Review

Neonatal Sepsis

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Abstract

Neonatal sepsis is associated with severe morbidity and mortality in the neonatal period. Clinical manifestations range from subclinical infection to severe local or systemic infection. Neonatal sepsis is divided into three groups as early-onset neonatal sepsis, late-onset neonatal sepsis and very late-onset neonatal sepsis according to the time of the onset. It was observed that the incidence of early-onset neonatal sepsis decreased with intrapartum antibiotic treatment. However, the incidence of late-onset neonatal sepsis has increased with the increase in the survival rate of preterm and very low weight babies. The source of the causative pathogen may be acquisition from the intrauterine origin but may also acquisition from maternal flora, hospital or community. Prematurity, low birth weight, chorioamnionitis, premature prolonged rupture of membranes, resuscitation, low APGAR score, inability to breastfeed, prolonged hospital stay and invasive procedures are among the risk factors. This article reviews current information on the definition, classification, epidemiology, risk factors, pathogenesis, clinical symptoms, diagnostic methods and treatment of neonatal sepsis.

Keywords: Early-onset; late onset; neonatal; sepsis; diagnosis; treatment.

Please cite this article as "Ozmeral Odabasi İ, Bulbul A. Neonatal Sepsis. Med Bull Sisli Etfal Hosp 2020;54(2):142–158".

Neonatal sepsis defines the systemic condition that arises from the bacterial, viral or fungal origin, associated with hemodynamic changes and clinical findings and causing severe morbidity and mortality. Its incidence varies depending on the definition of the case and the population studied and is between 1 and 5 in 1000 live births. The clinical manifestations range from subclinical infection to severe focal or systemic disease. While the infectious agent may arise from intrauterine or maternal flora, it may also be of the hospital or community origin. It is classified as early-onset, late-onset and very late-onset neonatal sepsis according to the time of onset of the findings. While early-onset neonatal sepsis describes cases where clinical manifestations occur in the first three days of life (<72 hours), some researchers consider this limit as the first seven days of life. In connection with this, late-onset neonatal sepsis describes cases diagnosed on 4th-30th days of life or cases diagnosed after the first seven days.[1, 2] Very late-onset neonatal sepsis, on the other hand, describes sepsis cases diagnosed in infants who are hospitalized in the neonatal intensive care unit from the first 30 days of life until discharge. Epidemiological studies related to neonatal sepsis since the early 1980s have shown a decrease in early-onset neonatal sepsis cases, especially with *Group B Streptococcus (GBS)*, with the improvement of obstetric care and the use of intrapartum antibiotic prophylaxis; while they show an increase in late-onset neonatal sepsis associated with increased survival rates and long hospitalization times of premature babies.[3, 4]

Terminology

Suspected sepsis: Regardless of whether there is a clinical symptom or not, the presence of sepsis risk factors in the

baby or findings suggesting sepsis in follow-up.[5]

Clinical sepsis: Clinical and laboratory findings are present, but the failure to show the causative microorganism.[5]

Proven sepsis: Clinical and laboratory findings are present, and demonstration of the pathogenic microorganism in cultures taken from the sterile field.[5]

Epidemiology Research shows that an average of 2.6 million newborns dies every year and three-quarters of these deaths occur in the first week of life.[6, 7] In a study conducted between 2000-2013, data of one hundred and ninety-four countries were evaluated in which the causes of death were investigated in the neonatal period, and the mortality rate due to sepsis was found to be 15%. In this study, it was determined that 2.8 million babies died in the neonatal period and 430.000 of these babies died due to sepsis and severe infections.[8] Neonatal sepsis ranks third among the causes of neonatal death following prematurity and intrapartum-related complications.[9] In the late neonatal period (7-27 days), the most common cause of death was sepsis, with a rate of 37.2%.[8] Neonatal death and sepsis frequency differ between populations. In their reports that Lawn et al. presented epidemiological data, 99% of newborn deaths occurred in low- and middle-income countries and 1% in high-income countries.[7]

The incidence of early-onset neonatal sepsis ranges from 1 to 5 per 1000 live births. This incidence has been shown to decrease with intrapartum antibiotic therapy.[10, 11] However, the incidence of late-onset neonatal sepsis increased with the increase in the survival rate of preterm and very low birth weight infants.[3] The incidence of late-onset neonatal sepsis is reported to vary between 0.61% and 14.2% in hospitalized newborn babies.[2] When classified by birth weight, the rate of early-onset neonatal sepsis in 1000 live births was reported to be 0.57 in babies over 2500 grams, and 10.96 in babies with a birth weight of between 401-1500 grams.[12] The incidence of late-onset neonatal sepsis was reported as 51.2% in infants between 501-750 grams of birth weight, 15-25% in infants below 1500 grams and 1.6% in infants above 2500 grams.[2]

Risk Factors

Risk Factors Associated with the Newborn

The most important risk factor causing sepsis development in the neonatal period is premature birth and low birth weight. Premature babies with low birth weight have a risk of developing sepsis three to ten times higher than full-term babies with normal birth weight.[13] In addition, low levels of transplacental maternal IgG levels in preterm babies are among the risk factors.[13] Fetal distress, low APGAR score, resuscitation of the baby and the multiple pregnancies increase the risk of early-onset sepsis, whereas invasive procedures, such as frequent blood sampling, intubation, mechanical ventilation, catheter/probe insertion, insufficient breastfeeding, long-term parenteral nutrition, low stomach acid and surgical interventions especially increase the risk of late-onset sepsis.[5] Early neonatal sepsis risk factors in developing countries also include inadequate antenatal care, high rate of home birth, unsanitary birth and umbilical cord care practices, and late recognition of conditions that pose a risk of infection in the mother or baby.[14]

