

Review Article

Neonatal Sepsis: Insight into Incidence, Classification, Risk Factors, Causative Organisms, Pathophysiology, Prognosis, Clinical Manifestations, Complications, Systemic Examination, and Treatment

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Abstract: **Background:** Neonatal sepsis is a clinical syndrome of bacteremia with systemic signs and is the most important cause of neonatal death in developing countries. Mortality increases with delay in diagnosis and initiation of antibiotics. So, improvements in diagnosis and management of sepsis can significantly decrease the sepsis complications and improve its outcome. **Objectives:** The current review aims to highlight the incidence of neonatal sepsis, classification, risk factors, causative organisms, pathophysiology, prognosis, clinical manifestations, complications, systemic examination, and treatment. The incidence of neonatal sepsis varies among the different geographic areas, the highest being registered in Africa and Asia and the lowest in the U.S.A and Australia. The sepsis has been classified as early-onset sepsis (EOS) and the late-onset sepsis. Late-onset infections onset after 1 month of life, which occurs particularly in VLBW preterm infants or term infants requiring prolonged neonatal intensive care for other chronic problems. Neonatal sepsis includes maternal risk factors and neonatal risk factors. Septic or traumatic risk factors delivery, maternal poverty, pre-eclampsia, cardiac disease and diabetes mellitus are significant risk factors. Prolonged rupture of fetal membranes before the onset of labour, chorio-amnionitis, and maternal genitourinary tract infections have been significantly associated with a wide range of adverse perinatal and maternal outcomes, including miscarriage, stillbirth, preterm birth, fetal growth restriction, neonatal and puerperal sepsis, neonatal encephalopathy and neonatal and maternal mortality. Improvement in the socio-economic status of the population and availability of affordable antenatal care would reduce the incidence of neonatal septicemia. Amniocentesis, cervical cerclage, transcervical chorionic villus sampling, or percutaneous blood sampling, can permit entry of skin or vaginal organisms, causing amnionitis and secondary fetal infection. Infection of skin abrasions after use of obstetric forceps increases the risk of infection in neonates. Infant birth weight is inversely related to risk of early-onset sepsis. Prematurity and low birth weights are the most important neonatal factors predisposing to infection. Endotracheal intubation, insertion of an umbilical vessel catheter, or both, is associated with an increased risk of bacterial infection. Prolonged intravascular access and mechanical ventilation, the use of intravenous intralipids, and the duration of total parenteral nutrition are among the proposed risk factors associated with NICU-acquired coagulase-negative staphylococcus infections. Males have an approximately two-fold higher incidence of sepsis than females. Systemic antibiotics potentiate the overgrowth of certain organisms as *Staphylococcus epidermidis*. Corticosteroid treatment increases the risk of invasive candidiasis among premature infants. Hospital Acquired Infections are one of the leading causes of mortality and morbidity in (NICU). Group B *Streptococcus* (GBS) and *E. coli* as the dominant early onset sepsis (EOS) pathogens and coagulase-negative staphylococci (CONS) as the dominant late onset sepsis (LOS) pathogen followed by GBS and *Staph aureus*. In developing countries, Gram negative organisms are more common and are mainly represented by *Klebsiella*, *E.coli* and *Pseudomonas* of the Gram positive organisms, *Staph aureus*, CONS, *Streptococcus pneumoniae*, and *Streptococcus pyogenes* are most commonly isolated. Agents that commonly cause nosocomial infection are coagulase-negative staphylococci, gram-negative bacilli, Enterococci, and *S. aureus*. Causative organisms are bacterial, fungal, viral, and protozoal organisms' infections. Mode of infection is prenatal, natal, and postnatal

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infections. Soon after birth, the newborns are exposed to the "hostile world" of bacteria, viruses, fungi and parasites, so they must immediately defend themselves. Necrotizing enterocolitis, Neonatal thrombocytopenia, endophthalmitis, abdominal aortic-iliac thrombosis, fatal complete atrioventricular block, and wide range of signs and symptoms that may be observed in neonates with septicemia frequently suggest a number of alternative diagnoses. Identification of infection may be made by isolating the etiologic agent from a sterile body fluid (blood, CSF, and urine), or by demonstrating endotoxin or bacterial antigen in a body fluid. Investigations are including isolation of the microorganism, blood culture, urine culture, tracheal aspirate culture, isolation of organisms from CSF, complete blood cell count, cerebrospinal fluid analysis, C-Reactive protein, and tissue release of procalcitonin. Purpose of supportive care is to normalize the temperature, stabilize the cardiopulmonary status, correct hypoglycemia, and prevent bleeding tendency. Treatment of sepsis includes antibiotics, antiviral, antifungal, intravenous immunoglobulin, granulocyte transfusion, granulocyte-macrophage colony stimulating factor, and double volume exchange transfusion. **Conclusion:** It can be concluded that this review highlighted the incidence of neonatal sepsis, classification, risk factors, causative organisms, pathophysiology, prognosis, clinical manifestations, complications, systemic examination, and treatment.

Keywords: Neonatal Sepsis, Incidence and classification, Risk factors, Pathophysiology, Clinical manifestations, Complications, Treatment.

INTRODUCTION

Neonatal sepsis is a clinical syndrome of bacteremia with systemic signs and symptoms of infection in the first 4 weeks of life. When pathogenic bacteria gain access into the blood stream, they may cause overwhelming infection without much localization (septicemia) or may be predominantly localized to the lung or the meninges (Paolucci *et al.*, 2012). It is the most important cause of neonatal death in developing country (Wynn *et al.*, 2014). It is divided into two categories: early-onset (EOS, sepsis presenting in the first 7 days of life) and late-onset sepsis (LOS, presentations of sepsis after 7 days) (Wynn *et al.*, 2014). Mortality increase with delay in diagnosis and initiation of antibiotics. So, improvements in diagnosis and management of sepsis can significantly decrease the sepsis complications and improve its outcome (Cohen *et al.*, 2011).

Objectives

The current review aims to high light on the incidence of neonatal sepsis, classification, risk factors, causative organisms, pathophysiology, prognosis, clinical manifestations, complications, systemic examination, and treatment.

Neonatal Sepsis Incidence

Sepsis is the commonest cause of neonatal mortality and is responsible for 30-50% of total neonatal deaths each year in developing countries. It is estimated that up to 20% of neonates develop sepsis and approximately 1% die from a sepsis related causes (Bhat *et al.*, 2016).

The World Health Organization (WHO) estimates that 1 million deaths per year (10% of all under-five years' mortality) are due to neonatal sepsis and 42% of these deaths occur in the first week of life (Lawn *et al.*, 2011).

The incidence of neonatal sepsis varies among the different geographic areas, the highest being registered in Africa and Asia (23-38/1,000 live births) and the lowest in countries such as the U.S. and Australia (range, 1.5-3.5 /1,000 live births). In South America and the Caribbean, the incidence of neonatal sepsis ranges between 3.5 and 8.9/1,000 live births (Leal *et al.*, 2012).

Neonatal Sepsis is classified into

Early-Onset Sepsis (EOS)

In many reports the sepsis has been classified as early-onset sepsis (EOS), if the infection starts before 72 hours of life. The infections with "early onset" originate from intrauterine colonization, but may also be acquired during delivery by contact with pathogens in the birth canal (Stoll, 2011).

In addition, they use "very early- onset" disease, if it starts <24 hours of life, when infection probably occurred in utero, justifying the classification of this group as a single entity (Haque *et al.*, 2004).

Late-Onset Sepsis (LOS)

The late-onset sepsis (LOS) defined if it occurred after 3 days of life. It is probably the result of nosocomial infection or community-acquired and neonates usually present with septicemia, pneumonia or meningitis. Factors that might increase the risk of community-acquired LOS include poor hygiene, poor cord care and bottle-feeding. In contrast, breastfeeding helps in prevention of infections (Sankar *et al.*, 2008)

Late-onset sepsis (LOS) is a common complication of prolonged admission to the neonatal intensive care unit

(NICU) following pre-term birth and is most often caused by Gram positive organisms. Late-onset infections (onset after 1 month of life) occur particularly in VLBW preterm infants or term infants requiring prolonged neonatal intensive care for other chronic problems (Stoll, 2011) for neonatal sepsis include: 1-Maternal Risk Factors. 2-Neonatal risk factors.

