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Original Article

Clinical and Laboratory Features of Culture-positive Neonatal Sepsis: A 5-year Single-center Experience at Tertiary Neonatal Intensive Care Unit in Turkey

Kültür Pozitif Neonatal Sepsisin Klinik ve Laboratuvar Özellikleri: Türkiye'de Üçüncü Düzey Bir Yenidoğan Yoğun Bakım Ünitesindeki 5 Yıllık Deneyim

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ABSTRACT

Objective: Neonatal sepsis is an important cause of neonatal mortality and morbidity, especially in low birth-weight premature babies. This study aimed to examine the clinical and laboratory features of cases with culture-positive neonatal sepsis.

Method: Medical records of 233 newborn infants with culture-positive sepsis among 4241 hospitalized patients between January 2013 and December 2017 were reviewed. Demographic and clinical data of these patients were retrospectively recorded.

Results: The majority of patients was extremely and moderately preterm infants (39.1% vs. 11.6%). These patients had a history of invasive mechanical ventilation (74.2%) or central catheterization (26.9%). The mostly isolated pathogens (56.2%) were Gram-negative bacteria, especially *Klebsiella pneumoniae* in 67 (28.8%) cases. Post-hoc test showed a statistically significant difference in the incidence rates of leukopenia between patients infected with Gram-positive, Gram-negative bacteria and fungi (12.3%, 16.8% and 17.2%, respectively) (p=0.021). Patients who developed leukopenia (n=36, 15.5%) had a higher mortality rate compared to those with leukocytosis (n=50, 21.5%) (72.2% vs. 50%, p<0.001). The duration of total parenteral nutrition was found to be a significant risk factor in terms of mortality (p=0.015).

Conclusion: Prolonged parenteral nutrition is an important risk factor for mortality in low-birth weight newborns and those with sepsis. It is noteworthy that the mortality rate is higher in newborns with sepsis who developed leukopenia and neutropenia.

Keywords: Newborn, microorganism, risk factors, sepsis, prognosis

ÖZ

Amaç: Neonatal sepsis yenidoğanda, özellikle de düşük doğum ağırlıklı prematüre bebeklerde önemli bir mortalite ve morbidite nedenidir. Bu çalışmada kültür pozitif neonatal sepsisli hastaların klinik ve laboratuvar özelliklerinin araştırılması amaçlandı.

Yöntem: Ocak 2013 ile Aralık 2017 tarihleri arasında hastanemizde yatan 4241 hasta arasında kültür pozitif sepsis tanısı alan 233 yenidoğan bebeğin tıbbi kayıtları incelendi. Bu hastaların demografik ve klinik verileri geriye dönük olarak kaydedildi.

Bulgular: Hastaların çoğunluğunu ileri derecede ve orta derecede prematüre bebekler oluşturmaktaydı (%39,1 vs. %11,6). Bunların %74,2'sinde invaziv mekanik ventilasyon, %26,9'unda santral kateterizasyon öyküsü vardı. En çok izole edilen patojenler (%56,2) Gram-negatif bakteriler, özellikle *Klebsiella pneumoniae* (n=67, %28,8) idi. Post-hoc analizi Gram-pozitif ve Gram-negatif bakteriler ile mantarlar arasında lökopeni yönünden istatistiksel olarak anlamlı bir fark olduğunu (sırasıyla %12,3, %16,8 ve %17,2) gösterdi (p=0,021). Lökopeni gelişen hastalarda (n=36, %15,5) ölüm oranı, lökositoz gelişen hastalara (n=50, %21,5) göre daha yüksekti (%72,2 vs. %50, p<0,001). Total parenteral beslenme süresinin mortalite açısından anlamlı risk faktörü olduğu belirlendi (p=0,015).

Sonuç: Uzun süreli parenteral beslenme, düşük doğum ağırlıklı ve septik yenidoğanlarda mortalite açısından önemli bir risk faktörüdür. Lökopeni ve nötropeni gelişen septik yenidoğanlarda ölüm oranının daha yüksek olması dikkat çekicidir.

Anahtar kelimeler: Yenidoğan, mikroorganizma, risk faktörleri, sepsis, prognoz

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INTRODUCTION

Neonatal sepsis is a clinical syndrome characterized by systemic symptoms and signs of infection in the first month of life, whether or not a specific microorganism was isolated in blood culture[1]. It has incidence rates ranging from 15/1000 to 49-170/1000, especially in very-low- birth-weight infants (VLBW) (<1500 g). Although perinatal and neonatal care has been improved thanks to recent medical and technological advances, many challenges still remain in the diagnosis and management of neonatal infections. Diagnosis of neonatal sepsis is difficult in some cases, particularly in preterm infants, because of the prevalence of sepsis-like non-infectious conditions and the lack of optimal diagnostic tests. These conditions will cause unnecessary use of both broad-spectrum antibiotics and prolonged treatment with empirical antibiotics which is associated with adverse outcomes and increase in antimicrobial resistance rates[2,3].

Neonatal sepsis is one of the leading causes of neonatal mortality and an important public health problem, especially in developing countries. According to the estimates made by the World Health Organization for 195 countries, neonatal bacterial infections cause the death of approximately 680,000 newborns, which corresponds nearly one quarter of all neonatal deaths[4]. Given the higher incidence and mortality rates of neonatal sepsis, especially in preterm infants, and its long-term consequences on growth and development, efforts to reduce infection rates in this vulnerable population appear to be one of the most important interventions in neonatal care. Therefore, in order to draw attention to the importance of neonatal sepsis, this retrospective study was conducted to examine the incidence, risk factors, clinical and laboratory features of culture-positive cases with neonatal sepsis that were referred to a tertiary neonatal intensive care unit (NICU) over four years, and the effects of clinical and laboratory parameters as well as the given treatments on mortality and morbidity.