Maternal Risk Factors

Chorioamnionitis, premature rupture of membranes (>18 hours), intrapartum maternal fever (>38 °C), delivery earlier than 37 weeks of gestation, maternal *group B streptococcal (GBS)* colonization and other conditions that increase the risk of GBS infection in the newborn (the mother's positive vaginal-rectal GBS screening cultures in the late stages of pregnancy, having a history of GBS-infected baby in a previous pregnancy, detection of GBS positive bacteriuria during pregnancy, positive intrapartum nucleic acid amplification tests for GBS) increases the risk of early neonatal sepsis.[15, 16] In the presence of early membrane rupture and chorioamnionitis, the incidence of early-onset sepsis is 1-3%, i.e., the risk of early neonatal sepsis is increased 10-fold.[17] While early-onset sepsis rate caused by *Group B streptococci* was 2 in 1000 live births and mortality was 50% in the 1960s, today, antibiotic prophylaxis and early treatment reduced morbidity and mortality rates.[18] However, GBS screening and intrapartum antibiotic prophylaxis do not eliminate but decrease the risk of GBS infection. The effectiveness of prophylaxis was determined between 86-89%.[19] In a study where approximately 400.000 babies were evaluated in the United States and the rate of early-onset sepsis was found to be 0.98 per 1000 live births, mothers of 57% of babies diagnosed with early neonatal sepsis were reported to have GBS prophylaxis.[12] CDC (Centers for Disease Control and Prevention) recommends GBS screening during 35,-37, gestational weeks and intrapartum prophylaxis in cases with the indication in preventing early neonatal sepsis.[19]

The incidence of GBS carriers in a study that evaluated 500 women in Turkey in 2016 was discovered as 13.6%.[20] In another study where a total of 150 pregnant women were screened, the prevalence of GBS carriers in pregnant women and frequency of colonization in newborns were evaluated, approximately 32% of the pregnant women and 17.3% of the newborns were found to be colonized with GBS.[21] In another study conducted in our country, 500 pregnant women and newborn babies of these pregnant women were evaluated, and maternal and infant coloniza-

tion rates were found as 9.2% and 1.6%, respectively, and vertical transition rate was determined as 15.2%.[22]

Pathophysiology and Causative Microorganisms

Causative microorganisms of early-onset neonatal sepsis are generally vertically transmitted from the mother. Microorganisms in the mother's birth canal, cervix, vagina, and rectum are known to cause chorioamnionitis by crossing intact or ruptured membranes before or during labor.[23] Nevertheless, severe clinical findings and bacteremia findings starting from birth, especially in babies without rupture in membranes and born by cesarean section, suggest placental transmission.[24] Chorioamnionitis, which is one of the most important risk factors in early-onset neonatal sepsis, is defined as an acute inflammation of fetal membranes and amniotic fluid. It often develops due to the microinvasion of amniotic fluid as a result of prolonged rupture of membranes. Fever, leukocytosis, foul-smelling or intense discharge, abdominal tenderness in the mother and fetal tachycardia are among the clinical findings of chorioamnionitis. However, chorioamnionitis may also present with a pathological laboratory finding without clinical findings.[13] Pathogens that cause sepsis vary according to geographical differences and countries. The most common pathogens in early-onset neonatal sepsis are GBS and *Escherichia coli (E. coli)* when *coagulase-negative staphylococci (CONS)* are excluded. Although some centers consider CONS growth as pathogens for disease, some centers consider this growth as contamination. In studies, cases with the CONS positivity in a single culture were excluded from the study,[12] while cases considered clinically significant in different studies were accepted as pathogens and included in the study.[25] Stoll et al. defined the cases with CONS growth in blood culture in three groups. Patients with CONS growth in two consecutive blood cultures taken within two days, or with CONS growth in single blood culture and C-reactive protein (CRP) elevation within two days after blood cultures were defined as a definitive infection. Patients with CONS growth at blood culture while receiving treatment with vancomycin, oxycycline or a semisynthetic antistaphylococcal agent for at least five days were defined as suspected infection; cases with blood culture positivity without accompanying CRP elevation or use of antibiotics were defined as contamination.[26] In data from the UK, GBS was detected in 58% of early-onset sepsis cases and E.coli in 18%.[25] These rates were found to be in the United States as 43% and 29%, respectively.[12] *Listeria monocytogenes (L. monocytogenes), Group A, C and G streptococci, Streptococcus pneumoniae (S. pneumoniae), Streptococcus viridans (S. viridans), Haemophilus influenzae (H. influenzae), Staphylo-*

coccus aureus (S. aureus), Klebsiella and CONS are less common. However, they are among the causative organisms for early-onset neonatal sepsis factors.[23, 27] In emerging countries, however, gram-negative bacteria *(Klebsiella, Enterobacter, Acinetobacter, E. coli)* are in the foreground.[28] Ureaplasma parvum and Ureaplasma urealyticum are the most common factors isolated from the placenta and amniotic fluid in patients with histologically detected chorioamnionitis; however, colonization of Ureaplasma strains in the respiratory system has been associated with bronchopulmonary dysplasia in preterm infants.[12] In our country, when the different studies are examined, the most common factors are found as K. pneumoniae, S. aureus and CONS.[29–31]

Late-onset neonatal sepsis often shows horizontal transmission from individuals responsible for the care of the baby, from environmental or nosocomial sources. In the case of vertical transmission, early colonization of the baby occurs as an infection in the late period. Metabolic factors, including hypoxia, acidosis, hypothermia, and hereditary metabolic disorders (e.g., galactosemia), are likely to contribute to the risk and severity of neonatal sepsis. These factors are thought to disrupt the host's immune response.[32] When late-onset neonatal sepsis is analyzed, CONS takes the first place as causative organisms in 53.7% -77.9% of cases in developed countries and 35.5% -47.4% of cases in developing countries.[2, 4, 25, 33–35] In the United States, *S. aureus, Candida spp., E. coli, Klebsiella spp., Enterobacter spp.* are listed as the most frequent factors, following CONS. The most frequently detected organisms in Australia are *CONS, S. aureus, Klebsiella spp., Pseudomonas spp., Candida spp., E. coli and Enterobacter spp.*, respectively. In the study by Aksoy et al. from Turkey, *K. pneumoniae* and *S. aureus* were found to be the most common factors in late-onset neonatal sepsis.[30] In the study of Türkmen et al., *S. epidermidis* was the most common agent and Candida was the second.[29] In a study by Özkan et al.[31] in preterm infants, CONS was found to be the most common factor in late-onset neonatal sepsis. In the study of Özdemir et al., *S. aureus* was the most common cause of late sepsis, followed by *K. pneumoniae* and *S. epidermidis*, respectively.[36] CONS was the most frequent factor in the study of Bülbül et al.,[37] in which they evaluated the nosocomial infections in the neonatal intensive care unit, whereas MSSA and *K. pneumonia* were found in the second place.