Maternal Risk Factors

General Condition and Delivery

Septic or traumatic Risk factors delivery, maternal poverty, pre-eclampsia, cardiac disease and diabetes mellitus are significant risk factors (Haque *et al.*, 2004).

Prolonged Rupture of Membranes

Prolonged rupture of membranes (PROM) is defined as rupture of fetal membranes before the onset of labour. Thus, the barrier between the fetus and the outside environment is disrupted which results in leakage of amniotic fluid. PROM may occur in response to untreated infection of the urinary tract or birth canal. (Mercer *et al.*, 2012).

Chorioamnionitis and Maternal Fever

The generally accepted definition for chorio-amnionitis is the presence of fever $>37.5^{\circ}\text{C}$ with two or more of the following findings: fetal tachycardia, uterine tenderness, foul vaginal discharge or maternal leukocytosis (TLC $> 18,000/\text{mm}^3$). Chorio-amnionitis is diagnosed by amniotic fluid analysis or histologically (Stoll, 2011). Maternal fever without signs of chorioamnionitis raises the risk of sepsis but may be confounded by non-infectious causes of maternal fever such as dehydration or epidural anesthesia. Another commonly accepted risk factor is the presence of foul smell of the amniotic fluid due to the presence of anaerobic bacteria (Riley *et al.*, 2011).

Maternal Genitourinary Tract Infections

Maternal genitourinary tract infections have been significantly associated with a wide range of adverse perinatal and maternal outcomes, including miscarriage, stillbirth, preterm birth, fetal growth restriction, neonatal and puerperal sepsis, neonatal encephalopathy and neonatal and maternal mortality. (Anne *et al.*, 2015).

Socioeconomic Status

Improvement in the socio-economic status of the population and availability of affordable antenatal care would reduce the incidence of neonatal septicemia.

Procedures Disturbing the Integrity of the Uterine Contents

Procedures disturbing the integrity of the uterine contents, such as amniocentesis, cervical cerclage, transcervical chorionic villus sampling, or percutaneous blood sampling, can permit entry of skin or vaginal organisms, causing amnionitis and secondary fetal infection (Chiesa *et al.*, 2004)

Traumatic or Septic Delivery

Infection of skin abrasions after use of obstetric forceps or infection of cephalhaematoma increases the risk of infection in neonates (Leal *et al.*, 2012).

Neonatal Risk Factors

Birth Weight

Infant birth weight is inversely related to risk of early- onset sepsis. The increased risk of early-onset sepsis in preterm infants is also related to complications of labor and delivery and immaturity of innate and adaptive immunity (Wynn and Levy, 2010).

Gestational Age

Prematurity and low birth weights are the most important neonatal factors predisposing to infection (Stoll *et al.*, 2012). Extremely preterm infants face a great risk of acquiring neonatal sepsis, with coagulase-negative staphylococci being the most common pathogen in this population. (Ohlin *et al.*, 2015). Premature neonates in particular lack an effective skin barrier, have an immature and often ineffective immune system, and often necessitate prolonged support and hospitalization. (Bizzarro, 2012).

Apgar score

Five minute score <7 carries 56 fold risk of sepsis for infants delivered vaginally higher than the infants with higher scores. Apgar score less than 5 at one minute may be due to sepsis, especially with presence of risk factors for infection (Shah *et al.*, 2006).

Resuscitation at Birth

Infants, who had fetal distress, were born by traumatic delivery or were severely depressed at birth and required

intubation and resuscitation are more vulnerable to develop EOS (Tricia, 2009). Endotracheal intubation, insertion of an umbilical vessel catheter, or both, is associated with an increased risk of bacterial infection. (Stoll, 2008)

Use of Invasive Procedures

Prolonged intravascular access and mechanical ventilation, the use of intravenous intralipids, and the duration of total parenteral nutrition are among the proposed risk factors associated with NICU-acquired coagulase- negative staphylococcus infections. (Bizzarro *et al.*, 2005)

Gender

Males have an approximately two-fold higher incidence of sepsis than females suggesting the possibility of a sex-linked factor in host susceptibility (Stoll, 2008).

Prolonged Use of Antibiotics

Systemic antibiotics potentiate the overgrowth of certain organisms as *Staphylococcus epidermidis* and also responsible for the emergence of antibiotic resistant strains. Also it interferes with colonization by normal flora and facilitates colonization of the infant skin, umbilicus, nasopharynx and gastrointestinal tract by pathogenic bacteria or fungi (Tripathi *et al.*, 2012).

Antibacterial therapy increases the density of *Candida* colonization by reducing the competitive pressure exerted by commensal bacteria, and receipt of broad-spectrum antibacterial antibiotics (e.g., third-generation cephalosporins) is among the most consistently identified risk factors for neonatal candidiasis. (Kelly *et al.*, 2015)

Prolonged Use of Corticosteroids

Corticosteroid treatment increases the risk of invasive candidiasis among premature infants. Corticosteroids alter the number and function of T lymphocytes and result in hyperglycemia, which facilitates growth and inhibits phagocytosis by *Candida* species. (Kelly *et al.*, 2015)

Hospital Acquired Infections (HAI)

Hospital Acquired Infections are one of the leading causes of mortality and morbidity in (NICU). Neonatal nosocomial infections are late-onset infections (appearing after the first 72 h of life) in hospitalized infants. The incidence of infections varies widely among NICUs (7% to 24.5%) depending on environmental factors and on differences in clinical practice. (Stoll, 2011). HAI are a major public health problem worldwide, but particularly in developing countries. Despite intensive surveillance and preventive measures, occur frequently in neonatal intensive care units where they are a leading cause of morbidity and mortality. There is a wide variation in the reported incidence rates of HAI between NICUs, but rates are generally higher in developing countries (Abdel-Wahab *et al.*, 2012).

Causative Organisms

Neonatal surveillance in developed countries generally identifies Group B *Streptococcus* (GBS) and *E. coli* as the dominant early onset sepsis (EOS) pathogens and coagulase- negative staphylococci (CONS) as the dominant late onset sepsis (LOS) pathogen followed by GBS and *Staph aureus*. In developing countries, Gram negative organisms are more common and are mainly represented by *Klebsiella*, *E.coli* and *Pseudomonas* of the Gram positive organisms, *Staph aureus*, CONS, *Streptococcus pneumoniae*, and *Streptococcus pyogenes* are most commonly isolated (Wattal *et al.*, 2011).

Agents that commonly cause nosocomial infection are coagulase-negative staphylococci, gram-negative bacilli, Enterococci, and *S. aureus* (Stoll, 2011).

Bacterial

Gram Positive Organisms

Gram-positive organisms account for 45–77% of infections. Of the Gram-positive organisms, coagulase-negative Staphylococci are the most prevalent (Stoll, 2011).

-Coagulase-Negative Staphylococci (CoNS)

It is the major pathogen involved in LOS, particularly in infants born at a lower gestational age (Marchant *et al.*, 2013). Levels of serum proteins, including transplacental anti- coagulase-negative staphylococci immunoglobulin and complement, correlate with gestational age, and this relative deficiency in preterm infants contributes to their suboptimal opsonization and impaired bacterial killing of coagulase-negative staphylococci. (Strunk *et al.*, 2007)

-Staphylococcus Aureus

Staphylococcus aureus is the causative agent of many nosocomial infections from minor skin abscesses to serious, potentially life threatening diseases such as bone and soft tissue intra-surgical infections, sepsis and invasive endocarditis.

The most infamous drug-resistant forms of these include MRSA (methicillin resistant *S. aureus*), VISA (vancomycin insensitive *S. aureus*), and VRSA (vancomycin resistant *S. aureus*) (Piper *et al.*, 2009)

-Group B Streptococci

It is a major cause of severe systemic and focal infections in the newborn. The organism is a common inhabitant of maternal genital and gastrointestinal tracts and colonized approximately 30 % of pregnant women. The early-onset infection is characterized by septicemia while late-onset GBS infection is characterized by the development of meningitis (Stoll *et al.*, 2012).

