Neonatal sepsis: a continuing disease burden

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Sepsis-related morbidity and mortality are increasing concerns in all Neonatal Intensive Care Units, with reported incidences that are dramatically high regardless of the improvements in the quality of neonatal assistance. Preterm neonates display clinical characteristics that make them prone to infections. Neonatal sepsis is one of the major causes of neonatal death in developing countries. Different microorganisms are responsible for disease according to the age at onset. Simple preventive and treatment strategies have the potential to save many newborns from sepsis-related death. This article is a review for understanding issues related to sepsis in the neonatal intensive care unit.

Key words: sepsis, neonatal, age at onset, neonatal intensive care unit.

Sepsis-related morbidity and mortality are increasing concerns in all Neonatal Intensive Care Units (NICUs), with reported incidences that are dramatically high regardless of the improvements in the quality of neonatal assistance.

Sepsis neonatorum is the term used to describe any systemic bacterial infection documented by a positive blood culture in the first month of life. Bacterial sepsis in the neonate is a clinical syndrome characterized by systemic signs of infection accompanied by bacteremia. Neonatal sepsis includes bloodstream, urine, cerebrospinal, and peritoneal infections and infections starting from any other usually sterile sites. Although most of the causative agents of neonatal sepsis are bacterial, viruses, fungi and parasites, though having a smaller role, must also be considered in the differential diagnosis of etiologies. Neonatal sepsis can be classified into two relatively distinct illnesses based on the postnatal age at onset: early-onset sepsis (EOS) occurs in the first 7 days of life and is usually a fulminate multisystem infection acquired by vertical transmission from the mother, while late-onset sepsis (LOS) is usually more insidious but may have an acute onset. LOS is a common complication of prolonged admission to the NICU following preterm birth. The distinction has clinical relevance, as EOS disease is mainly due to bacteria acquired before and during delivery, while LOS disease is due to bacteria acquired after delivery (nosocomial or community sources). In the literature, however, there is little consensus as to what age limits apply, with EOS ranging from 48 hours to 6 days after delivery[1]. Another type of sepsis has been recognized, very late-onset sepsis, which occurs after 3 months of life and affects premature infants who are of very low birth weight (VLBW).

Incidence and Mortality

The incidence of sepsis in the neonate is greater than at any other period of life and varies from hospital to hospital. Neonatal sepsis remains a major cause of death in developing countries. While some reports from developed countries demonstrated that the incidence of neonatal sepsis varies from 1 to 5 cases per 1000 live births, some other population-based studies from developing countries have reported clinical sepsis rates ranging from 49-170 per 1000 live births[2].

The median incidence of blood culture-confirmed sepsis was 16 per 1000 live births[3-5]. The incidence of culture-confirmed EOS ranged from 2.2 to 9.8 per 1000 live births, and the incidence of clinical sepsis ranged from 20.7 to 50 per 1000 live births[5,6]. Nosocomial sepsis frequency was 6.4%, ranging from 2.1-17%, in Turkey[7]. The case fatality rate for infectious neonatal illnesses has continued

to decline dramatically after the introduction of antimicrobial agents, and is now 5-20% for EOS. Documented mortality among very premature infants with EOS is much higher (over 30%)[8]. The Turkish Neonatal Society, Nosocomial Infections Study Group reported that sepsis-related mortality was 24.4 for 100 sepsis cases in Turkey[7].

While the true incidence is not known, a recent retrospective cohort study of 3,800 neonates admitted to the NICU over a 6-year period reported septic shock in 1.3%, with an associated mortality peaking at 71% for extremely low birth weight (ELBW) neonates <1000 g[9].

Etiology

Different microorganisms are responsible for the disease according to the age at onset. The predominant bacterial causes of sepsis have changed over time and may vary from hospital to hospital. Bacteria responsible for early-onset disease are acquired hours before delivery from overt or occult rupture of membranes (ROM) or from the birth canal during delivery. Gram-positive cocci were the most common pathogens before the introduction of antibiotics, but this predominance shifted to gram-negative enteric bacilli after antimicrobial agents came into common use. All serotypes of group B streptococci (GBS) and *Escherichia coli* K1 account for approximately 75% of EOS (10). Since the introduction of GBS intrapartum prophylaxis, several institutions in the United States have reported a shifted distribution of pathogenic agents responsible for neonatal sepsis towards a predominance of gram-negative rods, specifically *E. coli*[11].