S. aureus infections are reported to be more frequent, especially in patients with catheters. In a study conducted in the UK, where 117 sepsis episodes with S. aureus growth were evaluated, the central catheter was determined in 50% of the cases.[38] Especially in babies with long-term hospitalization due to prematurity, increased frequency of systemic infections caused by *Candida spp.* is seen. Although there

is a difference between the institutions, *Candida spp.* is reported to be the third most frequent agent of late-onset neonatal sepsis in babies weighing <1500 gr.[13]

Herpes Simplex Virus (HSV), which is often presented with late-onset neonatal sepsis is one of the most common factors when viral factors of neonatal sepsis are considered. Its frequency in the United States has been reported to be 1 in 3200 births.[39] Despite the progress in diagnosis and treatment, it remains important concerning morbidity and mortality. It has been reported that 85% of babies diagnosed with disseminated HSV disease before antiviral treatment and 50% of those with central nervous system involvement died before one year of age. Another viral factor in late-onset neonatal sepsis is enteroviruses. Enterovirus infections can present with nonspecific lethargy, poor nutrition, fever, restlessness, hypoperfusion, jaundice, meningoencephalitis, myocarditis and hepatitis.[40] The frequency of enterovirus in the newborn period is not known exactly.

Diagnostic Methods

Clinical Findings in Neonatal Sepsis

Signs and symptoms are generally non-specific in neonatal sepsis. Therefore, the differential diagnosis is important. While more than one organ or system findings may occur in early-onset neonatal sepsis, signs of infection in late and very late-onset neonatal sepsis may be multisystemic or focal (such as meningitis, pneumonia, omphalitis, osteomyelitis, septic arthritis).[23] Neonatal sepsis can present with groaning, contraction of the accessory muscles of respiration, nasal wing breathing, apnea, cyanosis, tachypnea in the respiratory system; bradycardia/tachycardia, peripheral circulatory disturbance, hypotension, prolonged capillary refill time in the cardiovascular system; nutritional intolerance, difficulty sucking, vomiting, diarrhea, abdominal distention, hepato-splenomegaly, jaundice in the digestive system; sclerema, cutis marmaratus, pustule, abscess, petechiae, purpura in the skin; and lethargy, hypotonicity, sleepiness, weak or high-pitched crying, bulging fontanelle, irritability, convulsion, hypoactivity, body temperature regulation problems and difficulty sucking in the central nervous system.[5, 13, 23, 27]

Laboratory Methods

Blood Culture

The gold standard for the diagnosis of neonatal sepsis is the growth of pathogenic microorganisms in body fluids (blood, urine, cerebrospinal fluid, pleural fluid, peritoneal fluid, joint fluid) that are expected to be sterile. Therefore, the amount of the sample and the method of obtaining

the sample are important. Minimum amount of blood required for blood culture should be 0.5-1 ml. It is recommended to take two different samples, preferably from two different regions.[41] 90% of growth takes place within the first 48 hours.[42] While the growth of the microorganism in blood culture is diagnostic in the neonatal period, the failure to produce it does not exclude the diagnosis.[23, 27] No growth in culture may be related to insufficient sample, mother's antibiotic use, antibiotic dose applied before sampling, low amount of bacteria in the blood or short term bacteremia.[5]

After the area that the blood culture will be taken is cleaned and prepared with an antibacterial solution, samples are taken from the arterial or venous route. Data on sterilization of intravenous catheter sites indicate that cleaning for 30 seconds or two consecutive cleansings is superior to a single, short (5-10 seconds) disinfection.[43] Simultaneous blood culture using catheter and periphery from patients with a central venous catheter is important in distinguishing catheter-related bloodstream infections.[13] Results obtained with manual laboratory methods showed that 96% of cultures taken before antibiotic administration was positive at the end of 48th hour and 98% at 72nd hour.[44] However, laboratory automation has significantly reduced the time required to detect positive cultures.[45] In automated laboratory methods, 94% of cultures taken before antibiotic treatment were positive within 24 hours (except for coagulase-negative Staphylococcus, Corynebacteria or yeast), and 97% were positive within 36 hours.[46]

Cerebrospinal Fluid (CSF) Culture

The use of CSF culture in newborns with suspected sepsis is controversial. Culture-proven bacterial meningitis occurs in about 0.25 per 1000 live births.[47, 48] Meningitis accompanies 20-25% of newborns with sepsis and 13% of early-onset neonatal sepsis.[49–51] Although there is no consensus on performing lumbar puncture in infants diagnosed with early neonatal sepsis, it should definitely be performed in infants with blood culture positivity and clinically considered meningitis.[23, 27, 47] Blood cultures cannot detect causative microorganisms in 15% to 50% of babies with bacterial meningitis.[52, 53] Taking CSF culture before or just after the administration of antibiotics may increase the likelihood of bacteriological diagnosis.[47, 54, 55] However, antibiotherapy should not be delayed to perform a lumbar puncture. On the other hand, although it is rare in asymptomatic term babies, meningitis is still seen as a complication of neonatal sepsis, and there are sources suggesting lumbar puncture in the assessment of all sick newborns.[56]

Urine Culture

In infants diagnosed with early-onset neonatal sepsis, urine culture does not need to be evaluated as part of early-onset neonatal sepsis since the amount of urine is limited and the rate of positivity in the urine culture is low, especially in the first 72 hours of life. Urinary tract infection assessment should be performed with the bladder catheter or suprapubic bladder aspiration since there is a high risk of contamination in samples taken with urine bags.[27] Urine culture in infants diagnosed with late-onset neonatal sepsis should be part of the evaluation of sepsis.[57]