-Enterococcus Species

Although accounting for only a small proportion of neonatal sepsis, enterococcus species deserve special mention because of the increasing incidence of neonatal enterococcal sepsis (4–15%) with a mortality rate of approximately 20% in several studies. Risk factors associated with nosocomial enterococcal infections were determined by multiple conditional logistic regression analyses of the cases and controls. Factors identified were placement of a central line, gastrointestinal tract pathology, and administration of multiple antimicrobial agents. The incidence of nosocomial enterococcal infections in children may be controlled by limiting the number of antimicrobial agents administered to hospitalized high risk patients. (Singh-Naz *et al.*, 2000).

-Streptococcus Pneumoniae

It is a rare cause of neonatal sepsis. Its prevalence is low but with a mortality of 50%. Measures to prevent *Streptococcus agalactiae* transmission could help to increase Invasive Pneumococcal Disease (IPD) in newborns. Transmission could be from mother intrapartum; or in those cases of late onset sepsis, the community carriers. Systematic vaccination is a measure that has demonstrated a reduction in the incidence of Invasive pneumococcal disease (Stoll *et al.*, 2012).

Gram Negative Organisms

Although less prevalent, Gram-negative organisms are associated with greater mortality (19% – 36%). Risk factors for Gm –ve blood stream infection are; central venous catheters, catheterization for more than 10 days, nasal continuous positive air way pressure, use of H2 blockers and proton pump inhibitors. GIT tract serves as a reservoir for Gm –ve organisms and colonization in this important system predisposes neonates to infection (Graham *et al.*, 2006).

-Pseudomonas Aeruginosa

Pseudomonas aeruginosa is associated with the highest mortality among premature infants, 45%-74%. *P. aeruginosa* has become an important cause of infection, especially in patients with compromised host defense mechanisms. It is among the most common gram-negative organisms causing nosocomial sepsis in NICU patients and it is the most common pathogen isolated from patients who have been hospitalized longer than 1 week. Pneumonia, urinary tract infections (UTIs), and bacteremia are a complication of Pseudomonal infections and can be life threatening (Lessnau, 2014).

-Escherichia Coli (E.coli)

E. coli is the second leading cause of EOS in neonates, accounting for about 24% of all EOS episodes, with 81% of cases occurring in preterm infants. (Shane and Stoll, 2013)

Escherichia coli associated neonatal meningitis is one of the most common infections that accounts for high mortality and morbidity rates (10–30 %) during the neonatal period (Wijetunge *et al.*, 2015).

-Nontypeable Haemophilus Influenzae

Isolation of Nontypeable *Haemophilus influenzae* from any obstetric or neonatal specimen is associated with chorioamnionitis, preterm birth, pregnancy loss, early-onset neonatal sepsis and neonatal death (Porter *et al.*, 2016). *Haemophilus influenzae* may be vertically transferred from mother to infant at the time of delivery and occasionally causes EOS in preterm infants. Mortality has been reported as high as 90% (Stoll, 2011).

-Enterobacter and Acinetobacter Species

Enterobacter species are common commensals of the human intestinal tract. Enterobacter accounts for 4% to 12% of the cases of bacteremia caused by Gram-negative bacteria and is associated with a relatively high mortality rate (Kuboyama *et al.*, 2003).

-Klebsiella Pneumoniae

It is a normal member of gastrointestinal tract flora has emerged as a significant nosocomial pathogen in neonatal units. Nosocomial klebsiella infections are also remarkably troublesome, particularly in premature infants and intensive

care units. Pediatric patients are easily colonized by klebsiella. Intestinal and oropharyngeal colonization acts as the main reservoir for nosocomial outbreaks. Most studies of bacteremia have not focused on klebsilla as a single entity, but rather have included it in the category of gram negative bacteremia. (Ghotaslou *et al.*, 2007). The high incidence of klebsiella pneumoniae infection is due to its presence in the delivery room suction apparatus and its incidence is markedly decreased after sterilization (De Benedetti *et al.*, 2007).

-Anaerobic Infections

Most episodes of clinically significant anaerobic bacteremia are associated with either in utero infection, gastrointestinal disease as perforation and Necrotizing Entero Colitis (NEC) or cord wound infection (Zaidi *et al.*, 2012).

Fungal Infection

Invasive fungal infection is an important cause of mortality and morbidity in very preterm or very low birth weight infants (Austin *et al.*, 2015).

The majority of invasive fungal infections in children occur in a hospital setting, and the majority of infections are caused by Candida species. In addition to the presence of Candida species in the blood (candidemia), Candida infections can disseminate to each main organ, including the brain, lung, liver, heart, kidneys, eyes and spleen (Liu *et al.*, 2015).

Fungal endocarditis remains an uncommon but life- threatening complication of invasive fungal infections. Neonatal fungal endocarditis (FE) most frequently occurs in very premature infants and is associated with central venous catheters. *C. albicans* is the predominant fungus. (Pana *et al.*, 2015).

Viral Infections

The incidence of viral infection in the NICU was 1%. Enteroviral infections were the most frequently diagnosed infections, occurred often in term infants born at home and presented with sepsis-like illness or seizures. Preterm infants hospitalized from birth mainly developed gastrointestinal disease caused by rotavirus and adenovirus infection or respiratory disease caused by RSV, parainfluenza and CMV infection. Enteroviruses were responsible for the highest mortality and development of serious sequelae (Verboon-Maciolek *et al.*, 2005).

Neonatal herpes simplex virus (HSV) is an uncommon but potentially devastating infection. The incidence of neonatal HSV was 9.6 per 100,000 births. Features suggestive of HSV disease, such as seizures, mucocutaneous lesions, or a sepsis- like syndrome, were sometimes absent on initial clinical presentation. Individually, seizures were seen on presentation in 20% of patients, mucocutaneous lesions in 41%, and critical illness in 43%. Temperature abnormality was not a reliable clinical finding, as 50% of our population had neither fever nor hypothermia on presentation. Delay in acyclovir initiation results in worse outcomes in neonatal HSV, therefore, acyclovir should be initiated as soon as HSV is suspected. (Curfman *et al.*, 2016). Enterovirus and herpes viral infections are important causes of neonatal morbidity and mortality (Stoll, 2011).

Protozoal Organisms

When a mother acquires the infection during gestation, the organism may disseminate hematogenously to the placenta. When this occurs, infection may be transmitted to the fetus transplacentally or during vaginal delivery of untreated maternal infections acquired in the first trimester, approximately 17% of the fetuses are infected usually with severe disease of untreated maternal infections acquired in the third trimester, approximately 65% of the fetuses are infected usually with disease that is mild or inapparent at birth (Stoll, 2011).

Mode of Infection

Prenatal Infection

Throughout pregnancy and until the membranes rupture, the fetus is relatively protected from the microbial flora of the mother by the chorioamniotic membranes, the placenta and the antibacterial factors in amniotic fluid however, there are many ways that infectious agents can reach the fetus to cause infection. Some microbial species cause intrauterine infections that present as congenital infections in the newborn (Klein *et al.*, 2011).

Intrauterine infection is a result of maternal infection with a variety of agents (cytomegalovirus (CMV), Treponemapallidum, Toxoplasma gondii, rubella virus, varicella virus, parvovirus B19) by hematogenous transplacental transmission to the fetus. Transplacental infection may occur at any time during gestation, and signs and symptoms may be present at birth or be delayed for months or years. Infection may result in early spontaneous abortion, congenital malformation, intrauterine growth retardation, premature birth, stillbirth, acute or delayed disease in the neonatal period or asymptomatic persistent infection with sequelae later in life (Stoll, 2011).

Natal Infection

The human birth canal is colonized with aerobic and anaerobic organisms. Vaginal delivery inevitably results in contamination and the beginning of colonization of skin and gut of the newborn. The commonest causative organisms are Group B Streptococci (GBS), gram-negative enteric organisms, Staphylococcus aureus and Streptococcus fecalis (Gomella *et al.*, 2009).

Factors influencing which colonized infant will develop disease are not well understood but include prematurity, underlying illness, invasive procedures, inoculum size, virulence of the organism, the innate immune system and host response and transplacental maternal antibodies. Aspiration or ingestion of bacteria in amniotic fluid may lead to congenital pneumonia or systemic infection, with manifestations becoming apparent before delivery (fetal distress), at delivery (perinatal asphyxia), or after a latent period of a few hours (respiratory distress, shock). Resuscitation at birth, particularly if it involves endotracheal intubation, insertion of an umbilical vessel catheter, or both, is associated with an increased risk of bacterial infection (Stoll, 2008).