Vaginal colonization with coagulase-negative staphylococci (CONS) was reported in approximately 50% of 465 women cultured[12]. In spite of this, less commonly, coagulase-negative staphylococci cause EOS.

Hyde et al[13]. reported that the most common etiologic agents in EOS were GBS, *E. coli* and *Streptococcus viridians,* while CONS, *Staphylococcus aureus* and *Candida albicans* were the most common etiologic agents in LOS. Zaidi et al[14]. showed that 25% of all episodes of EOS were caused by Klebsiella, 15% were caused by *E. coli*, 18% were caused by *S. aureus*, 7% were caused by GBS, and 12% were caused collectively by Acinetobacter and Pseudomonas. Yapıcıoğlu et al[15]. reported that the most frequent pathogens were gram-negative pathogens followed by gram-positive microorganisms in health-care-associated infections. Baş et al[16]. reported that a total of 106 nosocomial sepsis attacks were found in 100 patients, and gram-negative bacteria were isolated at a rate of 70.8% and gram-positive at a rate of 22.6% in a center in Turkey.

Pathogenesis

Early-onset neonatal sepsis (EOS) may have its onset in utero, secondary to maternal hematogenous infection or, more often, chorioamnionitis. Inhalation of infected amniotic fluid can produce pneumonia and sepsis in utero, manifested by fetal distress or neonatal asphyxia; in addition, aspiration of infected amniotic fluid or secretions in the birth canal can produce pneumonia and sepsis[10].

Infections occurring in utero can cause resorption of the embryo, abortus, stillbirths, congenital malformations, intrauterine growth retardation, premature births, acute illnesses in the neonatal period, or asymptomatic persistent infections producing neurologic sequelae in advanced stages of life.

Neonates are highly susceptible to infectious diseases because of their immature immune systems and poorly developed skin barrier. Innate immunity is compromised in neonates and results in decreased production of proinflammatory cytokines, particularly interleukin (IL)-1β tumor necrosis factor (TNF)-α, interferon-γ, and IL-12[17]. The impaired humoral defenses of the newborn play a significant role in the pathogenesis of neonatal sepsis. The neonate receives no immunoglobulins (Ig) of the Ig A and Ig M classes in fetal life. Specific bactericidal and opsonic antibodies against gram-negative bacteria are predominantly in the Ig M class; thus, the newborn infant lacks optimal humoral protection from infection with enteric organisms. In premature infants, cord Ig G levels are directly proportional to gestational age. Synthesis of fresh Ig G is impaired secondary to constraints in VH gene repertoire among newborns [18]. Neonates also exhibit decreased levels of complement proteins and complement-mediated opsonic capabilities compared with adults. There is

essentially no transfer of complement from the maternal circulation. The fetus synthesizes complement components as early as the first trimester[10]. Full-term infants have slightly diminished classic pathway complement activity and moderately diminished alternative pathway activity[10]. Neonatal antigen-presenting cells, including monocytes and macrophages, exhibit deficit in pathogen recognition, activation after stimulation, phagocytic function, and bactericidal function. The number of circulating monocytes in neonatal blood is normal, but the mass or function of the macrophages in the reticuloendothelial system (RES) is apparently diminished in the newborn. In the early neonatal period, their mononuclear phagocytes are hyporesponsive to the physiologic and pathologic signaling mechanism[19]. Significantly impaired intracellular oxidative burst in response to opsonized bacteria has been documented in neutrophils from preterm neonates[20]. Neonates also have a low storage pool of neutrophils, which have a reduced capacity to migrate from blood to sites of infection[21]. Studies in a hemorrhagic shock model revealed decreased lung inflammation and injury in immature mice versus adult mice[22]. Wynn et al[23]. compared the host inflammatory response and subsequent mortality rate in a fecal slurry model of generalized peritonitis between neonatal and adult mice. Compared with young adult mice, sepsis in neonatal mice was associated with a markedly attenuated systemic inflammatory response, decreased bacterial clearance and increased mortality rate.