MATERIALS and METHODS

Before starting the study, study approval was obtained from the Ethics Committee of Fırat University (decision no: 03, date: 19.01.2016). Among 241 newborns hospitalized in the Medical Faculty Hospital NICU of Fırat University between January 1, 2013 and December 31, 2017, 233, infants diagnosed with culture-positive sepsis were included in the study. Demographic data (gestational age, birth weight, gender, mode of delivery, Apgar scores), invasive procedures (tube thoracostomies, peripheral insertion of central venous catheters, umbilical catheterization and mechanical ventilation), and surgical procedures performed, length of hospitalization, duration of parenteral nutrition, laboratory test results [complete blood count, C-reactive protein, cerebrospinal fluid (CSF) analyzes, if any], reports of diagnostic imaging modalities (echocardiography, transfontanelle and abdominal ultrasonography, and cranial magnetic resonance imaging), complications and patient outcomes were evaluated retrospectively.

Patients with suspected sepsis were evaluated according to the "Töllner sepsis scoring system". Accordingly, <5 points were evaluated as "no suspected sepsis", 5-10 points as "suspected sepsis" and ≥10 points as "probable sepsis"[5]. Among the cases with "probable sepsis", the patients were considered to have "clinical sepsis" and "culture-positive sepsis" if a causative agent could not, and could be isolated in their blood cultures, respectively[6]. Isolation of coagulase-negative streptococci (CoNS) was accepted as contamination, except in cases of clinical sepsis characterized by symptoms of hyperthermia, hypothermia, apnea, or bradycardia and also excluding patients whose two or multiple blood cultures obtained during the same study period demonstrated growth of CoNS on separate occasions or patients with an intravascular line whose at least one blood culture revealed the presence of CoNS despite appropriate antibiotherapy, as well[7] .

According to the onset time of neonatal sepsis, early-onset neonatal sepsis (EOS) was defined as sepsis occurring at ≤72 h of life and late-onset sepsis (LOS) was defined as sepsis occurring 72 h after birth. In accordance with our treatment protocol of our unit, until the antibiogram results were obtained, empirical antibiotherapy was started with ampicillin + amikacin (or gentamicin) for patients with suspected EOS, while vancomycin + amikacin (or ceftazidime) was used as initial antibiotherapy for cases with suspected LOS[8].

Preterm birth refers to all deliveries occurring before $37^{0/7}$ gestational weeks; and extremely preterm (<28 weeks), very preterm ($28^{+0/7}$ -$31^{+6/7}$ weeks), moderately preterm ($32^{+0/7}$-$33^{+6/7}$ weeks), and late preterm births ($34^{+0/7}$-$36^{+6/7}$ weeks) are classified as preterm births. Term births refer to deliveries occurred between $37^{+0/7}$-$42^{+0/7}$ gestational weeks, and post-term births refer to any delivery occurring after $42^{+0/7}$ gestational weeks. According to the weight of a baby at birth, birth weights <1000 g was defined as extremely low birth-weight

(ELBW), 1000-1500 g as very low birth-weight (VLBW), 1500-2500 as low birth-weight (LBW), and >2500 g as normal birth-weight (NBW)[9,10].

Hemoglobin (Hb) levels lower than the normal range for birth weight and postnatal age were defined as "anemia"[11]. Platelet counts <150.000/mm^3, and >450.000/mm^3 were defined as thrombocytopenia "and thrombocytosis, respectively[12]. White blood cell (WBC) counts of 6.000-30.000/mm^3 in the first 24 hours of life and 5.000-20.000/mm^3 in the postnatal >3 days were considered to be within normal range[13]. Neutropenia was defined as an absolute neutrophil count (ANC) below 1.5×10^9/L (1500/mm^3)[14].

Bronchopulmonary dysplasia was defined as the condition where the oxygen requirement still continues at the postconceptional 36th week or during discharge in babies with a gestational age of <32 weeks, and between the postnatal 28-56th days in babies with a >32-week gestational age or at discharge[15]. Patients with suspected necrotizing enterocolitis were evaluated according to the Modified Bell staging[16]. In addition, patients were screened for retinopathy of prematurity at appropriate postconceptional weeks[17].

Prelabor rupture of the membranes (PROM) refers to rupture of the fetal membranes prior to the onset of regular uterine contractions. It may occur at term (≥37^{+0} gestational weeks) or preterm (<37^{+0} gestational weeks) (preterm PROM)[18].

Complete blood count analysis was performed with the ADVIA 2120i analyzer (Siemens AG, Erlangen, Germany). Approximately 0.5-1 mL of venous blood sample was inoculated into pediatric BACTEC culture media which was placed in the oven of the BACTEC 9240 hemoculture device (Becton Dickinson, USA). The samples were checked for bacterial growth every day. Antibiotic susceptibility tests were performed for the isolated microorganisms. The culture media kept in an oven for at least 7 days without any growth of pathogenic microorganism was considered to be culture negative. Micro-C-reactive protein (CRP) measurements were made using the QuikRead go® instant diagnostic system (Orion, Finland) installed in our unit.