Postnatal Infection

It occurs in the delivery room or the newborn nursery via respiratory tract, gastrointestinal tract, umbilical stump, infected circumcision wound. These infections may be transmitted through:

1. Umbilical or peripheral venous catheters, equipments of resuscitation, inhalation therapy, total parenteral nutrition (TPN), or exchange transfusion.
2. Direct transmission of organisms through the hands of nursery or other adult personnel when hand washing techniques are inadequate. (Edmond and Zaidi, 2010).

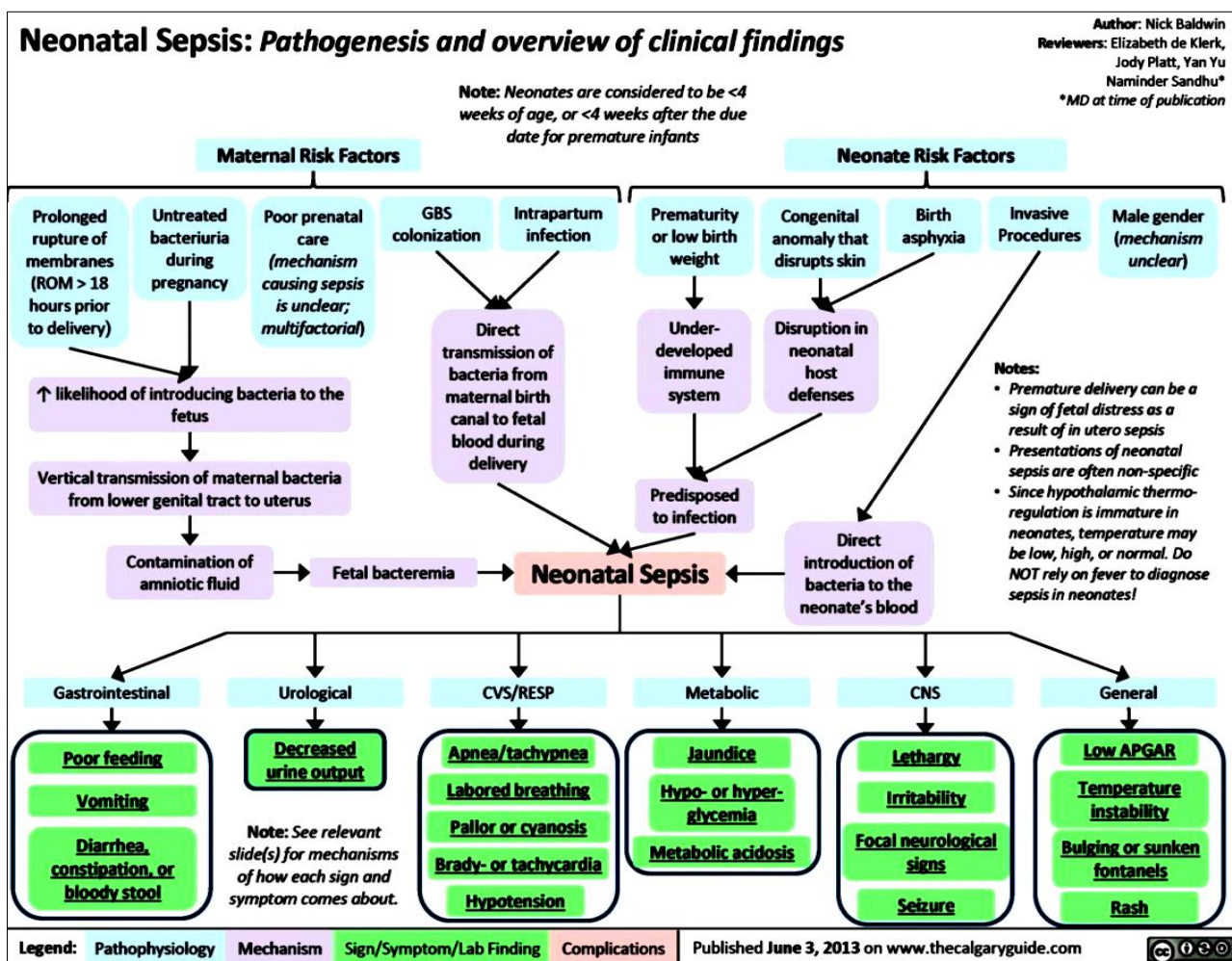


Figure I: Neonatal sepsis and overview of clinical finding (Stoll, 2011).

Pathophysiology of Neonatal Sepsis

Neonates have a low neutrophil storage pool and their existing neutrophils have impaired capacity to migrate from the blood to sites of infection. Decreased fibronectin and reduced cytokine production further impair the innate defensive

mechanisms. Antigen specific T- and B-cell functions are deficient, due to lack of prior antigenic exposure. Response to polysaccharide antigen is poor. T- cells are impaired in both helper and cytotoxic activities and restricted in their production of cytokines (*Basha et al., 2014*).

The fetal immune system develops in a sterile and protected environment and therefore lacks antigenic experience. Soon after birth, the newborns are exposed to the "hostile world" of bacteria, viruses, fungi and parasites, so they must immediately defend themselves. The immunologic competence of the neonate progresses rapidly in the first three months of life, as the cells involved in acquired immunity mature and gain antigenic experience. During this period, the neonate mainly depends upon components of the innate or antigen independent immune system, including phagocytes, natural killer cells, antigen presenting cells, humoral mediators of inflammation and complement (*Marodi, 2006*).

Systemic Response to Infection in Newborns:

The term systemic inflammatory response syndrome (SIRS) is most frequently used to describe this unique process of infection and the subsequent systemic response (*Stoll, 2011*).

SIRS may be a result of a variety of immunologic, endocrinologic, traumatic, surgical, chemotherapeutic, and infectious insults. Sepsis is considered when there is a systemic response to a possible infection. Evidence of bacteremia or an infectious focus is not required (*Angus and Wax, 2011*).

Table 1: The systemic inflammatory response to an infectious process (Stoll, 2011)

Temperature instability <35c or >38c
Respiratory dysfunction
Tachypnea > -2SD above the mean for age.
Hypoxaemia (pa o2 < 70mmHg on room air)
Cardiac dysfunction
Tachycardia > 2SD above the mean for age
Delayed capillary refill >3sec
Hypotension > 2SD below the mean for age
Perfusion abnormalities
Oligurea (urine output <0.5ml/kg/hr)
Lactic acidosis (elevated plasma lactate and/or arterial PH <7.25)
Altered mental status

Patients with SIRS have a spectrum of clinical symptoms that represent progressive stages of the pathologic process. In neonates and pediatric patients, it is manifested as temperature instability, respiratory dysfunction (altered gas exchange, hypoxemia, acute respiratory distress syndrome “ARDS”), cardiac dysfunction and perfusion abnormalities (oliguria and metabolic acidosis). Increased vascular permeability results in capillary leak into peripheral tissues and the lungs with resultant pulmonary and peripheral edema. DIC results in the more severely affected cases. The cascade of escalating tissue injury may lead to multisystem organ failure and death (*Stoll, 2011*).

The American College of Chest Physicians and the Society of Critical Care Medicine convened a Consensus Conference in an attempt to provide a conceptual and practical framework to define the systemic inflammatory response to infection, which is a progressive injurious process that falls under the generalized term “sepsis” and includes sepsis-associated organ dysfunction as well SIRS to a variety of Clinical insults manifested by two or more of the following conditions (*Stoll., 2008*).

Pathogenesis

Host Immunological Factors

Some components of the immune response are particularly important in preventing sepsis. The immune system is traditionally described in terms of the innate and the adaptive immune systems. The innate immune system is responsible for the naive, more rapid and first-line response to infection. At birth, the neonate's own adaptive immune system is largely uneducated. To protect against infection, neonates must therefore rely heavily on innate immune responses and on passive adaptive immune mechanisms acquired from the mother which are deficient in preterm neonates (Sharma *et al.*, 2012).

Mucosal Barriers

The outermost layer of the skin (stratum corneum) acts as a physical barrier and first line of defense against bacterial invasion. The skin secretes AMP, which are early-response factors creating a microbicidal shield particularly effective against CoNS. In preterm neonates, the immature stratum corneum only fully matures at one to two weeks after birth (Marchant *et al.*, 2013).

