failure and persistent hypotension[10,11]. Mortality varies up to 16% in newborns with sepsis and 40-60% in newborns with septic shock. The research of new biochemical markersenabling a precocious identification of neonates at risk of neonatal diseases, allowing a close monitoring of thedisease and providing information about prognosis,represents a strategic objective of several currentresearches[12]. One promising biochemical marker has been procalcitonin (PCT), whose concentration has been found to be elevated in sepsis. During infection, it is produced by the extrathyroid tissue. Mononuclear leukocytes and liver are the most likely sources of PCT in sepsis. Endotoxin and septic-bound proinflammatory cytokines have a stimulating effect on PCT by expressing mRNA in human mononuclear leukocytes[13,14]. Therefore, the diagnosis of severe septic condition, as well as organic dysfunction, is strongly related to the concentration of PCT. Therefore, it is considered particularly useful in monitoring the effect of anti-infective therapy in sepsis[15,16].

Materials and Methods

Patients: In a prospective study, 110newborns with proven sepsis (blood culture positive for bacteria) were included for the time period November 2018, until May2020.
Diagnosis of sepsis in newborns was diagnosed according to the standard protocols for disease diagnosis.Sepsis was defined as the identification of two or more Systemic Inflammatory Response Syndromecriteriain addition to present source of infection or suspected infection. The clinical criteria taken as indicative of sepsis in newborns was:respiratory distress,lethargy, apnea,tachypnea, bradycardia, seizures,poor perfusion,lethargy,feeding intolerance, temperature instability, low birth weight, gestational age, gender, and preterm newborns.
All the laboratory examination were analyzedin the Clinical Laboratory at the PHI University Children's Hospital–Skopje. A samplefor blood culture, PCT CRP and WBCobtained by peripheral venous puncturewas takenat the first admission,before initiation of antibiotic therapy in newborns suspected of sepsis, the second on 3 day and the third on day 7. Blood culture media were incubated at 37°C for 5days in BactAlert 3D 360.

Equipment: Procalcitonin was determined by immunoassay: patented ELFA (Enzyme-linked fluorescent assay) technology, automated Vidas Biomerieux immunoassay (ng/ml) CRP levels were

determined by using immunoturbidimetric method Architect c4000 Abbott(mg/L). White blood cells (WBCs) were determined by using Flow citometry method on Sysmex xs 800i/1000i.
Positive blood culture was proven with the new multiplex polymerase chain reaction–based rapid diagnostic test (BioFire FilmAray Blood Culture Identification).
Medical data records of admitted neonates suspected for sepsis injury were analyzed.

Statistical analysis The SPSS program was used for statistical analysis,to compare the means of the variables, one-way ANOVA test. Categorical variables between groups were analyzed using the Chi-square test. The results were presented as percentage (%), mean, standard deviation (SD), median, and minimum-maximum (min-max). A *P*-value< 0.05 was considered as significant.

Results

In the study,designed as a prospective study, we included 110 (M:F=67:43) newborns with proven sepsis hospitalized at the Intensive Care Unit at the PHI University Children's Hospital–Skopje for the time period November 2018 until May2020 (Figure.1).

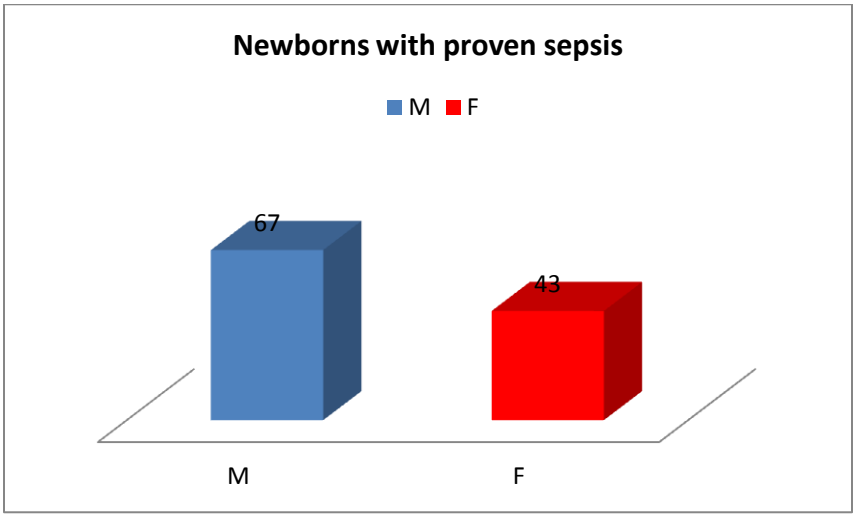

Figure 1. Distribution of newborns with proven sepsis depending on gender

The mean gestational age of newborns with proven sepsis was 37.21 ± 3.1 weeks. The mean birth weight of newborns with proven sepsis was 2856.5 ± 723.6 grams.