Statistical Analysis

For data analysis, the 22.0 version of the SPSS for Windows; statistical software program was used. Data were shown as mean ± standard deviation for normally distributed data, and median (maximum and minimum) values for non-normally distributed continuous variables, while nominal variables were expressed as numbers (n), and percentages (%). Student's t-test was used to compare mean, and Mann-Whitney U test to compare median values, while for the comparison of percentage values chi-square test was employed. Non-parametric multiple comparison tests included in one-way analysis of variance (ANOVA) were used to determine the conditions that caused the difference between groups. A p-value <0.05 was considered statistically significant.

RESULTS

A total of 4241 patients were hospitalized in the NICU of our hospital during the study period. Accordingly, the prevalence of blood culture-positive neonatal sepsis was calculated as 5.5 percent. The majority of the patients (67.8%) were low birth-weight babies. The mean birth-weight was 1999.72±905.41 g (range, 550 g-4200 g). According to the gestational age, the majority of the patients were extremely/very preterm and moderately preterm infants (39.1% vs. 11.6%). The mean gestational age of the patients was 33.13±4.67 weeks (range, 24-42 weeks).

The median 5th minute Apgar score was 8 in 171 patients whose data could be obtained from their medical records. There was no significant difference between EOS and LOS cases in terms of median Apgar scores. Respiratory distress syndrome (RDS) was present in 19 (22%) cases with EOS and 47 (31.9%) cases with LOS. The mean duration of total parenteral nutrition (TPN) was 15.3±14.63 days (range, 0-85 days). Cardiac pathology was detected on echocardiography in 76 (32.6%) patients. Main cardiac defects were patent ductus arteriosus (PDA) (n=51, 67.1%), ventricular septal defect (n=14, 18.4%), atrial septal defect (n=7, 9.2%) and atrioventricular septal defect (n=4, 5.3%). Medical PDA closure treatment was applied to 21 (41.2%) of those patients diagnosed with PDA. Demographic and clinical characteristics of the patients are summarized in Table 1.

Table 1. Demographic and clinical characteristics of the patients

Gender	**n (%)**
Male	125 (53.6)
Female	108 (46.4)
Type of birth	**n (%)**
Cesarean section	172 (73.8)
NSVY	61 (26.2)
Gestational age	**n (%)**
Extremely/very preterm babies	91 (39.1)
Moderately preterm babies	27 (11.6)
Late preterm babies	39 (16.7)

Table 1. Continued	
Gestational age	**n (%)**
Early term babies	9 (3.9)
Full-term babies	66 (28.3)
Post-term babies	1 (0.4)
Birth weight	**n (%)**
Normal birth-weight babies	75 (32.2)
Low birth-weight babies	73 (31.3)
Very- low- birth-weight babies	50 (21.5)
Extremely low- birth-weight babies	35 (15)
Onset time of neonatal sepsis	**n (%)**
Early-onset neonatal sepsis	86 (36.9)
PROM	20/86 (23.3)
Late-onset neonatal sepsis	147 (63.1)
Respiratory distress syndrome	**n (%)**
Yes	66 (28.3)
Mechanical ventilation	**n (%)**
Invasive	173 (74.2)
Non-invasive	21 (9.1)
Thoracic tube insertion	**n (%)**
Yes	15 (6.4)
Surgical operation	**n (%)**
Yes	15 (6.4)
Central catheterization	**n (%)**
Yes	58 (24.9)
TPN	**n (%)**
Yes	211 (90.6)
Lumbar puncture	**n (%)**
Yes	27 (11.6)
Meningitis	6/27 (22.2)
Complications	**n (%)**
Hydrocephalus	17 (7.3)
ARF	12 (5.2)
Cholestasis	11 (4.7)
PVL	9 (3.9)
GMH	8 (3.4)
NEC	8 (3.4)
BPD	7 (3)
Outcomes	**n (%)**
Survival without sequelae	107 (45.9)
Death	102 (43.8)
Survival with sequelae	24 (10.3)

NSVY: Normal spontaneous vaginal delivery, PROM: Prelabor rupture of the membranes[18], ARF: Acute renal failure, PVL: Periventricular leukomalacia, GMH: Germinal matrix hemorrhage, BPD: Bronchopulmonary dysplasia, NEC: Necrotizing enterocolitis

The most common pathogens in blood culture were Gram-negative bacteria (n=131, 56.2%), Gram-positive bacteria (n=73, 31.3%), and fungi (n=29, 12.4%). The most common pathogens in both EOS and LOS cases were Gram-negative bacteria (61.6% vs. 53.1%). There was no statistically significant difference between the cases with EOS and LOS in terms of distribution of causative pathogens including Gram-positive bacteria and fungal strains (p=0.453). The most common pathogens in blood cultures of patients diagnosed with neonatal sepsis was *K. pneumoniae* (n=67, 28.8%). Causative pathogens isolated from blood cultures of the septic patients are given in Table 2. Of the patients with accompanying meningitis, 2 (33.3%) had EOS and 4 (66.6%) had LOS. There was no statistically significant difference between the cases with EOS and LOS in terms of accompanying meningitis (p=0.458). The most commonly isolated pathogen in CSF culture media was *K. pneumoniae* (50%). Similarly, *K. pneumoniae* was isolated most frequently (30%) in urine cultures, (30%), and 10 (4.3%) patients with positive urine cultures were considered to have urosepsis.