Tracheal Aspirate Culture

Tracheal aspirate culture may help diagnosis in babies who are diagnosed with sepsis and need mechanical ventilation due to respiratory failure; however, the risk of colonization and contamination should be considered when evaluating the result.[27] It can be taken as a sample in patients with ventilator-associated pneumonia or in cases whose amount and characteristics of secretion varies, but it should be known that its diagnostic value is low.[32] It is not recommended to take tracheal aspirate cultures in prolonged intubation due to rapid colonization following intubation.[58]

Superficial Swab Cultures

Cultures obtained from superficial regions, such as the axilla, umbilical cord, outer ear canal, nasopharynx and orogastric tubes, show poor correlation with pathogens isolated from sterile areas. Routine collection of superficial swab cultures is not recommended in neonatal sepsis, as it has a low predictive value and can lead to erroneous assumptions in determining the factor.[59]

Complete Blood Count Components and Peripheral Smear

Many studies have been conducted on the diagnosis of neonatal sepsis, such as complete blood count, white blood cell count (WBC), absolute neutrophil count and the ratio of immature neutrophil count to total neutrophil count (I/T). The WBC upper limit is set at 30.000-40.000/mm³ in many sepsis screening protocols. However, it is noteworthy that leukocytosis was not detected in one-third of cases diagnosed with sepsis.[27, 60, 61] Although the normal value of WBC has a very wide range, it can be affected by the time and place of collection of the sample, the gestational week of the baby, and factors other than sepsis.[27, 62–64] Among the factors other than sepsis that change the value of WBC are conditions, such as preeclampsia, intraventricular hemorrhage, perinatal asphyxia, meconium aspiration, pneumothorax, convulsions and prolonged crying.[65]

The sensitivity of the complete blood count samples taken immediately after birth was found to be low in the evaluation of sepsis. Due to its weak positive and negative predictive value, the benefit of the use of complete blood count as a biomarker in neonatal sepsis has not been proven. However, studies show that serial normal complete blood count measurements can be reliable in excluding sepsis.[61, 66, 67]

Another parameter used in sepsis assessment among complete blood count is neutrophil count. The presence of neutropenia is more valuable than neutrophilia, especially in the first postnatal 48 hours in the diagnosis of sepsis.[60] It should be noted that as the gestational age decreases, the lower limit of absolute neutrophil count decreases.[68, 69] In addition, hypertension, maternal fever, asphyxia, meconium aspiration syndrome, mode of delivery, periventricular hemorrhage, reticulocytosis, hemolytic disease and pneumothorax are known to affect the neutrophil count.[62]

In the evaluation of peripheral smear, vacuolization, Döhle bodies and toxic granulation are guiding in the diagnosis of bacterial sepsis.[27, 64, 70] The I/T ratio drops from 0.16 at birth to 0.12 at 60 hours. I/T ratio of ≥0.2 is considered significant in the diagnosis of sepsis.[68] However, I/T ratio may cause erroneous interpretation in cases, such as perinatal asphyxia, maternal hypertension and long-term oxytocin induction.[15, 71] It should also be kept in mind that the technique of peripheral smear, the knowledge and experience of the investigator can affect the results.

Thrombocytopenia is a non-specific late finding of neonatal sepsis. It was found that the platelet count below 100000/mm³ for the first 10 days of the postnatal period and below 150000/mm³ in later periods are associated with sepsis.[72] In 50% of the cases with the bacterial infection, platelet count was found to be below 100.000/mm³.[73] Accompanying bacterial infections more frequently, thrombocytopenia is also seen in viral infections.[74, 75]

C-Reactive Protein (CRP)

CRP, which is a pentameric structure, containing 187 amino acids and synthesized from hepatocytes, and an acute-phase protein, is one of the most easily available and most frequently used laboratory tests in the diagnosis of neonatal sepsis.[76, 77] Its synthesis is stimulated by cytokines, primarily interleukin-6 (IL-6), IL-1 and tumor necrosis factor-α (TNF-α). Its half-life is between 24-48 hours. The normal lower limit is considered as 1 mg/dL in the neonatal period.[78, 79] It takes 10-12 hours for it to reach the measurable level in the serum, so its reliability is low in the early diagnosis of neonatal sepsis. Serial CRP measurements have been shown to increase sensitivity in the diagnosis of sepsis 24 to 48 hours after the onset of symptoms.[80] Serial CRP mea-

surements are also used to evaluate the antibiotic response.[81] Although CRP serum level rises mainly with infections, it may also rise due to non-infectious causes, such as premature rupture of the membranes, maternal fever, fetal distress, difficult birth, and perinatal asphyxia. This causes low specificity of CRP for early neonatal sepsis.[23, 27, 64]

High Sensitivity-CRP (hs-CRP)

High Sensitivity CRP (hs-CRP) is more sensitive than conventional CRP in the diagnosis of neonatal sepsis. While the normal lower value of conventional CRP is accepted as 1 mg/dL, this value is 1 mg/L for hs-CRP.[82] In studies conducted, infected newborns with high hs-CRP values were reported to have a significant increase in hs-CRP compared to non-infected newborns.[83] In another study, it was reported that there is no risk of infection when the hs-CRP value is detected as <0.5 mg/L, there is low infection risk when the value is between 0.5–1 mg/L, moderate infection risk when between 1–3 mg/L, and there is a high risk of infection at values >3 mg/L.[84] As a result, more studies are needed to evaluate the role of hs-CRP in use as a neonatal sepsis marker.