The vernix caseosa, a waxy coating on neonates' skin, provides additional antimicrobial protection in mature neonates. It is mainly formed during the last trimester of gestation, leaving extremely premature neonates far more vulnerable to infection. (Stoll, 2011) Immunity against CoNS is also limited in other mucosal surfaces in preterm neonates, for example, because of a thinner glycocalyx layer coating the intestinal epithelium, lower secretory IgA, and reduced AMP production by Paneth cells (Marchant *et al.*, 2013).

Cell

Neutrophils also play a major role in protection against neonatal sepsis as first responder leukocytes in the blood. Certain characteristics of neonatal neutrophils have been proposed as mechanisms of increased susceptibility to sepsis: their relatively inefficient recruitment and extravasation to the site of infection; their reduced bacterial killing capacity, in part due to the failure to up regulate their oxidative burst response; and the reduced ability of neonatal neutrophils to form "extracellular traps". (Yost *et al.*, 2009).

Pattern Recognition Receptors (PRR)

PRR detect the presence of microorganisms in the tissue through the recognition of conserved molecular structures specific to microbes (known as pathogen-associated molecular patterns: PAMP). To date, the best characterized PRR are the Toll-like receptors (TLR), which include ten receptors in humans (Marchant *et al.*, 2013).

Recent studies in mice have suggested that Toll-like receptor 2 (TLR2), an extracellular member of the TLR family, plays an important role in the immune recognition of CoNS (Strunk *et al.*, 2011).

Additionally, *S. epidermidis* induces an up regulation of TLR2 and MyD88, and a systemic increase in proinflammatory cytokines (e.g., interleukin (IL)-6) (Kronforst *et al.*, 2012).

Interestingly, activation of TLR2 by a yet unidentified product of *S. epidermidis* triggers the enhanced production of the human AMP family of β -defensins from keratinocytes and underscores a potential role of AMP in the control of staphylococcal infections. Reliance on TLR-induced CoNS immunity has important implications, since preterm neonates exhibit marked defects in TLR signaling cascades and cytokine responses. (Marchant *et al.*, 2013). Indeed, monocytes of premature neonates display a gestational age- dependent reduction in TLR-induced production of proinflammatory cytokines, whereas other monocyte functions related to phagocytosis and intracellular bacterial killing develop earlier, well before 30 weeks of gestation (Strunk *et al.*, 2012).

Bacterial Virulence Factors

CoNS lack several of the virulence factors shared with the closely related *S. aureus* species. Compared with *S. aureus*, *S. epidermidis* produces lower levels of cytolytic toxins. Therefore, *S. epidermidis* must rely on other mechanisms, such as biofilms and the anionic polymer poly- γ -DL-glutamic acid (PGA) to evade hosts' immune responses. (Remington *et al.*, 2011)

Biofilm formation serves as the primary mode of immune evasion of CoNS. These multilayered bacterial aggregates strongly adhere to inanimate objects such as indwelling medical devices. CoNS are particularly adept at biofilm formation, and this capacity is a key mechanism of their pathogenesis, particularly in relation to catheter- related infections. Biofilms act as nonselective physical barriers that obstruct antibiotic diffusion and hinder the cellular and humoral host immune responses. In addition, biofilms provide protection from antimicrobial therapy (Marchant *et al.*, 2013).

Poly-*N*-acetylglucosamine surface polysaccharide, also termed polysaccharide intercellular adhesion (PIA), is

crucial in facilitating cellular aggregation during biofilm formation and is the most extensively studied biofilm molecule. In rat models, PIA defective mutants have been shown to exhibit decreased virulence (Remington *et al.*, 2011).

Sepsis Cascade

Sepsis disturbs the harmonious balance that exists in healthy state between pro and anti-inflammatory cytokines, coagulant and anti-coagulant elements, and between endothelial integrity and circulating cells. Infection by a pathogen disturbs this balance. Body deals with infection by activating many of host defense systems simultaneously to regain the balance. If the balance is regained then outcome is recovery, but if this balance is either not restored or accentuated then the outcome is poor (Shuveksha *et al.*, 2013).

During the inflammatory process, cells of the haemopoetic system and immune modulating mediators are activated to move towards the affected site for destroying the pathogen. Activation of the inflammatory response is initiated by release of endotoxin (LPS) from Gram-negative or exotoxins (peptoglycans) from Gram-positive organism and other cellular antigenic components of the pathogen/s. From then on initiation and maintenance of inflammatory cascade result from a complex array of interactions between pathogen and host defence systems (Shuveksha *et al.*, 2013).

Transcription factors up-regulate the production of pro-inflammatory cytokines such as TNF- α , INF α , IL-6 and anti-inflammatory cytokines IL-10, IL-18 (Haque, 2007).

Preterm VLBW infants are either deficient or inefficient in generating these responses in an adequate manner. In particular, poor transmigration of neutrophils and chemotaxis results in lack of localization of infection hence the neonate is prone to more frequent generalized blood stream infections. The process of activated inflammatory cells producing range of pro-inflammatory mediators like TNF- α , IL-1, IL-6, and IL-8, platelet activating factor (PAF), leukotrienes and thromboxane A₂ accentuate endothelial damage (Kovatchev *et al.*, 2003).

Leak of granulocytes and other mediators through the injured endothelium cause the clinical effects seen in sepsis which can be enumerated by the synonym CHAOS;

C = Cardiovascular; changes in the micro and macro-circulation, decrease vascular tone, poor tissue perfusion, hypotension and organ failure.

H = Haemopoetic; anaemia, neutropenia, disseminated intra-vascular coagulation (DIC).

A = Apoptosis; increase in planned cell death.

O = Organ dysfunction; renal, hepatic and cardiovascular system failure.

S = Suppression of the immune system; immune paralysis (usually transitory).

The process of CHAOS take place with varying degree of severity in every infant with sepsis and correction of CHAOS, the imbalance between pro-inflammatory and anti-inflammatory cytokines, hypercoagulation and fibrinolysis apart from killing the pathogen is required for adequate management of sepsis (Stoll, 2011).

Clinical Manifestations and Complications

One of the most challenging aspects in the management of neonates with sepsis is making the diagnosis. Many of the complications of prematurity, including respiratory distress syndrome and congenital heart diseases have similar manifestations to those associated with sepsis. Several physiological and laboratory parameters have been assessed to diagnose sepsis in neonates (Shane and Stoll, 2013).

Non Specific Manifestations

The manifestations of neonatal septicemia are often vague and therefore demand a high index of suspicion for early diagnosis. The most common and characteristic manifestation is an alteration in the established feeding behavior in late onset sepsis and respiratory distress in early onset sepsis. The baby, who had been active and sucking well, gradually or suddenly becomes lethargic inactive or unresponsive and refuses to suckle. Hypothermia is a common manifestation of sepsis, whilst fever is infrequent. Diarrhea, vomiting and abdominal distension may occur. Episodes of apneic spells or gasping may be the only manifestation of septicemia. In sick neonates, the skin may become tight giving a hide-bound feel (sclerema) and the perfusion becomes poor (capillary refill time of over 3 seconds). Cyanosis may appear. A critical neonate may develop shock, bleeding and renal failure (Carr *et al.*, 2009).

Jaundice is one of the presenting signs of bacterial infection in newborns. UTI was found in 12.5% of the asymptomatic jaundiced neonates with the onset of unconjugated hyperbilirubinemia in the first week of life (Shahian *et al.*, 2012). It is present in approximately one third of infants with sepsis (Wilson *et al.*, 2015). Change in mental status can help in identifying septic shock before hypotension occurs (Carcillo and Fields, 2002).

-Fever and Hypothermia: Hypothermia is more common in preterm and low birth weight infants (*Sankar et al., 2008*).

Skin Changes: Multiple pustules, abscess, mottling, umbilical redness and discharge (*Sankar et al., 2008*).

-Systemic Examination

Respiratory manifestations

Dyspnea (grunting, nasal flaring and/or chest retractions), tachypnea (respiratory rate >60/min during >1 hour), need for increased respiratory support (intensifying the modus, i.e. low flow, CPAP or endotracheal ventilation and/or degree of respiratory support), increasing need for supplemental oxygen & increasing frequency of apnoea (*Bekhof et al., 2013*).