The mean total WBC count of the patients was 12.350±9.067/mm^3 (range, 600-57.790/mm^3). Gram-positive bacteria (12.3%), Gram-negative bacteria (16.8%) and fungi (17.2%) caused leukopenia in respective percentages of patients, and the post-hoc test revealed the presence of a statistically significant difference between them (p=0.021). Twenty-six (72.2%) out of 36

Table 2. Causative pathogens isolated from blood cultures		
Pathogens causing infection	**n**	**%**
Klebsiella pneumoniae	67	28.8
Acinetobacter baumannii	32	13.7
CoNS	31	13.3
Staphylococcus haemolyticus	24	10.3
Escherichia coli	18	7.7
Candida parapsilosis	16	6.9
Candida albicans	13	5.6
Serratia marcescens	11	4.7
Streptococcus pneumoniae	11	4.7
MRSA	5	2.1
Stenotrophomonas maltophilia	3	1.3
Pseudomonas aeruginosa	1	0.4
Proteus mirabilis	1	0.4
Total	233	100

CoNS: Coagulase-negative staphylococci, MRSA: Methicillin-resistant *S. aureus*

(15.5%) patients with leukopenia, and half of the patients with leukocytosis (n=50, 21.5%) died, with a statistically significant difference between groups regarding mortality rates (p=0.001).

The mean ANC was 5.620±6.758/mm^3 (range, 90-48.270/mm^3). Gram-positive bacteria (17.8%), Gram-negative bacteria (19.1%), and fungi (24.1%) caused neutropenia in indicated percentages of patients, without any statistically significant difference between them (p=0.762). *Stenotrophomonas maltophilia* was the leading (100%) cause of neutropenia among all pathogens. Twenty-five (60%) out of 45 (19.3%) patients with neutropenia died (p=0.024). Accordingly, 70.6% of the infants who died due to sepsis developed neutropenia (p=0.003).

The mean platelet count of the patients was 132.000±195.676/mm^3 (range, 6000-1.190.000 mm^3). Gram-positive (42.5%), Gram-negative bacteria (58%), and fungal agents (65.5%) caused thrombocytopenia in indicated percentages of patients without any statistically significant difference between them in this respect (p=0.094). Most frequently, fungal agents (65.5%) caused neonatal sepsis, and among them the leading pathogen was *Candida parapsilosis* in 87.5% of the cases. However, *Serratia marcescens* (90.9%) was the leading cause of thrombocytopenia among all pathogens. Accordingly, a statistically significant difference was detected between *Serratia marcescens* and other pathogens in this regard (p=0.01). The mean platelet counts of patients with sepsis due to *Serratia marcescens* was 56.860/mm^3. In addition, among Gram-positive bacteria, most frequently thrombocytopenia was found in cases infected with *Streptococcus pneumoniae* (50%).

The mean Hb value of the septic newborn was 11.4±3.35 g/dL (range, 5.4-22 g/dL), while in 188 (80.7%) patients priorly CRP-positivity was detected.

The mean age at diagnosis of sepsis was 17.24±16.4 days (range, 0-85 days). Most of the patients (n=210, 90.1%) had used antibiotherapy before the development of sepsis and while 23 (9.9%) patients had not. The mean duration of antibiotherapy during sepsis attack was 22.5±12.5 days (range, 1-65 days). The mean total hospital stay was 39.3±32.1 days. Any statistically significant difference was not detected between patients infected with Gram-positive, Gram-negative bacteria and fungi in terms of length of hospital stay (110.53, 118.06 and 128.48 days, respectively), (p=0.461).

Mortality rates among ELBW (n=24; 68.6%) VLBW (n=27; 54%), LBW (n=22; 30.1%), and NBW (n=29; 38.6%) infants were also estimated. Post-hoc tests showed a statistically significant difference between birth weights and mortality rates. Mortality rates were significantly higher in patients with ELBW and VLBW infants than in LBW and NBW infants (p=0.02).

Mortality rates according to gestational ages were 58.2% (n=53) in extremely/very preterm, 25.9% (n=7) in moderately preterm, 38.5% (n=15) in late preterm, 22.2% (n=2) in early term and 37.3% (n=25) in term infants. The impact of gestational age on mortality rates was not statistically significant (p=0.062). Similarly, there was no statistically significant difference in mortality rates between patients with and without RDS (p=0.32). In addition, average mortality rate was statistically significantly higher (n=13, 61.9%) in patients who underwent medical PDA closure (p=0.036).

The mortality rate was statistically significantly higher (70%) in patients who had central venous catheter (p=0.01). The total duration of TPN in patients who died due to sepsis (n=102) was 16.48±15.7 days (range, 0-85 days), while it was 14.38±13.7 days (range, 0-65) in survivors (n=131) (p=0.015).

Sixty (45.8%) patients with Gram-negative, 24 (32.9%) patients with Gram-positive, and 18 (62.1%) patients with fungal sepsis died (p=0.094). However, among pathogenic agents, *Candida parapsilosis* was associated with the highest mortality rate (68.8%). Congenital anomalies were detected in 25 (24.5%) of the deceased patients.

Cranial MR images obtained in 173 (74.2%) patients, were unremarkable only in 129 (74.6%) cases, while pathological findings were reported for the remaining 44 cases (Table 1). Since the data related to the hearing test results could not be found in the medical records, it was not possible to conclude how many patients developed hearing loss in total. Unfortunately, the hearing test (audiogram) results of all patients could not be obtained.

DISCUSSION

Neonatal sepsis continues to be an important cause of mortality and morbidity among newborn infants. High incidence rates ranging from 1-5/1000 to 49-170/1000 have been reported, especially among LBW infants[3]. Getabelew et al.[19] reported a very high prevalence (77.9%) of "probable sepsis" in Ethiopia. In Taiwan, which is a developing country, its prevalence was reported as 3-9.3% in previous years[20,21]. Hacımustafaoğlu et al.[22]

reported the prevalence of culture-positive nosocomial sepsis as 12% in Turkey. As can be seen, prevalence rates between countries, and NICUs are highly variable according to the development level of the countries. We speculate that the low prevalence rate of neonatal sepsis (5.5%) in our study is a result of good compliance of our patients with infection control measures.