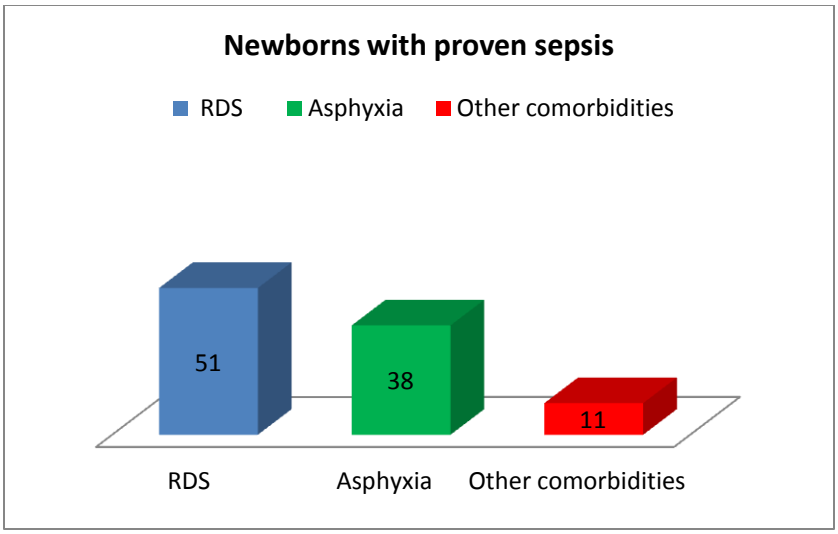

Figure2. Distribution of newborns with proven sepsis according to the comorbidity

Figure 2 show the newborns with proven sepsis,in 51 (46%) the cause was ±RDS, in 38 the cause was Asphyxia, and in 11the cause was other comorbidities.

The newborns with proven sepsis have been divided intotwo groups The first group included 50 proven sepsis pretermnewborns with positive blood culture and the second group included 50 proven sepsis full-term newborns .

We isolated forty-two that had two or three bacteria at the same time. The identified bacteria included Staphylococcusaureus (n=56) mecA, Streptococcus (n=6), Acinetobacter baumannii (n=18), Serratia marcescens (n=9) and Entrobacteriaceae (n=31), Candida albicans(n= 1) Candidaparapsilosis(n=1).

In the first group of preterm newborns with proven sepsis, 36(65,50%) were male and 19(34,50%)were female. The comparable values in the second group were 31(56,40%)malesand 24(43,60%) females.There is no significant difference in this parameter between the two groups ($p<0.01$).

In the firstgroup of preterm newborns with proven sepsis,thebirth weightwas 2656,2±792,6 grams and the second group of full-term newborns with proven sepsis was 2895,7±892,5 grams. There was no significant difference in this parameter between the two groups ($p<0.01$) .

Statistical analysis confirmed significantly different values of PCT in the analyzed time period in preterm newborns with proven sepsis $p<0.001$(Figure.3). The highest average values (43.37±53.79) were measured during admission with a subsequent sharp jump. After the second measurement at day 3, the average values of PCT slowly decreased(32.05±46.19), so that after the third measurement on day 7,they slowly began to normalize(11.76±15.58).