Cardiovascular Manifestations

Heart rate variability (HRV) analysis and noninvasive cardiac output have been shown to be useful adjuncts to sepsis detection in many patient groups. HRV are more sensitive than traditionally used vital signs, such as cardiac output and mean arterial pressure, in the confirmation of sepsis in extremely low birth weight neonates. HRV may allow for earlier identification of septic physiology (*Bohanon et al., 2015*).

Renal Manifestations

Sepsis is a cause of significant morbidity and mortality in neonates. Sepsis has been consistently shown to be a risk factor for the development of acute kidney injury (AKI) across neonatal populations. Those who developed AKI had a lower birth weight and were more likely to have meningitis, disseminated intravascular coagulation, and septic shock. Neonates who develop sepsis are classically thought to be predisposed to AKI secondary to the hypotension associated with systemic inflammation, but there also appears to be a direct impact on the kidneys (*Blatt et al., 2014*).

Furthermore, AKI may develop despite the maintenance of systemic blood pressures and renal blood flow, suggesting that sepsis may directly damage the kidney by effects on microvasculature (*Selewski et al., 2015*).

Central Nervous System

Clinical signs of neonatal meningitis are usually very ill defined and more than 30% of them occur in asymptomatic infants. Frequently, findings associated with neonatal sepsis (e.g., temperature instability, respiratory distress, jaundice, apnea) are manifest. Central nervous system (CNS) signs (e.g., lethargy, seizures (particularly focal), vomiting, irritability) more specifically suggest meningitis. A bulging or full fontanelle occurs in about 25% and nuchal rigidity in only 15%. Cranial nerve abnormalities may also be present (*Baud and Aujard, 2013*).

Disseminated Intravascular Coagulation (DIC)

It is uncontrolled, simultaneous bleeding and clotting occurring as a secondary disorder in sick neonates. Among critically ill patients, the risk of developing disseminated intravascular coagulation (DIC) is probably highest in neonates. Low plasma reserves in pro- and anticoagulant coagulation factors, intravascular volume contraction after birth, and a high incidence of hypoxia and sepsis in critically ill newborns rapidly lead to a decompensation of the coagulation system in this population (*Veldman et al., 2010*).

Complications of Neonatal Sepsis

Septic Shock

Septic shock is defined as sepsis with hypotension despite adequate fluid replacement and hypoperfusion that may lead to lactic acidosis, oliguria or an acute alteration in mental status. Septic shock may progress to multiple organ dysfunction syndrome (MODS) and death (*Sherlock, 2009*).

Brain Abscess: Complicating staphylococcus aureus sepsis in a premature infants (*Vartzelis et al., 2005*).

Hydrocephalus and/or Periventricular Leukomalacia

Pneumonia

Early onset has high mortality (50%) especially when due to GBS. Late onset has less mortality rate (15%) despite it is association with bacteremia and meningitis (*Linda and Bryan, 2004*).

Necrotizing Enterocolitis

Incidence ranges from 1-5% of admission to NICUs (*Gephart et al., 2014*). Multiple risk factors have been identified that predispose the infant to NEC. Many of these risk factors are related to neonatal problems that may reduce gastrointestinal blood flow as antenatal factors, multiple gestation, patent ductus arteriosus and polycythemia (*Stoll, 2011*).

Sepsis Induced Disseminated Intravascular Coagulation

Sick baby have petechia, GIT bleeding, oozing from venipunctures and hypoxia, decrease platelet count, prolonged PT, PTT, fragmented red blood cells seen in bloodsmear and decrease fibrinogen and increase fibrin split products (*Ng et al., 2006*).

Neonatal Thrombocytopenia (Roberts and Murray, 2006)

Endophthalmitis

It may be due to *Serratia marcescens* in the course of early onset neonatal sepsis or occur as a complication of late-onset sepsis in a premature infant in the form of bilateral endogenous endophthalmitis (*Matasova et al., 2003*).

Abdominal Aortic-Iliac Thrombosis: In a group B streptococcus sepsis with a renal vascular hypertension (*Stoll, 2011*).

Fatal Complete Atrioventricular Block: In a premature newborn (*Lucos et al., 2005*).

Differential Diagnosis

The non-specificity and wide range of signs and symptoms that may be observed in neonates with septicemia frequently suggest a number of alternative diagnoses (*Stoll, 2011*).

Table 2: Serious systemic illness in newborns (Stoll, 2011)

<p>Cardia Congenital Hypoplastic left heart - Acquired: Myocarditis, hypovolemic syndrome, other structural disease, PPHN or cardiogenic shock, PPHN</p> <p>Gastrointestinal</p> <ul style="list-style-type: none"> - Necrotizing enterocolitis - Spontaneous GI perforation - Structural abnormalities <p>Hematologic</p> <ul style="list-style-type: none"> - Neonatal purpurafulminans - Severe anemia - Immune-mediated neutropenia - Hereditary clotting disorders - Immune-mediated thrombocytopenia - Malignancies (congenital leukemia) <p>Metabolic</p> <ul style="list-style-type: none"> - Hypoglycemia - Adrenal disorders: Adrenal hemorrhage, adrenal insufficiency, congenital adrenal hyperplasia - Inborn errors of metabolism: Organic acidurias, lactic acidosis, urea cycle disorders, galactosemia <p>Neurologic</p> <ul style="list-style-type: none"> - Intracranial hemorrhage: spontaneous, child abuse - Hypoxic-ischemic encephalopathy - Neonatal seizures - Infant botulism <p>Respiratory</p> <ul style="list-style-type: none"> - Respiratory distress syndrome - Aspiration pneumonia: Amniotic fluid, meconium, or gastric contents - Lung hypoplasia - Tracheoesophageal fistula - Transient tachypnea of the newborn
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Investigations

Isolation of the Microorganism

Identification of infection may be made by isolating the etiologic agent from a sterile body fluid (blood, CSF, urine), or by demonstrating endotoxin or bacterial antigen in a body fluid (*Zaidi et al., 2012*).

Blood Culture

Early diagnosis of neonatal sepsis is challenging because clinical characteristics are non-specific and difficult to differentiate from those of non-infectious etiologies, and because the repertoire of ancillary laboratory tests is limited and not always reliable. Blood culture remains the gold standard for diagnosis of neonatal sepsis, but the rate of positivity is

low, influenced by factors such as intrapartum antimicrobial administration and limitations in blood volume per culture that can be obtained in neonates (Camacho-Gonzalez *et al.*, 2013).

One classic study, focusing on *E. coli* infection, found that neonates have high-colony-count bacteremia. However, a more recent study including other common neonatal-sepsis pathogens found that 68% of septic infants have low-level bacteremia (≤ 10 Colony-forming units (CFU)/ml) and 42% have counts ≤ 1 CFU/ml. In low-colony-count bacteremia, as many as 60% of cultures will be falsely negative with 0.5 ml sample volumes. Multiple blood cultures could help increase the yield of this test, but studies in the neonatal period have shown conflicting results (Zea-Vera *et al.*, 2015).

Blood cultures are done to detect the presence of bacteria or yeasts, which may have spread from a specific site in the body into the bloodstream. Blood culture usually done when having symptoms of septicemia or sepsis, which indicates that bacterium or their products are causing harm in the body. Blood cultures are drawn more frequently in newborns that may have an infection but may not have the typical signs and symptoms of sepsis. Similarly, blood cultures are collected in young children to detect serious infections (Stoll *et al.*, 2012).

Urine Culture

Sterile acquired bladder tap or catheterized specimens minimize false positive cultures but they are difficult to obtain in the low-urine state of the newborn and there is a very low yield in the first 72 hours of life. Therefore, a urine culture is not suggested as part of the work-up for EOS. It is most appropriate when investigating LOS (Ottolini *et al.*, 2003). Urine cultures (only useful after the 3rd day of life) should be obtained (Camacho-Gonzalez *et al.*, 2013)

Tracheal Aspirate Culture

Tracheal aspirate culture has proven to be useful when obtained within the first 12 hours of life. A positive tracheal aspirate culture may be found in 44% of infants with clinical pneumonia and negative blood cultures (Gerdes, 2004).

Isolation of Organisms from CSF

The diagnosis of meningitis is confirmed by examination of CSF and identification of a bacterium, virus, or fungus by culture, antigen, or the use of PCR (Stoll, 2011).