Procalcitonin

Procalcitonin (PCT), which is the prohormone of calcitonin and used as the acute-phase reactant protein, consists of 116 amino acids. PCT is encoded by the Calc-1 gene and synthesized by macrophage and hepatocytes.[85] The PCT level rises rapidly 2-4 hours after exposure to bacterial endotoxin, reaches a peak in 6-8 hours and remains high for 24 hours.[86] The half-life of PCT is 24-30 hours. Due to its rapid rise from the onset of bacterial sepsis, it is considered a better marker for early diagnosis of neonatal sepsis compared to CRP. In healthy newborns, plasma PCT concentrations increase gradually after birth, reaching peak levels in about 24 hours (in the range of 0.1-20 ng/mL), and then falls to normal values below 0.5 ng/mL in 48-72 hours.[87] Procalcitonin over 2-2.5 ng/ml after postnatal 72 hours should suggest infection.[88]

Prematurity, intracranial hemorrhage, asphyxia, neonatal hypoxemia, resuscitation, chorioamnionitis where neonatal infection does not develop, maternal GBS colonization, prolonged membrane rupture, prenatal antibiotic use, surfactant administration, postpartum antibiotic use and very low birth weight may cause false positives. Thus, increases in the PCT level should be interpreted correctly.[89-91] It was concluded that PCT is a useful biomarker for early diagnosis of newborn sepsis in critically ill patients in meta-analyses.[92]

Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF)

Matrix Assisted Laser Desorption Ionization-Time of Flight, MALDI-TOF) started to take place among the new diagnostic tests based on the identification of microorganism. MALDI-TOF is a mass spectrometer tool that allows rapid and accurate identification of the species within a wide range of gram-negative and gram positive bacteria, as soon as the organism is present in the culture.[93-95] It, therefore, allows organisms to be diagnosed earlier than blood culture and targeted antibiotic therapy to start earlier.[96] Its routine use is not yet common in the neonatal period.

Molecular Methods

Nucleic acid analysis methods are important in recognizing the morphological, metabolic or cytopathic features of microorganisms with no culture possibility or which grow slowly. Molecular technologies are being studied to enable rapid identification of gram-negative and gram positive bacteria in the diagnosis of sepsis. Amplification of nucleic acids can be done by methods, such as replication of the target nucleic acid (polymerase chain reaction), replication of the nucleic acid probe (ligase chain reaction) or signal duplication (branched-probe DNA determination).[97] Polymerase chain reaction targets conserved regions of the 16S ribosomal RNA gene, which is common to all bacteria and not found in organisms other than bacteria.[98] In the study conducted by Dutta et al.,[98] PCR analysis was performed before and after the initiation of antibiotherapy in clinically suspected sepsis cases; and the sensitivity, specificity, positive and negative predictive values of PCR were found as 96.2%, 96.3%, 87.7% and 98.8%, respectively. It was shown that the patients who had PCR positivity in the sample taken before antibiotherapy, continued their positivity at the 12th hour after antibiotherapy, but that there was no PCR positivity at the 24th and 48th hours. This test, which may be useful in early diagnosis, is not recommended for use in patients whose antibiotic treatment is continued for more than 12 hours.[98]

While it is advantageous that a small amount of sample is sufficient and the test can be run in body fluids, such as surgical tissues and effusions, the disadvantages are the weakness of clinical correlation due to the inability to distinguish between active infection and past infection and high probability of detecting contamination.[56] As a result, comprehensive studies evaluating a larger number of cases for routine clinical use in newborns with suspected sepsis are required.

Serum Amyloid A (SAA)

Serum Amyloid A (SAA) is an apolipoprotein synthesized by the liver, of whom the synthesis is controlled by IL-1, IL-6 and TNF-α.[99] It is secreted in response to injury or infection. It is known to play a role in inflammation and stimulate the release of IL-8 from neutrophils. In a study comparing SAA, CRP, IL-6 and WBC in preterm infants with sepsis, it was reported that mortality and SAA levels in newborn babies were inversely proportional.[100] In another study, SAA levels in septic babies were shown to be significantly higher at 0, 24 and 48 hours, compared to SAA levels in non-septic babies. However, compared to CRP, SAA has been reported to show a sharper and faster rise and also to return to normal faster.[101] As a result, SAA can be used as a useful marker in early detection of neonatal sepsis if rapid and reliable kits are used.

Lipopolysaccharide Binding Protein (LBP):

The lipopolysaccharide-binding protein interacts with endotoxins produced by Gram-negative bacteria species and transfers them to CD14 immune cells.[102, 103] LBP is produced by hepatocytes, epithelium, muscle cells, and its level rises approximately 6-8 hours after the onset of acute infection. Another advantage of LBP, which has high sensitivity and negative predictive value in the diagnosis of early-onset neonatal sepsis, is that it is less affected by physiological changes and obstetric events that occur within the first two postnatal days.[104, 105]

Cytokines and Chemokines

Cytokine levels change rapidly during the neonatal sepsis course. Cytokines, such as IL-6, IL-1b, SIL2R, IL-8 and TNF-a primarily increase in response to bacterial infections. This increase occurs before clinical symptoms or positive standardized diagnostic tests.[56] Also, the levels of cytokines in the newborn baby in the first few hours of life indicate the risk of infection.[106, 107]

Interleukin- 6 (IL-6)

Interleukin-6 stimulates the production of acute-phase reactants, such as CRP from the liver by releasing from B and T lymphocytes, monocytes, endothelial cells and fibroblasts in the acute-phase of infections.[108, 109] The advantage of using IL-6 as a marker of sepsis is that there is a rapid increase in concentration just before the CRP level rises and immediately after the onset of bacteremia. However, its half-life is being very short and its level is being normalized within 24 hours after the start of antimicrobials appear to be disadvantages. Thus, IL-6 has a narrow window of opportunity.[110] IL-6 levels obtained from umbilical cord were found to be high in early neonatal sepsis cases.[111, 112] Studies have shown that IL-6 has better sensitivity and negative predictive value compared to CRP when used as an early phase biomarker.[70] Also, its combination with other sepsis markers, such as TNF-a and CRP, has been shown to have higher sensitivity and negative predictive value in the diagnosis of early-onset neonatal sepsis.[113]

IL-8

IL-8 is a pro-inflammatory cytokine produced by monocyte, macrophage, fibroblast and endothelial cells. It is known to be responsible for the activation and chemotaxis of neutrophils.[114, 115] IL-8 guides both in the diagnosis and recognition of the severity of neonatal sepsis. Its sensitivity was detected as 80-91% and specificity as 76-100% in determining neonatal sepsis.[70, 116] The combination of IL-8 with CRP as a biomarker has been shown to have higher sensitivity and specificity in newborns considered suspicious sepsis and help reducing antibiotic use.[70] IL-8 level increases rapidly within 2-4 hours from the start of an infection and then decreases within four hours. This makes IL-8 similar to IL-6, to be used as only an early marker of infection.[117] As a result, there are studies indicating that IL-8 may play a role in the diagnosis of neonatal sepsis, but more studies are needed to provide strong evidence to support its use.