Risk Factors

Host susceptibility, socioeconomic factors, obstetric and nursery practices, and health and nutrition of the mother are all important in the pathogenesis of neonatal sepsis. Infants who develop particularly EOS usually have a history of risk factors associated with the pregnancy and delivery.

Maternal Risk Factors: Although the reason is not well defined, the rates of early-onset GBS infection are higher among blacks than in other racial groups. The rates of prematurity and LBW, which both predispose to neonatal infection, are inversely related to socioeconomic status. Asymptomatic bacteriuria has been associated with premature birth [24]. Other risk factors include premature birth, LBW, premature rupture of membranes (PROM), prolonged time of ROM, maternal peripartum infection, and septic or traumatic delivery [25]. Uncomplicated ROM lasting longer than 24 hours has been associated with a 1% incidence of neonatal sepsis above the baseline rate of 0.1% to 0.5% [24]. The risk of infection increases four-fold if chorioamnionitis and prolonged ROM coexist[24]. Newborns in developing countries are also exposed to external risk factors that put them at a greater risk for infections compared with neonates in the industrialized countries. Perinatal asphyxia has also been associated with increased incidence of sepsis. Greenberg and coworkers[26]. found that certain conditions were common in their prospective study of 229 infants with sepsis; 130 (57%) were premature, 64 (28%) were delivered by cesarean section or instrumental delivery, 43 (19%) had an Apgar score of less than 7 at the 5th minute, and 27 (2%) had a prolonged (>24 hours) interval after maternal ROM.

Neonatal Risk Factors: Possibly due to defects in X-linked immunoregulatory genes, male infants had a higher incidence of neonatal sepsis than female infants. Birth weight also affects the attack rates of EOS. It is reported that although birth weight and Apgar scores have been associated previously with risk of early-onset neonatal sepsis, Shin et al[27]. found no earlier reports suggesting that they predict mortality. Hypothermia in newborns, generally defined as a rectal temperature ≤35°C, is associated with a significant increase in the incidence of sepsis. Hypothermia is usually accompanied by abnormal leukocyte counts, acidosis and uremia, each of which can interfere with resistance to infection[25]. Metabolic disorders may predispose to infection. Infants with galactosemia have increased susceptibility to sepsis caused by gram-negative enteric bacilli, in particular *E. coli*. The umbilical cord is a particularly common portal of entry for systemic infection.

Furthermore, intrinsic factors and extrinsic factors in the antenatal, intrapartum and early neonatal period in developing countries are additional factors that put newborns at an even greater risk for developing EOS[17].

Clinical Manifestations

The clinical signs of infection in the newborn

infant vary from mild to severe. Variables affecting the manifestations include the duration of infection, virulence of the causative agent, and degree of maturity of host defense mechanisms. Signs and symptoms of neonatal sepsis are often nonspecific. The temperature may be elevated, depressed or normal. Fever in newborn infants may be due to elevated environmental temperature, dehydration or hematoma. A single temperature elevation is rarely associated with infection, but a sustained elevation is highly predictive. In premature infants with sepsis, subnormal temperatures and irregular fluctuations are observed as often as fever[10]. Signs of fetal distress can be the earliest indication of infection in neonates with sepsis, beginning at or soon after delivery. Cyanosis and apnea occur frequently in premature infants but may be signs of sepsis. Fetal tachycardia in the second stage of labor was evaluated as a sign of infection by Schiano and colleagues [28]. Neonatal tachycardia, bradycardia and hypotension are among the common signs of sepsis.