Inverse relationships existed between gestational age, birth weight, mortality and morbidity. As the gestational age and birth weight decrease, the incidence of neonatal sepsis increases inversely. In their study, Harris and Goldman[23] reported the incidence rates of neonatal sepsis in VLBW (15-20%), and ELBW (40%) infants as indicated. Seo et al.[24] indicated the incidence rates of neonatal sepsis as 0.6% in full-term and 16.6% in premature babies born before 28th gestational week. Similarly, approximately two-thirds of the cases included in our study consisted of LBW, VLBW and ELBW infants. Likewise, approximately two-thirds of our cases were preterm infants. Among these, especially preterm VLBW and ELBW babies constituted the highest risk group. These infants are at risk for healthcare-associated infections due to their innate immunodeficiency, exposure to highly invasive procedures and prolonged hospitalizations[1,21,22,24].

EOS is a community-acquired infection transmitted mainly from the mother, while LOS mostly refers to healthcare-associated infections. In a study investigating the epidemiology of neonatal sepsis during the last 10 years in the UK, the prevalence rates of EOS and LOS were found to be 24% and 76%, respectively[25]. Getabelew et al.[19] reported that 65% of their cases in Ethiopia were EOS and 35% of them were LOS. In consistent with the results of the above-mentioned study performed in the UK, infants with EOS, and LOS comprised of 40%, and 60% of our study population, respectively.

Patients hospitalized in the NICU frequently undergo invasive procedures. Central venous catheterization, mechanical ventilation, TPN, and time to start first enteral feeding are independent risk factors for LOS in VLBW infants. In addition, other invasive procedures such as thoracic tube insertion and surgical interventions increase the risk of infection[26,27]. Kung et al.[28] reported that 78% of their cases with neonatal sepsis had undergone tracheal intubation and invasive mechanical ventilation. In addition, thoracic tube insertion, and surgical operations had been performed in approximately 6%, and 15 % of their cases, respectively.

In the study of Osman et al.[29] the most frequently isolated microorganism was CoNS (17.5%), followed by *S. aureus* (12.5%) and *K. pneumoniae* (10%). According to a study by Kara et al.[30] from Eastern Turkey, the mainly isolated pathogenic agents in neonatal sepsis were ConNS (46.1%), *K. pneumoniae* (21.2%), *E. coli* (9.6%), and *A. baumannii* (3.8%). In their study, de Benedetti et al.[31] reported commonly seen pathogens of neonatal sepsis as *K. pneumoniae* (47.5%), *P. aeroginosa* (20%), *E. coli* (10%) and *C. albicans* (10%). Similarly, in our study, approximately 1/3 of the isolated pathogens of neonatal sepsis were *K. pneumoniae*, followed by *A. baumannii* (13.7%) and CoNS (13.3%). Fungal infections were mainly caused by *C. parapsilosis* (6.9%) and *C. albicans* (5.6%). The high prevalence of *K. pneumoniae* in our study may be due to its colonization in the environment and *klebsiella pneumoniae* outbreaks seen in certain periods of time. The fact that CoNS was not the leading pathogen in our study may be due to the acceptance of some isolated pathogens as contaminants. CoNS is a commensal microorganism of the skin, and therefore its isolation in blood culture may reflect contamination if blood samples are obtained appropriately.

Abnormal WBC counts were observed in only 2/3 of the patients at the onset of neonatal sepsis, and in some series, this rate increased to 80-90%[32,33]. Wu et al.[21] reported leukocytosis or leukopenia in 27% of their cases with neonatal sepsis. In our study, leukopenia, and leukocytosis were detected in 15.5%, and 21.5% of the cases, respectively. Although the most common cause of leukopenia was fungal infections, it was determined that the specific agent was mostly streptococci. In the bone marrow, both leukopenia and thrombocytopenia can occur due to cessation of maturation and insufficient bone marrow supply of progenitors[34]. Hornik et al.[33] reported a high odds ratio (5.38%) and specificity rate (73.7%) together with a sensitivity rate (0.3-54.5%) for leukopenia in neonatal sepsis. These data show that WBC count has an important place in the diagnosis of sepsis, but it will not be sufficient diagnostic criterion on its own.

In addition to leukocytosis or leukopenia, low ANC and high I/T neutrophil ratios help to diagnose sepsis. Hornik et al.[33] reported high odds ratios (6.84-7.97), high specificity (99.9% and >99.8%), and low sensitivity rates (0.3-54.5%) for low ANS and high I/T neutrophil ratios. In the present study, there was a statistically insignificant difference between Gram-positive and Gram-negative bacteria and fungi in terms of causing neutropenia. Similar to cases with leukopenia, the most common

pathogens causing neutropenia were fungal strains and *S.maltophilia* was isolated in all cases of neutropenia (100%).

Thrombocytopenia is a non-specific finding that occurs late in neonatal sepsis. In a study by Lim et al.[35] in which they investigated the prevalence of neonatal sepsis and the distribution of pathogens in VLBW infants, they found thrombocytopenia at a rate of 50% in EOS and 47.3% in LOS; however, thrombocytopenia -though not statistically significant- was observed more frequently in fungal sepsis. Arif et al.[36] showed that thrombocytopenia was more severe in Gram-negative septicemia than in Gram-positive septicemia. In our study, although fungi, Gram-negative and Gram-positive microorganisms were causative pathogens in cases with thrombocytopenia without any statistically significant difference among them in terms of incidence rates. Among all pathogens, *S. marcessens* was the leading cause of thrombocytopenia in neonatal sepsis.