Tumor Necrosis Factor Alpha (TNF- α)

It is a proinflammatory cytokine produced by activated phagocytes during systemic infection and inflammation. It has pharmacokinetic properties similar to IL-6.[116] The increase in TNF-α level is independent of the pregnancy week of the newborn and postnatal age.[70] In studies comparing septic newborns and healthy newborns, TNF-α levels have been shown to be significantly higher in septic cases.[118, 119] When combined with IL-6, it shows 60% sensitivity and 100% specificity in the diagnosis of sepsis.[120] In a meta-analysis study, TNF-α was found to show a moderate accuracy both in the diagnosis of early-onset neonatal sepsis (sensitivity 66%, specificity 76%) and in the diagnosis of late-onset neonatal sepsis (sensitivity 68%, specificity 89%).[121]

Other Chemokines

Chemokines that can be used with other inflammatory markers in the diagnosis of neonatal sepsis include monokines, RANKES, MCP-1 and IP-10.[122] While the use of chemokine as a biomarker provides an advantage since it rises very early compared to CRP after the onset of infection; causes, such as decreased levels within approximately 24 hours after the start of treatment, need of special tools for

the study of the test and difficulty to reach the test limit its use.[107] However, more studies are needed for their widespread and safe use.

Cell Surface Markers

Cell surface markers can be determined by flow cytometry. [64] Studies are conducted on the use of cell surface antigens as biomarkers, such as CD11b (100% specificity, 100% sensitivity), CD64 (83% specificity and 82% sensitivity), sCD163. CD64 is a high-affinity Fc receptor for immunoglobulin G, of which its expression is increased in response to infection.[123] Its level increases ten-fold in activated neutrophils 4-6 hours after the onset of bacterial infection. Its level decreases to normal within a few days after the infection is suppressed.[124, 125] The combination of CD64 with PCT, CRP and WBC has been shown to increase sensitivity in the early diagnosis of sepsis.[126]

CD11b (Mac-1, CR3) is the α subunit of the b2-integrin adhesion molecule and is expressed at low concentration in inactivated neutrophils. Its level rises five minutes after the onset of bacterial infection and it is thought to be a good biomarker for early detection of neonatal sepsis.[70] In the study of Weirich et al.,[124] CD11b was detected in all babies with proven sepsis, but not in newborns without sepsis. In the diagnosis of neonatal infection, negative predictive value, positive predictive value, sensitivity and specificity of CD11b were detected as 100%, 99%, 96% and 100%, respectively. Nupponen et al.[125] showed that CD11b has 100% sensitivity and specificity in the detection of neonatal sepsis in their studies comparing newborns with suspected infections and healthy newborns. Adib et al. showed that the sensitivity and negative predictive value of the combination of CD11b with CRP reached 100% in the diagnosis of

neonatal sepsis.[127] Current studies have shown that CD11b has good diagnostic accuracy as a biomarker for neonatal sepsis. However, the insufficiency and high cost of the centers where the examination can be carried out are among the factors limiting their routine use.

Soluble CD163 (sCD 163) reduces the oxidative damage that arises from hemolysis by clearing circulating free hemoglobin with the help of haptoglobin.[128] It binds to gram-negative and gram-positive bacteria, stimulating the synthesis of proinflammatory cytokines, such as TNF-α, IL-1b, IL-6 and IL-10.[129] In a study comparing CRP, IL-6, IL-8, TNF-α and sCD163, it is concluded that sCD163 is the strongest predictive marker for the differentiation of infected and uninfected newborns before antibiotherapy is started.[130]

Other Biomarkers

Other biomarkers that draw attention in the diagnosis and treatment of sepsis are suPAR, angiopoietins, pentraxin 3 (PTX3), sTREM-1 and inter alpha inhibitory proteins (IaIp). [131-135] However, further studies are needed to support their routine use.

Diagnostic Algorithms

Early diagnosis of sepsis is difficult due to the absence of specific symptoms and the suboptimal diagnostic value of the laboratory findings. This causes high levels of empirical antimicrobial use. In the literature, sepsis scores were tried to be created with different combinations of inflammatory response parameters, laboratory tests and physical examination findings. Töllner developed the first known newborn sepsis scoring system in 1982 to identify sepsis using clinical and basic laboratory evaluations (Table 1).[136] The Pediatric Committee (PDCO) of the European Medicines

Table 1. Töllner sepsis scoring system

Score	0	1	2	3
Change in skin color	Absent		Moderate	Evident
Peripheral circulatory disorder	Absent		Impaired	Evident
Hypotonia	Absent	Moderate	Evident	
Bradycardia	Absent	Present		
Apnea	Absent	Present		
Respiratory distress	Absent	Present		
Hepatomegaly	Absent	>4cm		
GIS finding	Absent	Present		
Leukocyte count	Normal	Leukocytosis		Leukopenia
Left shifting	Absent		Moderate	Evident
Thrombocytopenia	Absent		Present	
Metabolic acidosis	Normal	>7.2	<7.2	

If the total score is below 5, it is normal, between 5-10 it is suspected, and above 10 points, it is considered as definite sepsis.

Agency (EMA) proposed EMA sepsis criteria for standardization of the definition of neonatal sepsis in 2010 (Table 2).[137] However, the adequacy and reliability of the use of a single scoring system in the diagnosis of sepsis have not been proven yet.

Today, online early-onset neonatal sepsis calculators are also used to predict the possibility of early-onset neonatal sepsis and to guide decisions regarding initiation of antibiotherapy (https://neonatalsepsiscalculator.kaiserpermanente.org).

Treatment

Antimicrobial treatment of neonatal infections is divided into two as the treatment of suspected (empirical) or known (definitive) pathogens. Whether there is early or late-onset of symptoms, and the infection is nosocomial or community-acquired, affects antimicrobial selection. Although it is important to take appropriate culture samples before starting antibiotherapy, this should not delay starting treatment.