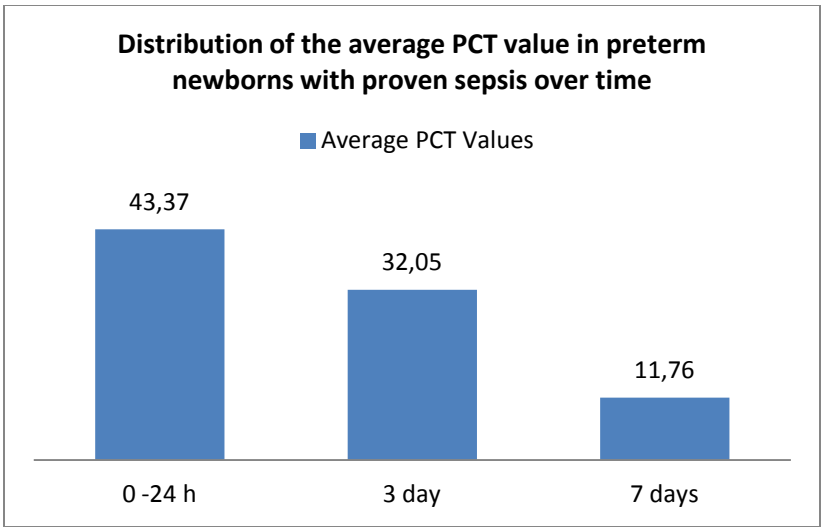

Figure3. Distribution of the average PCT value in preterm newborns with proven sepsis over time

Statistical analysis confirmed significantly different values of CRP in the analyzed time period in preterm newborns with proven sepsis $p<0.001$(Figure.4). At the first measurement, the average values of CRP slowly increased (21.4±44.37).The highest average values were measured (42.17±61.84) after the second measurement on day 3 with a subsequent sharp jump. At the third measurement on day 7, the average values of CRP slowly decreased (23.53±29.59)

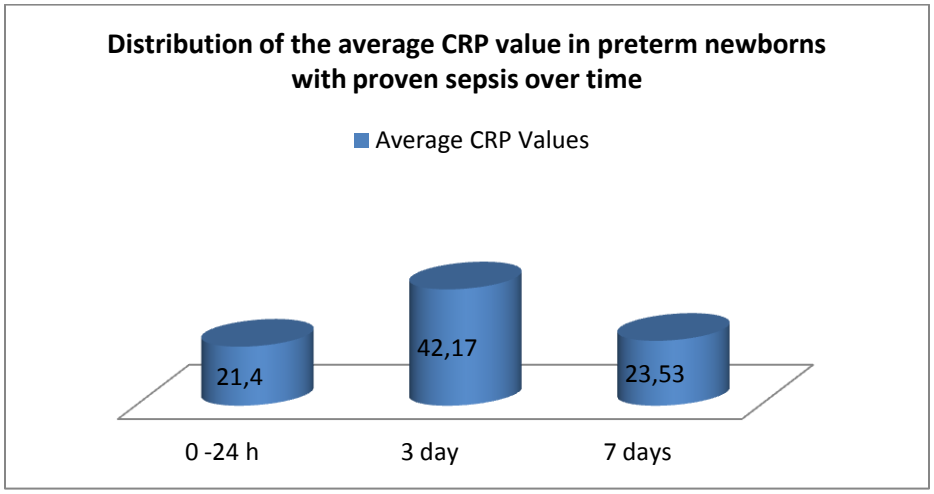

Figure 4. Distribution of the average CRP value in preterm newborns with proven sepsis over time

Statistical analysis confirmed insignificantly values of WBC in the analyzed time period in preterm newborns with proven sepsis (Figure.5). In the three measurements they had an average value WBC (15.839±8.35, 15.85±9.64. 16.01±11.72)

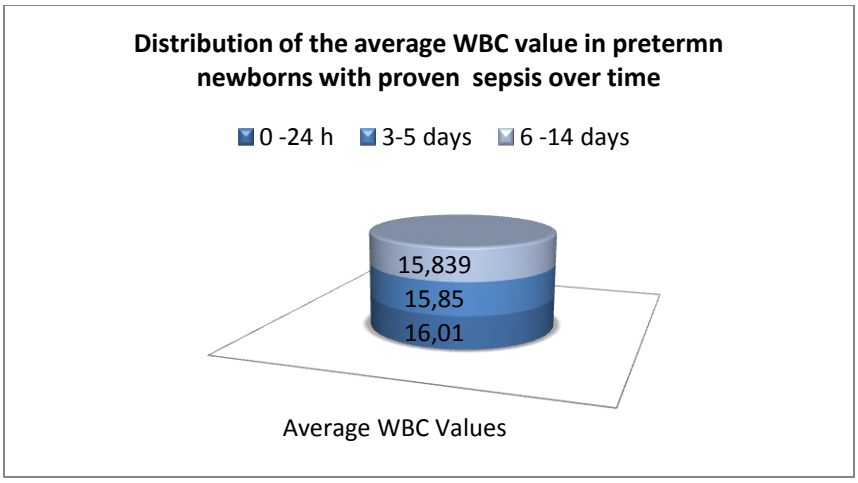

Figure5. Distribution of the average WBC value in preterm newborns with proven sepsis over time