The earliest signs of sepsis often are subtle and nonspecific. Feeding pattern, level of activity, level of alertness, muscle tone, and peripheral perfusion are all affected. The nonspecific and subtle nature of the signs of sepsis in newborns is even more problematic in identifying sepsis in VLBW infants [29]. Septic infants can present with neurologic findings such as seizures and full fontanel even in the absence of meningitis. Gastrointestinal disturbances, including regurgitation, vomiting, large gastric residuals in infants fed with tube, and abdominal distention, are common and significant early signs of sepsis[25]. A variety of skin lesions accompany bacteremia, including abscesses, sclerema, and petechia, etc. Complications of neonatal sepsis include metastatic foci of infection, disseminated intravascular coagulation (DIC), congestive heart failure, and shock.

Diagnosis

The diagnosis of systemic infection in the newborn is difficult to establish on the basis of clinical findings alone. The newborn infant responds similarly to a variety of stresses, regardless of their nature or location. A high index of suspicion is required to identify and evaluate at-risk infants. Rapid identification of the septic infant is important for effective treatment.

Ideally, the mother should be evaluated and appropriate specimens obtained if she is suspected of having an infection.

A definitive diagnosis of neonatal sepsis can be made only with a positive blood culture[24]. Blood, urine and cerebrospinal fluid (CSF) should be obtained from all infants suspected of having sepsis. Obtaining more than one blood culture can be helpful in distinguishing blood culture contaminants from true pathogens[24]. Bacterial growth is evident in most cultures of blood from neonates within 48 hours. The majority of sepsis cases were not confirmed by culture: among inborn patients, 14.6% of EOS was culture-confirmed. Among outborn patients, the confirmation rate was much lower, at only 6.2%[27].

Other Laboratory Tests

Peripheral Blood Count: Leukocytosis and leukopenia, defined as more than 20,000/mm^3 and fewer than 5000/mm^3, respectively, were believed to be reliable indicators of infection but are now known to be insensitive and nonspecific. An absolute neutrophil count outside the normal range, with a ratio of immature (I) to total (T) neutrophils, the I: T ratio, higher than the established norm for age, has been investigated as an early predictor of sepsis. The maximal I: T ratio in uninfected neonates is 0.16 in the first 24 hours of life, decreasing to 0.12 by 60 hours. An I: T ratio of more than 0.2 is considered to be significant in terms of infection. Metsvaht et al[30]. found that neither neutrophil count or I: T ratio, but rather the total white blood cells (WBC), with the cut-off values of $<8.25x10^9$ L-1 at 24 hours and $<3.5x10^9$ L-1 or >39.8 $x10^9$ L-1 at 72 hours of age, has the best discriminative power in the prediction of antibiotic treatment failure in neonates with high risk of EOS. Thrombocytopenia due to bone marrow suppression or DIC may be present. Results of the study of Metsvaht et al[30]. showed that thrombocytopenia ≤ 94.5 $x10^9$ L-1 had excellent discriminative power in infants needing concomitant vasoactive support by 72 hours of age.

Acute Phase Reactants: C-reactive protein (CRP):

CRP is a protein synthesized and secreted by the liver in response to inflammatory cytokines, particularly IL-6. Normal concentration of CRP in neonates is 1 mg/dl or lower. CRP level begins to increase 4-6 hours after an inflammatory trigger and peaks at 36-50 hours. Levels decrease rapidly with the resolution of inflammation. CRP is commonly used for bacterial sepsis detection in neonates, but it is not useful as an early phase infection marker and it lacks specificity [31]. Serial determination of CRP at 12-hour intervals after the onset of signs of sepsis increased the sensitivity of CRP in detecting sepsis [24]. Non-infectious processes can also have an elevated CRP up to 10 times the normal concentration.

Procalcitonin (PCT): PCT is a precursor peptide of the hormone calcitonin. The physiological function of PCT is unknown. PCT has proven to be superior to CRP in children but its value in the first days of life is limited by a marked physiological increase after birth[32]. After injection of endotoxin, PCT concentrations rise within 6-8 hours and reach a plateau after approximately 12 hours. This is a faster response than that seen in CRP.

Erythrocyte Sedimentation Rate (ESR): A micro-ESR test has been developed for use in infants. Clinically, slightly elevated micro-ESR has been noted with superficial infections and with noninfectious processes.