Empirical Treatment

Empirical treatment of early-onset bacterial infections should include ampicillin and an aminoglycoside antibiotic (usually gentamicin). Renal function tests should be evaluated at the beginning of treatment with gentamicin, and serum gentamicin level should be checked in infants whose antibiotherapy will be completed. If renal function tests are normal in babies whose treatment is completed after 48 hours, gentamicin level examination is not necessary.[23] The use of third and fourth generation cephalosporins should only be added to the treatment in case of suspected gram-negative meningitis.[13] The use of third-generation cephalosporins and vancomycin has been associated with an increase in vancomycin-resistant enterococci and extended-spectrum β-lactamase (ESBL)-producing gram-negative bacteria (GNB).[138] Empirical use of third-generation cephalosporins is not recommended, as it causes an increased risk of invasive candidiasis in long-term administration as well as resistance development.[139] Ampicillin and third-generation cephalosporin regimen have been shown to be no more ef-

Table 2. EMA sepsis scoring system

Clinical Findings	Laboratory Findings
Body temperature: >38.5 ºC or <36 ºC and/or temperature irregularities	**Leukocyte count:** <4.000/mm³ or >20.000/mm³
Cardiovascular instability: Bradycardia or tachycardia and/or rhythm irregularity Urine amount <1 ml/kg/hour Hypotension Impaired peripheral perfusion	**Immature/total neutrophil ratio:** ≥0.2
Skin and subcutaneous lesions: Petechiae Sclerema	**Platelet Count:** <100.000/mm³
Respiratory instability: Apnea or Tachypnea or Increased oxygen demand or Increased need for ventilation support	CRP >15mg/L (1.5 mg/dL) or procalcitonin ≥2 ng/mL
Gastrointestinal: Nutritional intolerance Insufficient breastfeeding Abdominal distention	**In blood sugar monitoring (at least twice):** Hyperglycemia (>180 mg/dL or 10 mMol/L) or Hypoglycemia (<45 mg/dL or 2.5 mMol/L)
Non-specific: Irritability Lethargy Hypotonia	**Metabolic acidosis:** Base deficit >10 mEq/L or Serum lactate >2 mMol/L

Positivity in at least two of the clinical categories and at least two of the laboratory categories is considered as clinical sepsis. It can be used up to postnatal 44 weeks.

fective than the combination of ampicillin and gentamicin.[140] Ampicillin + gentamicin is synergistic in the treatment of infections that arise from GBS and *L. monocytogenes*, but cephalosporins are not effective against *L. monocytogenes*. Empirical treatment of late-onset neonatal sepsis usually includes vancomycin and an aminoglycoside antibiotic group, effective for *coagulase-negative Staphylococci, S. aureus* and gram-negative organisms. However, as in early-onset sepsis, if gram-negative meningitis is suspected, the addition of third-generation cephalosporins should be considered.[139] Carbapenem group antibiotic use can be an option considering local resistance levels or if the patient has previously used a third-generation cephalosporin antibiotic.[141] The use of piperacillin + tazobactam and ampicillin + sulbactam is gradually increasing in the treatment of infections that occur during neonatal intensive care unit hospitalization; however, penetration of tazobactam into the central nervous system is unreliable and it should not be used to treat meningitis. However, β-lactamase inhibitor sulbactam is known to reach high concentrations in CSF when combined with ampicillin.[142] Rapid and aggressive treatment should be initiated when fungal infections, such as candidiasis, aspergillosis and zygomycosis, are suspected. Empirical antifungal therapy with amphotericin B deoxycholate can be considered in high-risk babies with risk factors for invasive candidiasis.[13]

Treatment should be continued for 7-10 days in the diagnosis of clinical sepsis. The clinical condition of the baby, laboratory examinations and response to the treatment are monitored. The improvement of clinical findings in the first 24-48 hours from the start of treatment, the normalization of CRP level, I/T ratio and white blood cell count in 48-72 hours indicates an appropriate response is received.[5] It is often difficult to determine an appropriate antibiotic treatment period for suspected sepsis when cultures are negative. Standard practice in babies who are fine and have no clinical or hematological evidence for infection is to stop antibiotherapy if there is no culture growth after 48 hours.[139]

Pathogen-Oriented Treatment

Once the pathogens have been identified, treatment should be reorganized according to the type and sensitivity. When looking at the treatment regimens, in babies with bacteremia and sepsis that arise from GBS, gentamicin is often used in combination with ampicillin or penicillin, but there are insufficient data to suggest that the aminoglycoside addition improves the result. However, it is common practice to use a combination of these two drugs in the first few days of treatment, and then continue treatment with just ampicillin or penicillin.[24] Although ampicillin alone is sufficient in the treatment of L. monocytogenesis, aminoglycosides show synergistic effects. Enterococci should be treated with

an antibiotic containing penicillin, and aminoglycoside can be added to the treatment if the synergistic effect is documented. Aminoglycoside therapy may be discontinued when cultures result as sterile. Ampicillin-resistant enterococcal infections can be treated with vancomycin without the addition of aminoglycosides. In *S. aureus* infections, vancomycin is used for treatment until the susceptibility profile is concluded, while it is continued in patients with MRSA. If MSSA is detected, cefazolin can be used as an alternative treatment in conditions other than CNS infections and endocarditis. *Coagulase-negative staphylococcal* infections require treatment with vancomycin. Ampicillin (if sensitive) or an aminoglycoside is sufficient for the treatment of gram-negative enteric bacterial infections. However, if meningitis is suspected, third-generation or fourth-generation cephalosporin (for example, cefotaxime, ceftazidime, or cefepime if Pseudomonas spp is the causative agent) or carbapenem should be used. Carbepenem is the best option in the treatment of Enterobacteriaceae strains that produce extended-spectrum beta-lactamase (ESBL), while cefepime may also be considered. Infections that arise from *Enterobacteriacaea* strains that produce carbapenemase are treated with colistin in addition to carbapenem, or high-dose tigecycline, or a regimen containing aminoglycoside. It is appropriate to use clindamycin, ampicillin + sulbactam or metronidazole in the treatment of anaerobic infections; if CNS involvement is present, metronidazole is preferred. When fungal infections are evaluated, Amphotericin B deoxycholate is the first choice for the treatment of invasive candidiasis.[143] Fluconazole can be used as an alternative therapy in the treatment of patients with sensitive fungal infections and patients without prophylaxis given.[144] Liposomal amphotericin or echinocandin (caspofungin or micafungin) can be used in the treatment of hepatic or splenic candidiasis. Antibiotics and their frequently used doses in the neonatal period are summarized in Table 3.[145, 146]