Statistical analysis confirmed significantly different values of PCT in the analyzed time period in newborns with proven sepsis with asphyxia p<0.001(Figure.6). The highest average values (44.68±62.50) were measuredin newborns with proven sepsis with asphyxia during admission, with a subsequent sharp jump compared to newborns with proven sepsis with RDS and other comorbidities. After the second measurement at day 3, the average values of PCT in newborns with proven sepsis with asphyxia slowly decreased(39.95±49.35), so that after the third measurement on day 7in newborns with proven sepsis with asphyxia,they slowly began to normalize(32.55±38.16).

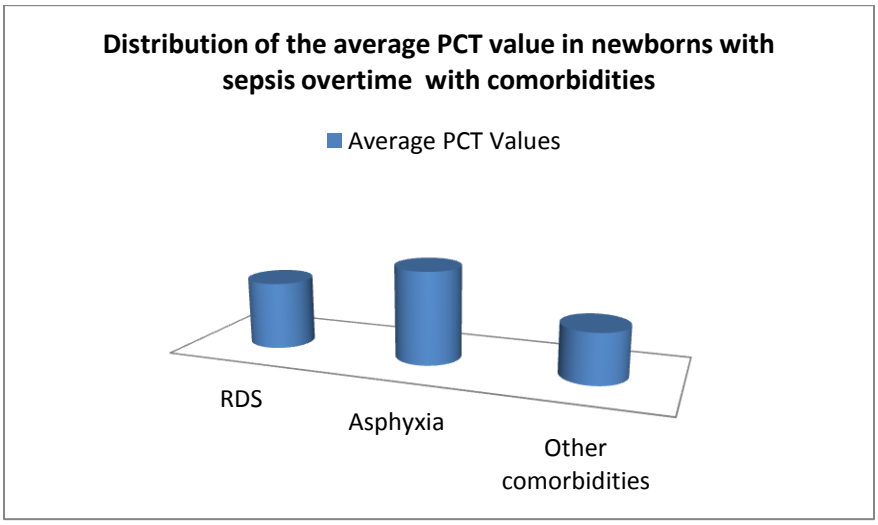

Figure 6. Distribution of average PCT value in newborns with proven sepsis over time compared to comorbidities

Statistical analysis confirmed significantly different values of CRP in the analyzed time period in newborns with proven sepsis with asphyxiaand RDS p<0.001(Figure.7).. At the first measurement, the average values of CRP in newborns with proven sepsis with asphyxia and RDSslowly increased (36.67±50.43).The highest average values in newborns with proven sepsis with asphyxia were measured (52.72±85.69) after the second measurement on day 3, with a subsequent sharp jumpcompared with newborns with proven sepsis with other comorbidities.At the third measurementin newborns with proven sepsis with asphyxiaon day 7, the average values of CRP slowly decreased (55.68±72.52).