Long-term hospitalization of LBW premature babies in the NICU increases the risk of neonatal sepsis. In a study conducted in Spain, the average LOS of patients with nosocomial infections in the NICU was reported to be 30 days[37]. Hacımustafaoğlu et al.[22] reported that the mean length of stay in the NICU of patients diagnosed with nosocomial infection was 67 days. The mean duration of hospitalization of our patients was 39.3±32.1 days. Although the longest hospital stay was in sepsis caused by fungi followed by Gram-negative and Gram-positive bacteria, any statistical difference was not found among them in terms of LOS.

Parenteral nutrition is an important risk factor for the development of nosocomial infections. Sohn et al.[38] and Kawagoe et al.[39] reported that TPN increased the risk of neonatal sepsis by 5.7, and 4 times, respectively. In our study, a significant relationship was found between the duration of TPN and the development of sepsis, which is consistent with the literature. In addition, mortality rates were higher in patients with central catheters and on long-term TPN.

The mortality rates of neonatal sepsis generally range from 10-50%[40,41]. As a result of the 6-year analysis by Wu et al.[21], the mortality rates of EOS and LOS were 10% and 7%, while Martius et al.[42] a mortality rate of 10.5% for neonatal sepsis. Şahin and Şahin[43], reported that 7 (29%) of their 24 patients with neonatal sepsis died. However, the high mortality rate of 44% in our patients may be due to the inclusion of only culture-proven sepsis cases in this study and the fact that only cases with poor clinical condition were referred to our reference center hospital. In addition, multi-drug resistance may explain our high mortality rate compared to previous years.

Mortality and morbidity rates in LBW preterm infants are inversely proportional to gestational age and birth-weight[44]. About half of our patients were ELBW infants, and 60% of these infants died.

We have revealed statistically significant effect of birth-weight on mortality. Mortality rates in Gram-negative septicemia and fungal infections are higher compared to Gram-positive septicemia[45]. Similarly, sepsis caused by fungi, Gram-negative bacteria and Gram-positive bacteria, respectively, although not statistically significant, had the worst prognosis among our cases. In addition, patients with leukopenia and neutropenia had a worse prognosis compared to patients with leukocytosis.

Study Limitations

Limitations of our study were i) the prevalence of neonatal sepsis could not be estimated precisely because cases with clinical sepsis were not included in the study; ii) Some data concerning CRP levels, Apgar scores and blood gas analyzes of some patients could not be obtained from their medical records; iii) the prevalence of cases with meningitis could not be determined due to extremely low rates of lumbar puncture which could not be performed at an optimal frequency probably because of the hemodynamic instability of the patients and/or the severity of the prematurity not allowing the procedure; iv) since micro-CRP, one of the infection markers, is studied in our unit, patients are not routinely asked for PCT examination; v) Results of interleukin-6 assay were not available because this test is not routinely performed in our hospital.

CONCLUSION

In conclusion, neonatal sepsis is an important health problem of the newborn infants, which causes prolonged hospitalization, mortality and morbidity. The prevalence of culture-positive neonatal sepsis in NICU is 5.5%. Preterm birth and LBW are crucial risk factors for neonatal sepsis and mortality. *K. pneumoniae* is one of the leading pathogens causing neonatal sepsis. However, *C. parapsilosis* is the pathogen with the highest mortality in neonatal sepsis. Fungal strains and Gram-negative bacteria cause leukopenia more frequently. However, *S. marcescens* is the most common pathogen causing thrombocytopenia. Mortality rate is higher in septic neonates who develop leukopenia and neutropenia. Prolonged parenteral nutrition increases the risk of sepsis and mortality.

Ethics

Ethics Committee Approval: Study approval was obtained from the Ethics Committee of Fırat University (decision no: 03, date: 19.01.2016).

Informed Consent: Retrospective study.

Author Contributions

Surgical and Medical Practices: M.A., I.Ö., S.B., N.H., E.T., Concept: M.A., I.Ö., E.T., Design: M.A., I.Ö., E.T., Data Collection or Processing: M.A., I.Ö., A.O., Analysis or Interpretation: M.A., I.Ö., A.O., S.B., N.H., Literature Search: M.A., I.Ö., A.O., S.B., N.H., Writing: M.A., I.Ö., A.O., N.H.