The duration of treatment is determined by the site of infection and the clinical response of the patient. Bacteremia without infection focus is usually treated for 7-10 days. Although there are few randomized controlled studies on antibiotherapy periods in premature babies with very low birth weight, duration of antibiotherapy can be extended until day 10-14 in infants younger than 32nd gestational weeks.[147] Gram-negative bacteremia treatment is also extended until 10th-14th days. The duration of treatment in uncomplicated GBS meningitis is usually until day 10-14, while the duration is extended in focal complications.[139] In gram-negative bacterial meningitis, treatment is continued for 21 days or for another two weeks after the first negative CSF culture.[148]

Table 3. Antibiotic doses

Antibiotic	Administration Route	Dosing
AMIKACIN	IM, IV	**Gestational age <30 weeks:** PNA ≤14 days: 15 mg/kg/dose every 48 hours PNA ≥15 days: 15 mg/kg/dose every 24 hours **Gestational age between 30-34 weeks:** PNA ≤60 days: 15 mg/kg/dose every 24 hours **Gestational age ≥35 weeks:** PNA ≤7 days: 15 mg/kg/dose every 24 hours PNA ≥8 days: 17,5 mg/kg/dose every 24 hours
AMPICILLIN	IM, IV	**Gestational age ≤34 weeks:** PNA ≤7 days: 50 mg/kg/dose every 12 hours PNA 8-28 days: 75 mg/kg/dose every 12 hours **Gestational age >34 weeks:** PNA ≤28 days: 50 mg/kg/dose every 8 hours **Meningitis:** PNA ≤7 days (IV): 200- 300 mg/kg/days every 8 hours PNA >7 days (IV): 300 mg/kg/days every 6 hours
CEFOTAXIME	IM, IV	**Gestational age <32 weeks:** PNA <14 days: 50 mg/kg/dose every 12 hours PNA 14-28 days: 50 mg/kg/dose every 8 hours **Gestational age ≥32 weeks:** PNA ≤7 days: 50 mg/kg/dose every 12 hours PNA 8-28 days: 50 mg/kg/dose every 8 hours
MEROPENEM	IV	**Birth weight ≤ 2 kg** PNA ≤14 days: 20 mg/kg/dose every 12 hours PNA 15-28 days: 20 mg/kg/doz every 8 hours PNA 29-60 days: 30mg/kg/dose every 8 hours **Birth weight > 2 kg** PNA ≤14 days: 20 mg/kg/dose every 8 hours PNA 15-60 days: 30 mg/kg/dose every 8 hours
PIPERACILLIN - TAZOBACTAM	IV	**Birth weight ≤ 2 kg** PNA ≤7 days: 100 mg/kg/dose every 8 hours PNA 8-28 days: PMA ≤ 30 GH 100 mg/kg/dose every 8 hours PMA >30 GH 80 mg/kg/ dose every 6 hours PNA 29-60 days: 80mg / kg/dose every 6 hours **Birth weight > 2 kg** PNA ≤ 60 days: 80 mg / kg/dose every 6 hours
VANCOMYCIN	IV	Loading dose: 20mg/kg/dose **Gestational age <28 weeks:** Serum Creatinine <0.5 mg/dL 15 mg/kg/dose every 12 hours Serum Creatinine 0.5-0.7 mg/dL 20 mg/kg/dose every 24 hours Serum Creatinine 0.8- 1 mg/dL 15 mg/kg/dose every 24 hours Serum Creatinine 1.1- 1.4 mg/dL 10 mg/kg/dose every 24 hours Serum Creatinine >1.4 mg/dL 15 mg/kg/dose every 48 hours **Gestational age >28 weeks:** Serum Creatinine <0.7 mg/dL 15 mg/kg/dose every 12 hours Serum Creatinine 0.7-0.9 mg/dL 20 mg/kg/dose every 24 hours Serum Creatinine 1-1.2 mg/dL 15 mg/kg/dose every 24 hours Serum Creatinine 1.3- 1.6 mg/dL 10 mg/kg/dose every 24 hours Serum Creatinine >1.6 mg/dL 15 mg/kg/dose every 48 hours
TEICOPLANIN	IV	Loading dose: 16 mg/kg/dose Maintenance dose: 8 mg/kg/dose every 24 hours

Supportive Treatments

Treatments that increase the number or function of neutrophils, including granulocyte transfusions, granulocyte-macrophage colony-stimulating factor (GM-CSF), G-CSF, and intravenous immune globulin (IVIG) treatments, are also considered in the treatment of neonatal sepsis. However, many studies have failed to show that GM-CSF or G-CSF has a significant effect on the reduction in mortality. [149–152] Considering the use of IVIG, in the Cochrane review of the International Neonatal Immunotherapy Study (INIS Collaborative Group), which included over 7000 babies, IVIG infusions used in neonatal sepsis have been shown to have no effect on morbidity or long-term mortality. [153, 154] The use of pentoxifylline, which works by reducing TNF-α concentrations associated with sepsis and improving microcirculation, has been evaluated in two studies with randomized control and it was shown to cause an improvement in survival rates in babies with proven sepsis.[155] However, further studies are needed on this subject.

Disclosures

Peer-review: Externally peer-reviewed.

Conflict of Interest: None declared.

Authorship Contributions: Concept – İ.Ö.O., A.B.; Design – İ.Ö.O., A.B.; Supervision – A.B.; Materials – İ.Ö.O.; Data collection &/or processing – İ.Ö.O.; Analysis and/or interpretation – A.B.; Literature search – İ.Ö.O.; Writing – İ.Ö.O.; Critical review – A.B.

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