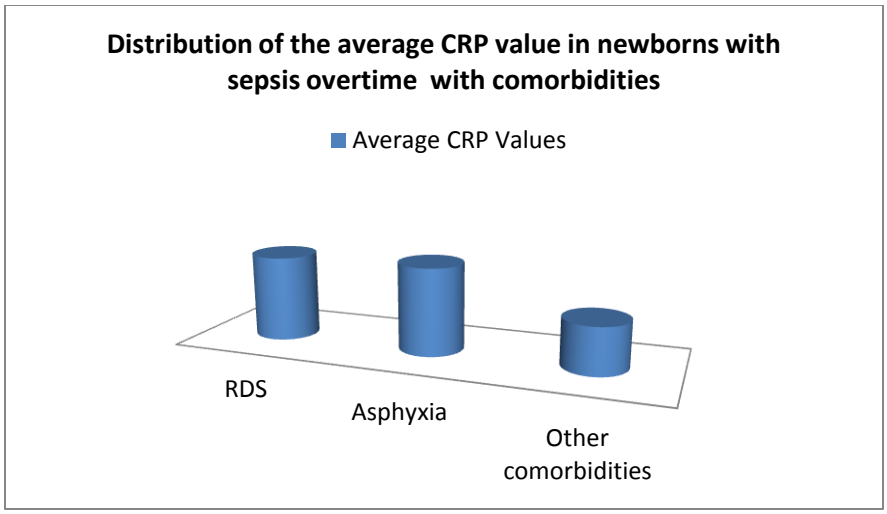

Figure 7. Distribution of average CRP value in newborns with proven sepsis over time compared to comorbidities

Statistical analysis confirmed insignificant values of WBC in the analyzed time period in newborns with proven sepsis with asphyxia (Figure.8). In the three measurements, they had an average value WBC (15.72±9.26, 16.81±9.64. 16.81±12.71)in newborns with proven sepsis with RDS and other comorbidities.

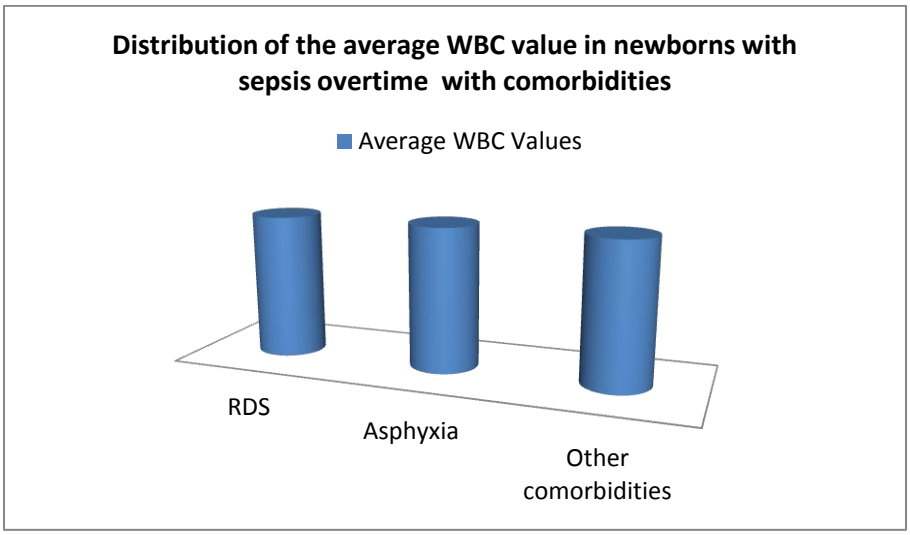

Figure 8. Distribution of average WBC value in newborns with proven sepsis over time compared to comorbidities

Capillary blood test findings show lower mean pH in preterm newborns with proven sepsis compared with the control group (7,18±0,14 versus 7,23±0,12).

Average serum albumin values in newborns with sepsis showed lower values compared with the control group (25,21±3,6g/L versus 32.82±5,2g/L).

Average total serum protein values in newborns with sepsis showed lower values compared to the control group (46,01±6,1g/L versus 50.41±7,1g/L).

Hypoxia was reported in 32/55 preterm newborns with proven sepsis and 20/55 full-term newborns with proven sepsis, with mean partial pressure values of O_2 5,21±0,7кPa versus O_2 5,25±1,1кPa.

Hypercapnia was reported in 35/55 preterm newborns with proven sepsis and 29/55 full-term newborns with proven sepsis with mean partial pressure values of CO_2 6,71±1,3кPa versus CO_2 5,15±1,1,3кPa.

Discussion

Perinatal asphyxia is the leading cause of death and neurological damage in neonates. Many surviving infants with severe asphyxia have a clinical picture of hypoxemic ischemic encephalopathy. There are limited data on the long-term outcome after perinatal asphyxia, such as developmental delay, cerebral palsy, and visual and hearing impairment.

The clinical diagnosis of perinatal asphyxia is made on the basis of several criteria: apgar score less than 7 to 5 minutes after birth (cardiorespiratory and neurological depression) and metabolic acidosis from gas analyzes pH <7.0.