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REFERENCES

1. Shane AL, Sánchez PJ, Stoll BJ. Neonatal sepsis. Lancet. 2017;390(10104):1770-80. doi: 10.1016/S0140-6736(17)31002-4.
2. Zea-Vera A, Ochoa TJ. Challenges in the diagnosis and management of neonatal sepsis. J Trop Pediatr. 2015;61(1):1-13. doi: 10.1093/tropej/fmu079.
3. Shah BA, Padbury JF. Neonatal sepsis: an old problem with new insights. Virulence. 2014;5(1):170-8. doi: 10.4161/viru.26906.
4. Liu L, Oza S, Hogan D, Chu Y, Perin J, Zhu J, et al. Global, regional, and national causes of under-5 mortality in 2000-15: an updated systematic analysis with implications for the Sustainable Development Goals. Lancet. 2016;388(10063):3027-35. doi: 10.1016/S0140-6736(16)31593-8.
5. Töllner U. Early diagnosis of septicemia in the newborn. Clinical studies and sepsis score. Eur J Pediatr. 1982;138:331-7. doi: 10.1007/BF00442511.
6. Aydin M, Barut S, Akbulut HH, Ucar S, Orman A. Application of flow cytometry in the early diagnosis of neonatal sepsis. Ann Clin Lab Sci. 2017;47:184-90.
7. Garner JS, Jarvis WR, Emori TG, Horan TC, Hughes JM. CDC definitions for nosocomial infections, 1988. Am J Infect Control. 1988;16(3):128-40. doi: 10.1016/0196-6553(88)90053-3.
8. Satar M, Arısoy AE, Çelik İH. Turkish Neonatal Society guideline on neonatal infections-diagnosis and treatment. Turk Pediatri Ars. 2018;53(Suppl 1):S88-100. doi: 10.5152/TurkPediatriArs.2018.01809
9. Quinn JA, Munoz FM, Gonik B, Frau L, Cutland C, Mallett-Moore T, et al. Preterm birth: Case definition & guidelines for data collection, analysis, and presentation of immunisation safety data. Vaccine. 2016;34(49):6047-56. doi: 10.1016/j.vaccine.2016.03.045.
10. Hamilton BE, Martin JA, Osterman MJ. Births: Provisional Data for 2021. National Center for Health Statistics. National Vital Statistics System, Vital Statistics Rapid Release Program, no 20. Hyattsville, MD. National Center for Health Statistics. 2022.
11. Henry E, Christensen RD. Reference Intervals in Neonatal Hematology. Clin Perinatol. 2015;42(3):483-97. doi: 10.1016/j.clp.2015.04.005.
12. Patel RM, Josephson C. Neonatal and pediatric platelet transfusions: current concepts and controversies. Curr Opin Hematol. 2019;26(6):466-72. doi: 10.1097/MOH.0000000000000542.
13. Schelonka RL, Yoder BA, desJardins SE, Hall RB, Butler J. Peripheral leukocyte count and leukocyte indexes in healthy newborn term infants. J Pediatr. 1994;125(4):603-6. doi: 10.1016/s0022-3476(94)70018-4.
14. Newburger PE, Dale DC. Evaluation and management of patients with isolated neutropenia. Semin Hematol. 2013;50(3):198-206. doi: 10.1053/j.seminhematol.2013.06.010.
15. Hwang JS, Rehan VK. Recent Advances in Bronchopulmonary Dysplasia: Pathophysiology, Prevention, and Treatment. Lung. 2018;196(2):129-38. doi: 10.1007/s00408-018-0084-z.
16 Battersby C, Santhalingam T, Costeloe K, Modi N. Incidence of neonatal necrotising enterocolitis in high-income countries: a systematic review. Arch Dis Child Fetal Neonatal Ed. 2018;103(2):F182-9. doi: 10.1136/archdischild-2017-313880.
17. Bancalari M A, Schade R. Retinopatía del prematuro: Actualización en detección y tratamiento [Retinopathy of the premature: Update in screening and treatment]. Rev Chil Pediatr. 2020;91(1):122-30. doi: 10.32641/rchped.v91i1.1079.
18. Hannah ME, Ohlsson A, Farine D, Hewson SA, Hodnett ED, Myhr TL, et al. Induction of labor compared with expectant management for prelabor rupture of the membranes at term. TERMPROM Study Group. N Engl J Med. 1996;334(16):1005-10. doi: 10.1056/NEJM199604183341601.
19. Getabelew A, Aman M, Fantaye E, Yeheyis T. Prevalence of Neonatal Sepsis and Associated Factors among Neonates in Neonatal Intensive Care Unit at Selected Governmental Hospitals in Shashemene Town, Oromia Regional State, Ethiopia, 2017. Int J Pediatr. 2018;2018:7801272. doi: 10.1155/2018/7801272.
20. Jiang JH, Chiu NC, Huang FY, Kao HA, Hsu CH, Hung HY, et al. Neonatal sepsis in the neonatal intensive care unit: characteristics of early versus late onset. J Microbiol Immunol Infect. 2004;37(5):301-6.
21. Wu JH, Chen CY, Tsao PN, Hsieh WS, Chou HC. Neonatal sepsis: a 6-year analysis in a neonatal care unit in Taiwan. Pediatr Neonatol. 2009;50(3):88-95. doi: 10.1016/S1875-9572(09)60042-5.
22. Hacımustafaoğlu M, Çelebi S, Köksal N, Kavurt S, Özkan H, Çetinkaya M, et al. Nosocomial infections in neonatology clinic and neonatal intensive care unit. Turk Arch Ped. 2011;46:293-8. doi: 10.4274/jcp.36025.
23. Harris JS, Goldman DA. Infections acquired in the nursery; epidemiyoloji and control. In: Remington JS, Klein JO (Eds). Infectious Diseases of the Fetus and Newborn Infant. Philadelphia: WB Saunders 2001:1371-418.
24. Seo K, McGregor JA, French JI. Preterm birth is associated with increased risk of maternal and neonatal infection. Obstet Gynecol. 1992;79(1):75-80.
25. Cailes B, Kortsalioudaki C, Buttery J, Pattnayak S, Greenough A, Matthes J, et al. Epidemiology of UK neonatal infections: the neonIN infection surveillance network. Arch Dis Child Fetal Neonatal Ed. 2018;103(6):F547-53. doi: 10.1136/archdischild-2017-313203.
26. Sundaralingam A, Bedawi EO, Harriss EK, Munavvar M, Rahman NM. The frequency, risk factors, and management of complications from pleural procedures. Chest. 2022;161:1407-25. doi: 10.1016/j.chest.2021.11.031.
27. García H, Torres-Gutiérrez J, Peregrino-Bejarano L, Cruz-Castañeda MA. Factores de riesgo asociados a infección nosocomial (IN) en

una Unidad de Cuidados Intensivos Neonatales (UCIN) de tercer nivel. Gac Med Mex. 2015;151:711-9.