CD64 is expressed at low concentration on the surface of non-activated neutrophils but can be markedly unregulated at the onset of infection. Expression of CD64 on neutrophils has been found to be a promising new marker for bacterial sepsis. Several studies reported its potential utility for diagnostic assessment of sepsis or infection in neonates[33,34].

Increased lipopolysaccharide-binding protein (LBP) levels in bacterial sepsis have already been reported in neonates[35,36]. Groselj-Grenc et al.[37] showed that both CD64 and LBP are good markers for bacterial sepsis in critically ill neonates and children with systemic inflammatory response syndrome (SIRS). They also reported that diagnostic accuracy of CD64 in their study was higher than the diagnostic accuracy of routine laboratory markers PCT and CRP in neonates at the time of suspected sepsis.

Cytokines: Recent evidence shows that neonates react to bacterial sepsis with an exaggerated inflammatory response, which may contribute to the high mortality observed in neonatal sepsis[38]. Cytokines are endogenous chemical mediators that play an important role in the inflammatory cascade. IL-1, IL-6, TNF, and platelet-activating factor (PAF) may be elevated in plasma and in CSF of older children and adults with sepsis. The detection of IL-6 in serum, especially in combination with an elevated CRP measurement, may be useful in the early diagnosis of neonatal infection. Hotoura et al[39]. demonstrated in their study an increase in CRP and in IL-1b, IL-6, and TNF-α in the group of neonates with sepsis to values higher than those in neonates with suspected infection or healthy control subjects with no signs of infection. Ng et al[31]. found that IL-6 levels decreased by 83% 48 hours after the introduction of treatment in VLBW neonates with sepsis. The levels of colony stimulating factor, IL-1 receptor antagonists, serum PCT, and IL-8 are all elevated in newborns with sepsis, but how these laboratory findings can best be utilized for the diagnosis of neonatal sepsis is yet to be defined[41,42]. In one study, IL-1 receptor antagonist and IL-6 levels were found to be elevated 2 days before a diagnosis of sepsis was made[43].

Few clinical studies have reported on lymphocyte subsets in neonates with infection. Juretic et al[44]. showed that preterm neonates with sepsis had lower CD3+ and CD+4 than uninfected premature neonates. Godula-Stuglik et al[45]. showed that full-term neonates with sepsis during the first week of life have a significant increase in CD3+.

Upregulation of many surface activation markers on peripheral blood-derived T cells, monocytes and Natural Killer (NK) cells was shown to be a sensitive marker of neonatal infection[46].

Excluding cultures, none of the previously mentioned tests, when used alone, is sensitive or specific enough to reliably diagnose or exclude neonatal sepsis.

Treatment

If neonatal sepsis is suspected, treatment should be initiated as rapidly as possible after specimens for diagnostic studies have been obtained. The

choice of empirical treatment should be based on several factors: timing and setting of the disease, the microorganisms most frequently encountered, the susceptibility profiles for those organisms, site of the suspected infection, and the safety of the antibiotic. The initial empiric treatment of early-onset infections should consist of ampicillin and an aminoglycoside by intravenous or intramuscular route. Serum concentrations of aminoglycoside should be monitored in LBW premature infants because of erratic absorption and elimination of the drugs in these infants. After the pathogen is identified and its antimicrobial susceptibilities are known, the most appropriate drug or drugs should be selected. The dosage and frequency of administration of antimicrobial agents vary with the newborns' gestational age, postnatal age, birth weight, and status of hepatic and renal function.

Guidelines for determining the duration of therapy in the neonatal period often are lacking, because objective evidence of illness may be minimal. If the neonate appears to be well and there is reason to believe that infection was unlikely, treatment can be discontinued at 48 hours. However, if treatment for infection is deemed necessary, parenteral administration for 10 days is recommended[25].