Currently, newborn sepsis is a life-threatening conditionand still represents an important cause of mortality and morbidity. For pediatricians,early identification of infections is still a challenge.Theetiology of sepsis is not always clear and an organism that initiates disease in one newborn may not in another(17,18). Bacterial infections are the most common cause of sepsis in newborns. The infection can be located in any of number of places throughout the body.One promising biochemical marker has been procalcitonin (PCT), whose concentration has been found to be elevated in sepsis [19,20].Many authors found that procalcitonin is a promising marker for the diagnosis of sepsis in newborns [21,22].An ideal biomarker for sepsis should have high sensitivity and specificity, with early phase elevation, low cost and quick result.

The diagnostic performance of PCT in numerous studies from literature has suggested PCT to be a useful marker in the diagnosis of sepsis [23,23,25]. In our study, we examined three parameters PCT, CRP, and WBCin newborns with proven sepsis.Statistical analysis confirmed significantly different values of PCT in the analyzed time period in preterm newborns with proven sepsis $p<0.001$.The highest average values (43.37±53.79) were measured during admission with a subsequent sharp jump. Statistical analysis confirmed significantly different values of CRP in the analyzed time period in preterm newborns with proven sepsis $p<0.001$[26,27]. The highest average values were measured (42.17±61.84) after the second measurement on day 3 with a subsequent sharp jump. Statistical analysis confirmed insignificant values of WBC in the analyzed time period in preterm newborns with proven sepsis [28]. PCT have a higher discriminative ability than theWBC in distinguishing a bacterial infection from another inflammatory process in the early infection diagnosis, and also found to have been more reliable than that of the CRP.Statistical analysis confirmed significantly different values of PCT in the analyzed time period in newborns with proven sepsis with asphyxia $p<0.001$[29,30]. The highest average values (44.68±62.50) were measuredin newborns with proven sepsis with asphyxia during admission with a subsequent sharp jump compared with newborns with proven sepsis with RDS and other comorbidities.

Conclusion

We examined three parameters in newborns with proven sepsis, the values of PCT increased at the moment of admission in the group of preterm newborns compared with the group of full-term newborns, while the values of of C-reactive protein gradually increased. The values of PCT havean important clinical significance in predicting the prognosis of asphyxed newborns with sepsis.

Procalcitonin (PCT) is a useful biochemical marker in asphyxedpreterm newborns with sepsis, whose values can prevent the development of severe sepsis and septic shock.