28. Kung YH, Hsieh YF, Weng YH, Lien RI, Luo J, Wang Y, et al. Risk factors of late-onset neonatal sepsis in Taiwan: A matched case-control study. J Microbiol Immunol Infect. 2016;49(3):430-5. doi: 10.1016/j.jmii.2013.10.001.
29. Osman AS, Awadallah MG, Tabl HAEM, Abed NT, Goudah ESS. Presepsin as a novel diagnostic marker in neonatal septicemia. Egypt J Med Microbiol. 2015;24:21-6.
30. Kara H, Ertuğrul S, Gündoğuş N, Akpolat N, Özmen Ö. [An evaluation of patients with culture-proven sepsis in a neonatal intensive care unit]. Dicle Medical Journal. 2015;42:355-60. doi: 10.5798/diclemedj.0921.2015.03.0589.
31. de Benedetti F, Auriti C, D'Urbano LE, Ronchetti MP, Ravà L, Tozzi A, et al. Low serum levels of mannose binding lectin are a risk factor for neonatal sepsis. Pediatr Res. 2007;61:325-8. doi: 10.1203/pdr.0b013e318030d12f.
32. Benuck I, David RJ. Sensitivity of published neutrophil indexes in identifying newborn infants with sepsis. J Pediatr. 1983;103:961-3. doi: 10.1016/s0022-3476(83)80731-8.
33. Hornik CP, Benjamin DK, Becker KC, Benjamin DK Jr, Li J, Clark RH, et al. Use of the complete blood cell count in early-onset neonatal sepsis. Pediatr Infect Dis J. 2012;31:799-802. doi: 10.1097/INF.0b013e318256905c.
34. Belok SH, Bosch NA, Klings ES, Walkey AJ. Evaluation of leukopenia during sepsis as a marker of sepsis-defining organ dysfunction. PLoS One. 2021;16:e0252206. doi: 10.1371/journal.pone.0252206.
35. Lim WH, Lien R, Huang YC, Chiang MC, Fu RH, Chu SM, et al. Prevalence and pathogen distribution of neonatal sepsis among very-low-birth-weight infants. Pediatr Neonatol. 2012;53:228-34. doi: 10.1016/j.pedneo.2012.06.003.
36. Arif SH, Ahmad I, Ali SM, Khan HM. Thrombocytopenia and bacterial sepsis in neonates. Indian J Hematol Blood Transfus. 2012;28:147-51. doi: 10.1007/s12288-011-0118-7.
37. Mireya UA, Martí PO, Xavier KV, Cristina LO, Miguel MM, Magda CM. Nosocomial infections in paediatric and neonatal intensive care units. J Infect. 2007;54:212-20. doi: 10.1016/j.jinf.2006.03.023.
38. Sohn AH, Garrett DO, Sinkowitz-Cochran RL, Grohskopf LA, Levine GL, Stover BH, et al. Pediatric Prevention Network. Prevalence of nosocomial infections in neonatal intensive care unit patients: Results from the first national point-prevalence survey. J Pediatr. 2001;139:821-7. doi: 10.1067/mpd.2001.119442.
39. Kawagoe JY, Segre CA, Pereira CR, Cardoso MF, Silva CV, Fukushima JT. Risk factors for nosocomial infections in critically ill newborns: a 5-year prospective cohort study. Am J Infect Control. 2001;29:109-14. doi: 10.1067/mic.2001.114162.
40. Stoll BJ, Hansen NI, Higgins RD, Fanaroff AA, Duara S, Goldberg R, et al. National Institute of Child Health and Human Development. Very low birth weight preterm infants with early onset neonatal sepsis: the predominance of gram-negative infections continues in the National Institute of Child Health and Human Development Neonatal Research Network, 2002-2003. Pediatr Infect Dis J. 2005;24(7):635-9. doi: 10.1097/01.inf.0000168749.82105.64.
41. Makhoul IR, Sujov P, Smolkin T, Lusky A, Reichman B; Israel Neonatal Network. Pathogen-specific early mortality in very low birth-weight infants with late-onset sepsis: a national survey. Clin Infect Dis. 2005;40:218-24. doi: 10.1086/426444.
42. Martius JA, Roos T, Gora B, Oehler MK, Schrod L, Papadopoulos T, et al. Risk factors associated with early-onset sepsis in premature infants. Eur J Obstet Gynecol Reprod Biol. 1999;85:151-8. doi: 10.1016/s0301-2115(99)00018-4.
43. Şahin Y, Şahin DA. [The role of interleukin-6 and C-reactive protein in the early diagnosis of neonatal sepsis]. Turk Pediatr Arş. 2004;39:171-7. doi: 10.1016/s0301-2115(99)00018-4.
44. Stoll BJ, Hansen N, Fanaroff AA, Wright LL, Carlo WA, Ehrenkranz RA, et al. Late-onset sepsis in very low birth weight neonates: the experience of the NICHD Neonatal Research Network. Pediatrics. 2002;110(2 Pt 1):285-91. doi: 10.1542/peds.110.2.285.
45. Steinbach WJ, Roilides E, Berman D, Hoffman JA, Groll AH, Bin-Hussain I, et al. Results from a prospective, international, epidemiologic study of invasive candidiasis in children and neonates. Pediatr Infect Dis J. 2012;31(12):1252-7. doi: 10.1097/INF.0b013e3182737427.