Complete Blood Cell Count

Neutropenia is more common than neutrophilia in severe NS (Gotoff, 2000). Early-onset neutropenia (ANC) in premature infants has previously been correlated with sepsis, maternal hypertension (HTN), severe perinatal asphyxia and periventricular hemorrhage. Late-onset neutropenia in stable, growing premature infants; defined as ANC $< 1500/\text{mm}^3$ at a postnatal age of ≥ 3 weeks has been recently reported (Gamal El-Din, 2005).

Timing is also important (Gerdes, 2004). However, a normal WBC and differential can be initially seen. A second WBC performed at 12 to 24 hours of age can be helpful in making the diagnosis of infection (Cloherty *et al.*, 2004).

The Absolute neutrophil count, determined by multiplying the WBC count by the fraction of neutrophils plus bands in the differential, is only slightly better predictor of sepsis than the total WBC count. The specificity of the WBC parameters as an indicator of sepsis is increased by calculating the ratio of the concentration of immature cells of neutrophilic series (band forms) to total cells of neutrophil series, known as the I:T ratio. An increased concentration of immature neutrophil series cells and an I:T ratio of greater than 0.2 has moderately increased specificity for sepsis. The I:T ratio takes into consideration the nonnative values over the first days of life and is less than or equal to 0.12 by the third day of life in noninfected infant. It has only moderate sensitivity, but good negative predictive value if normal (McMillan *et al.*, 2006). Morphological changes in the neutrophil are often severe and late signs of infection (Ng, 2004).

Microscopic evaluation to determine the presence of reactive morphologic changes in neutrophils such as toxic granules, Döhle bodies and cytoplasmic molecules has a high sensitivity (80%) but a low specificity (58%) in predicting infection (Ahmed, 2003).

Thrombocytopenia is generally observed after sepsis has been diagnosed and usually lasts 1 week, though it can last as long as 3 weeks. Only 10-60% of infants with sepsis have thrombocytopenia because of the appearance of newly formed platelets. Mean platelet volume (MPV) and platelet distribution width (PDW) are shown to be significantly higher in neonatal sepsis after 3 days (Remington *et al.*, 2005). A decrease in the platelet count, usually less than 50 000, has a known association with infection often from classes of organisms but not a specific organism (Tappero and Johnson, 2010).

Thrombocytopenia and evidence of DIC (prolonged PT, PTT, elevated international normalized ratio (INR) and decreased fibrinogen) can be seen in severely ill infants (Cloherty *et al.*, 2004).

HSS assigns a score of 1 for each of seven findings significantly associated with sepsis: Abnormal total leukocyte count, abnormal total PMN count, elevated immature PMN count, elevated immature to total (I:T) PMN ratio, immature to mature (I:M) PMN ratio ≥ 0.3 , platelet count $\leq 150,000/\text{mm}^3$, and pronounced degenerative or toxic changes in PMNs. An abnormal total PMN count is assigned score of two instead of 1, if no mature polymorphs are seen on the peripheral smear to compensate for the low I:M ratio (*Manisha Makkar et al.*, 2013)

Cerebrospinal Fluid Analysis

Lumbar puncture should be done in all cases of late onset (> 72 hours) and symptomatic early onset sepsis because 10-15 percent of them may have associated meningitis. In early onset sepsis, a lumbar puncture is indicated in the presence, of either a positive blood culture or presence of clinical picture of septicemia (*Sankar et al.*, 2008).

The CSF findings in infectious neonatal meningitis are elevated WBCs (especially polymorphnuclear leucocytes), elevated protein level, depressed glucose level and positive culture. The CSF abnormalities are more severe in LOS and with Gram-negative organisms (*Ali*, 2006).

C- Reactive Protein

CRP was first described in the 1930's and since then multiple studies have shown elevation of the CRP in several infectious and non-infectious etiologies that share a common background of inflammation or tissue injury. In neonates, serial measurements of the CRP in the first 24–48 hours of symptoms increases the sensitivity of the test, with suggestion that normal CRP values during this period of time have a 99% negative predicted value for determination of infection. In contrast, elevated levels of CRP may be more difficult to interpret, especially for diagnosis of EOS because factors such as PROM, maternal fever, pregnancy-induced hypertension, prenatal steroid use, and fetal distress may also cause elevation of the CRP (*Camacho-Gonzalez et al.*, 2013)

IL-6 is the major stimulus for the production of CRP along with IL-1 and tumor necrosis factor- α . CRP is released 4 to 6 hours after the onset of stimulus, reaches a peak at 24 to 48 hours and then diminishes over time as the inflammation resolves (*Gerdas*, 2004).

As the concentrations of CRP increase rather slowly in the initial phase, the sensitivity at the time of sepsis evaluation is only 60%. Serial measurements at 24 and 48 hours after the onset of illness considerably improve the sensitivity (82% and 84% respectively) (*Ng*, 2004).

The specificity and positive predictive value of CRP range from 93% to 100% throughout the study period. Thus, CRP can be considered as a “specific” but “late” marker of neonatal infection (*Badrawi et al.*, 2005).

Grella, (2007) have considered a CRP value >10 mg/L combined with an immature: total neutrophil ratio >0.25 as a criterion to start antibiotic therapy even in babies with no symptoms of infection.

Gestational age influences CRP kinetics, with preterm infants having a lower and shorter CRP response compared to healthy term infants. Studies suggest that CRP is best used as part of a group of ancillary diagnostic tests to help determine if an infant has infection, rather than as a single test (*Camacho-Gonzalez et al.*, 2013). CRP concentration increases within 6 to 8 hours of an infectious episode in neonates and peaks at 24 hours (*Benitz*, 2010).

Procalcitonin

Tissue release of procalcitonin (PCT) increases with infection making it a potential marker for early detection of sepsis. PCT differs from CRP, in that PCT levels increase more rapidly and may be more useful for detection of EOS. Auriti and colleagues in a multicenter, prospective observational study of 762 neonates showed a significant increase in the median value of PCT level in neonates with sepsis compared with those without sepsis (3.58vs 0.49 ng/ml; $P<0.001$). In addition, a cut-off value of 2.4 ng/ml was suggested as the most accurate level for differentiation of sepsis in neonates regardless of gestational age, with a sensitivity of 62% and a specificity of 84% (*Auriti et al.*, 2012).

Mannose-Binding Lectin (MBL)

It is a plasma protein, primarily produced by the liver with an important role in the innate immune defense. MBL activates the lectin pathway of the complement system increasing opsonization and enhancing phagocytosis (*Neth et al.*, 2000).

Genetic polymorphisms in the MBL gene have been associated with an increased risk of sepsis. In a recent study in which MBL levels were measure in 93 neonates, development of sepsis was associated with lower levels of MBL and with the presence of BB genotype in exon 1 of the MBL gene. MBL remains a research tool with further studies needed to confirm diagnostic utility (*Özkan et al.*, 2012).

Cytokine Profile

Multiple cytokines have been studied for diagnosis of neonatal sepsis including IL-6, IL-8, IL-10 and TNF alpha. IL-6 and IL-8 increase very rapidly with bacterial invasion but they promptly normalize in serum levels (within the first 24 hours), limiting their ability to be used as clinical markers. TNF alpha has not shown to have high sensitivity, but the ratio of IL-10 and TNF alpha has been used for diagnosis of LOS in VLBW neonates with some success. Evaluating a combination of cytokine profiles may increase likelihood of identifying infection than single measurements (Camacho-Gonzalez *et al.*, 2013).

Neutrophil CD64 and Neutrophil/Monocyte CD11B

These specific markers are cell surface antigens whose production increases after activation of leukocytes by bacteria and therefore can potentially be used for diagnosis of neonatal sepsis. Their up-regulation precedes that of CRP suggesting potential use in EOS. A recent study by Genel showed that CD64 had a sensitivity and specificity to accurately identify neonatal sepsis of 81 and 77% respectively, with a negative predictive value (NPV) of 75%. Similarly, CD11b had a sensitivity and specificity of 66 and 71%. Cost and processing time may be barriers to use of these markers in clinical practice (Genel *et al.*, 2012).