References

1. Thornberg E, Thiringer K, Odeback A, et al. Birth asphyxia: incidence, clinical course and outcome in a Swedish population. Acta Paediatr 1995;84:927–932.
2. Airede AI. Birth asphyxia and hypoxic-ischaemic encephalopathy: incidence and severity. Ann Trop Paediatr 1991;11:331–335.
3. Oswyn G, Vince JD, Friesen H. Perinatal asphyxia at Port Moresby General Hospital: a study of incidence, risk factors and outcome. P N G Med J 2000;43:110–120.
4. Birth asphyxia, neonatal risk factors for hypoxic ischemic encephalopathy Pálsdóttir K, Thórkelsson T, Hardardóttir H, Dagbjartsson A.Laeknabladid. 2007 Oct;93(10):669-73.
5. Kliegman RM, Stanton B, Geme JS, St Schor NF, Behrman RE. Nelson textbook of pediatrics. 20th ed. Philadelphia (PA): Elsevier Health Sciences; 2015
6. Whicher J, Bienvenu J, Monneret G. Procalcitonin as an acute phase marker. Ann Clin Biochem. 2001;38(Pt 5):483–493.
7. Gogos CA, Drosou E, Bassaris HP, Skoutelis A. Pro- versus anti-inflammatory cytokine profile in patients with severe sepsis: a marker for prognosis and future therapeutic options. J Infect Dis. 2000;181:176–180.
8. Müller B, White JC, Nylén ES, Snider RH, Becker KL, Habener JF. Ubiquitous expression of the calcitonin-i gene in multiple tissues in response to sepsis. J Clin Endocrinol Metab. 2001;86:396–404.
9. Chiesa C, Panero A, Rossi N, Stegagno M, De Giusti M, Osborn JF, et al. Reliability of procalcitonin concentrations for the diagnosis of sepsis in critically ill neonates. Clin Infect Dis. 1998;26:664–672.
10. Christ-Crain M, Müller B. Procalcitonin in bacterial infections: hype, hope, more or less? Swiss Med Wkly. 2005;135:451–460.
11. Uzzan B, Cohen R, Nicolas P, Cucherat M, Perret GY. Procalcitonin as a diagnostic test for sepsis in critically ill adults and after surgery or trauma: a systematic review and meta-analysis. Crit Care Med. 2006;34:1996–2003.
12. Lapillonne A, Basson E, Monneret G, Bienvenu J, Salle BL. Lack of specificity of procalcitonin for sepsis diagnosis in premature infants. Lancet. 1998;351:1211–1212.
13. Guibourdenche J, Bedu A, Petzold L, Marchand M, Mariani-Kurdjian P, Hurtaud-Roux MF, et al. Biochemical markers of neonatal sepsis: value of procalcitonin in the emergency setting. Ann Clin Biochem. 2002;39(Pt 2):130–135.
14. Turner D, Hammerman C, Rudensky B, Schlesinger Y, Goia C, Schimmel MS. Procalcitonin in preterm infants during the first few days of life: introducing an age related nomogram. Arch Dis Child Fetal Neonatal Ed. 2006;91:F283–F286.]
15. Assumma M, Signore F, Pacifico L, Rossi N, Osborn JF, Chiesa C. Serum procalcitonin concentrations in term delivering mothers and their healthy offspring: a longitudinal study. Clin Chem. 2000;46:1583–1587.
16. Barbara S. Infections of the neonatal infant. In: Behrman Re, Kliegman R, Jensen HB., editors. Nelson Textbook of Pediatrics. Philadelphia: WB Saunders CO; 2008. pp. 794–811.
17. Young LS. Sepsis syndrome. In: Mandell GL, Bennett JE, Dolin R, editors. Principles and Practice of Infectious Diseases. Philadelphia: Churchill livingstone; 2005. pp. 910–920.
18. Remington JS, Klein JO. Bacterial sepsis and meningitis. Infectious diseases of the fetus and newborn infant. Philadelphia: W.B.Saunders Company; 2001.
19. Brun-Buisson C. The epidemiology of the systemic inflammatory response. Intensive Care Med. 2000;26:S74–S79.
20. Black S, Kushner I, Samols D. C-reactive protein. Minireview. J Biol Chem. 2004;279:48487–48490.
21. Ng P. Diagnostic markers of infection in neonates. Arch Dis Child Fetal Neonatal Ed. 2004;89:229–235.

22. Weinschenk NP, Farina A, Bianchi DW. Premature infants respond to early-onset and late onset sepsis with leukocyte activation. J Pediatr. 2000;137:345–350.
23. Chiesa C, Panero A, Rossi N. Reliability of procalcitonin concentrations for the diagnosis of sepsis in critically ill neonates. Clin Infect Dis. 1998;26:664–672.
24. Vincent JL. Procalcitonin: The marker of sepsis? Crit Care Med. 2000;28:1226–1228.
25. Athhan F, Akagunduz B, Genel F, Bak M, Can D. Procalcitonin: A marker of neonatal sepsis. J Trop Pediatr . 2002;48:10–14.
26. Carrol ED, Newland P, Riordan FAI, Thomson APJ, Curtis N, Hart CA. Procalcitonin as a diagnostic marker of meningococcal disease in children presenting with fever and a rash. Arch Dis Child. 2002;86:282–285.
27. Carrol ED, Thomson AP, Hart CA. Procalcitonin as a marker of sepsis. Int J Antimicrobe Agents. 2002;20:1–9.
28. Schuetz P, Albrich W, Mueller B. Procalcitonin for diagnosis of infection and guide to antibiotic decisions: past, present and future. BMC Med 2011;9:107
29. Ugarte H, Silva E, Mercan D, De Mendonça A, Vincent JL. Procalcitonin used as a marker of infection in the intensive care unit. Crit Care Med 1999;27:498–504.
30. Hansson LO, Lindquist L. C-reactive protein: its role in the diagnosis and follow-up of infectious diseases. Curr Opin Infect Dis 1997;10:196–201.