Resistance to antibiotics is increasing as the uncontrolled antibiotic use pervades in ICUs. It is reported that resistance to ampicillin was 100%, to cefotaxime 88% and to amikacin 23% in gram-negative organisms in a study from a center in Turkey[16]. Thus, antibiotic treatment has to be suitable for the specific care unit, the disease of the neonate and possible etiologic agent of the sepsis[15].

Although appropriate antimicrobial therapy is crucial, supportive care is equally important. Ventilator support, intravenous hydration and parenteral nutrition with close monitoring of electrolytes and glucose should be considered.

Neonates with sepsis may present in or progress to septic shock, exemplified initially by cardiovascular dysfunction requiring fluid resuscitation or inotropic support. Hypotension refractory to fluid load and high-dose catecholamines is associated with a high mortality rate in critically ill neonates.

Dopamine is generally the first-line vasoactive drug, with a starting dose of 5–10 μg/kg/min and dose escalation as needed. For neonates with shock, which is unresolved with volume resuscitation and dopamine, several possibilities exist for additional therapy, including glucocorticoids, other catecholamines, and inotropes/vasodilators. Epinephrine or norepinephrine infusions for refractory shock in neonates have been studied to a very limited extent. Dobutamine also has chronotropic actions, and severe tachycardia may lead to decreased cardiac output that may be corrected by decreasing the dose. Milrinone, a phosphodiesterase inhibitor and inodilator, has not been studied in neonatal septic shock but has been used in pediatric patients with septic shock[47,48].

In hypotensive term and preterm infants, hydrocortisone is increasingly being used to treat refractory hypotension with rapid increase in blood pressure and diuresis. Cortisol production in the neonate is significantly increased early in septic shock. However, very preterm neonates can have relative adrenal insufficiency that may contribute to hemodynamic instability and hypotension. In many clinical practices, hydrocortisone is the third-line agent in treatment of neonatal shock after volume resuscitation and dopamine. In addition to its cytokine-suppressing effects, hydrocortisone has been shown to increase the sensitivity of the cardiovascular system to endogenous or exogenous catecholamines, resulting in improvements in myocardial contractility, stroke volume, effective circulating blood volume, systemic vascular resistance, and urine output[49].

Vasopressin has been used as a rescue therapy in neonates with catecholamine-refractory shock[50,51].

Deficiency of Ig G, which binds to cell surface receptors, provides opsonic activity, activates fixation of complement, promotes antibody-dependent cytotoxicity, improves neutrophil phagocytosis, and can improve neutropenia by enhancing the release of stored neutrophils, enhancing tendency to infections. There have been a number of studies evaluating intravenous immunoglobulin (IVIG) for treatment of infected neonates. A Cochrane systematic review of the prophylactic use of non-specific IVIG in 19 randomized controlled trials (RCTs)

with preterm or LBW infants has demonstrated that IVIG administration resulted in a 3% reduction in sepsis and a 4% reduction in any serious infection, one or more episodes, but is not associated with reductions in other important outcomes: sepsis, necrotizing enterocolitis, intraventricular hemorrhage, or length of hospital stay. Most importantly, IVIG administration does not have any significant effect on mortality from any cause or from infections[52]. Further studies are needed before routine IVIG use in infections.

Pentoxifylline inhibits production of TNF, preserves microvascular blood flow, prevents circulatory failure and intestinal vasoconstriction, and improves survival[53]. Among 107 with positive blood cultures, pentoxifylline was associated with an 86% reduction in risk of mortality[54].

White cell transfusions may also be a clinical approach but there are potential risks from transmission of infection or from graft-versus-host disease, and the technology is not widely available [55]. Exchange transfusion with fresh whole adult blood appeared effective in septic infants, but may also transmit infection[56]. In another RCT, in 776 infants of less than 32 weeks' gestation, there was no evidence that prophylactic fresh frozen plasma reduced risk of mortality from all causes or of disability in survivors at two years[57].

Newborn sepsis is still a major cause of child mortality across the world. Although developed countries have made remarkable progress in reducing newborn sepsis, in developing countries, there are a number of low-cost proven interventions and promising approaches that have the potential to significantly reduce the burden of neonatal sepsis worldwide.

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