Molecular techniques for Early Detection of Neonatal Sepsis

Important advances have been made in molecular diagnostics, and studies of real time PCR (RT-PCR) and a broad range of conventional PCR assays suggest improved sensitivity and specificity for sepsis diagnosis. A meta-analysis done by Pammi *et al.*, (2011) found that sensitivity and specificity of RT-PCR was 0.96 (95% C.I for sensitivity: 0.65–1.0 and 95% C.I. for specificity: 0.92–0.98). Similarly, broad range PCR had a sensitivity of 0.95 (95% C.I 0.84-0.98) and specificity of 0.98 (0.95–1.0). However, neither test achieved the minimum limits of sensitivity or specificity set up by the study and results were insufficient to replace blood cultures for diagnosis of neonatal sepsis.

Genomics and Proteomics

Exciting alternatives for detection of neonatal sepsis include the use of genomics and proteomics for identification of host response biomarkers. Genomics targets genes that are up regulated with infection and proteomics analyzes the structure, function and interactions of proteins produced by a particular gene. Early studies in neonates have suggested potential utility in these techniques for identification of sepsis and necrotizing enterocolitis. A score based on proapolipoprotein CII (Pro-apoC2) and a des-arginine variant of serum amyloid, was used to withhold antibiotics in 45% of patients with suspected infection and to discontinue antibiotics in 16% (Camacho-Gonzalez *et al.*, 2013).

Treatment of Neonatal Sepsis

The Supportive Care

Purpose of supportive care is to normalize the temperature, stabilize the cardiopulmonary status, correct hypoglycemia and prevent bleeding tendency. The septic neonate should be nursed in a thermo neutral environment. If hypothermic, the temperature should be raised using a heat source. An intravenous line should be established. If perfusion is poor as indicated by a capillary refill time of more than 3 seconds, normal saline bolus should be infused immediately. A dextrose bolus will help correct hypoglycemia which is often present in septic infants. Vitamin K should be given to prevent bleeding. Oxygen should be provided if the infant is having retractions, grunt or cyanosis. Apneic neonates should be given physical stimulation and bag-mask ventilation, if required. Enteral feeds are avoided if infant is very sick or has abdominal distension. Appropriate maintenance intravenous fluids are administered. In neonates with sclerema, exchange transfusion with fresh whole blood may be contemplated (MacKay, 2010).

Monitoring of blood pressure, vital signs, hematocrit, platelets, and coagulation studies is vital. Not uncommonly, blood product transfusion, including Packed Red Blood Cells (PRBCs), platelets, and Fresh Frozen Plasma (FFP), is indicated (Manzoni *et al.*, 2010).

Pressor agents as dopamine or dobutamine may be needed (Gotoff, 2004). Surgical consultation for central line placement may be necessary in infants who require prolonged IV antimicrobial therapy for sepsis, if peripheral IV access cannot be maintained (Davis *et al.*, 2008). Surfactant replacement therapy is given in PT infants who are mechanically ventilated for respiratory insufficiency of bacterial sepsis in a dose of 50 or 100 mg / kg (Elmikkawy, 2000).

Antimicrobial Treatment of Sepsis

Antibiotics

Current approach to the treatment of early-onset neonatal sepsis includes combined IV aminoglycoside and expanded-spectrum penicillin antibiotic therapy. This provides coverage for gram-positive organisms, especially group B *Streptococcus* (GBS), and gram-negative bacteria, such as *Escherichia coli*. The specific antibiotics to be used are chosen on the basis of maternal history and prevalent trends of organism colonization and antibiotic susceptibility in individual

nurseries (Tzialla *et al.*, 2015).

Reserving broad-spectrum therapy for high-risk infants and quickly de-escalating once culture results are available is one strategy for improving neonatal outcomes. If an infection appears to be nosocomial (late-onset sepsis), antibiotic coverage should be directed at organisms implicated in hospital-acquired infections, including *S aureus*, *Sepidermidis*, and *Pseudomonas* species. Most strains of *S aureus* produce beta-lactamase, which makes them resistant to penicillin G, ampicillin, carbenicillin, and ticarcillin. Vancomycin has been favored for this coverage; however, concern exists that overuse of this drug may lead to vancomycin-resistant organisms, thereby eliminating the best response to penicillin-resistant organisms. For this reason, some clinicians prefer oxacillin therapy in this setting (Shipp *et al.*, 2015).

Cephalosporins are attractive in the treatment of nosocomial infection because of their lack of dose-related toxicity and their ability to reach adequate serum and cerebrospinal fluid (CSF) concentrations; however, their use has led to resistance in gram-negative organisms. Ceftriaxone displaces bilirubin from serum albumin and should be used with caution in infants with significant hyperbilirubinemia. Resistance and sensitivities for the organism isolated from cultures are used to select the most effective drug (Tzialla *et al.*, 2015).

Aminoglycosides and vancomycin both have the potential to produce ototoxicity and nephrotoxicity and should therefore be used with caution. The serum drug level is assessed around the third dose or at 48 hours after the start of treatment to determine whether levels are within the therapeutic range. The drug dosage or interval may have to be adjusted to optimize the drug serum levels. Infants who received aminoglycosides should undergo audiology screening before discharge. If the infant's clinical condition has not improved, a serum level may also be warranted to ensure that a therapeutic level has been reached. In addition, renal function and hearing screening should be considered after completion of the therapeutic course to determine whether any short- or long-range toxic effects of these drugs have occurred (Zaidi *et al.*, 2012).

If culture results are negative but the infant is at significant risk for or has clinical signs of sepsis, the clinician must decide whether to provide continued treatment. In most cases, 2-3 days of negative culture results should allow the clinician to be confident that sepsis is absent; however, a small number of infants shown to have had sepsis by postmortem examination had negative culture results during their initial sepsis (Tzialla *et al.*, 2015). Treatment for 7-10 days may be appropriate, even if culture results remain negative at 48-72 hours. (Shipp *et al.*, 2015). Because of its excellent CSF penetration, empirical or therapeutic use of cefotaxime should be restricted for use in infants with meningitis attributable to Gram-negative organisms (Richard and Polin, 2012).

Bacteremia without an identifiable focus of infection is generally treated for 10 days (Nizet, 2010). Uncomplicated meningitis attributable to GBS is treated for a minimum of 14 days (Pickering, 2009).

Other focal infections secondary to GBS (eg, cerebritis, osteomyelitis, endocarditis) are treated for longer durations. Gram negative meningitis is treated for minimum of 21 days or 14 days after obtaining a negative culture, whichever is longer (Pickering *et al.*, 2009). Treatment of Gram-negative meningitis should include cefotaxime and an aminoglycoside until the results of susceptibility testing are known (Nizet *et al.*, 2010).

The duration of antimicrobial therapy in infants with negative blood cultures is controversial. Many women receive antimicrobial agents during labor as prophylaxis to prevent early-onset GBS infections or for management of suspected intra-aminiontic infection or PPROM. In those instances, postnatal blood cultures may be sterile (false negative) (Richard and Polin, 2012).

Antiviral

There are several antiviral agents that can be used for the treatment of neonatal viral infections. Acyclovir (60 mg/kg/d IV divided every 8 hours) is the treatment of choice for term infants with herpes simplex virus (HSV) and varicella-zoster infections. After parenteral therapy with acyclovir, it is recommended to give HSV suppressive regimen (300 mg/m²/dose po tid), which improves neurodevelopmental outcomes of infants with central nervous system involvement (Santos and Tristram, 2015).

Antifungal

For candidiasis, IV amphotericin B deoxycholate (1 mg/ kg/ d) and IV fluconazole (loading dose 25 mg/d on day 1, then 12 mg/kg/d on 2 day) may be used for susceptible isolates. Lipid-based amphotericin (3– 5mg/kg/d) can be used if there is no renal involvement because of inadequate kidney penetration. For aspergillosis, voriconazole (loading dose 18 mg/kg/d divided every 12 hours on day 1, then 16 mg/kg/d on day 2) is the drug of choice (Santos and Tristram, 2015).

Fluconazole prophylaxis (6 mg/kg/d twice a week) may be indicated in high-risk infants with birth weight of less than 1000g from institutions with high incidence of candidiasis (above 10%).

Luconazole prophylaxis (25 mg/kg once weekly) may be offered to young infants younger than 4 months old on extracorporeal membrane oxygenation (Santos and Tristram, 2015).

Intravenous Immunoglobulin

Maternal transport of immunoglobulins to the fetus mainly occurs after 32 weeks' gestation, and endogenous synthesis begins several months after birth. Administration of intravenous immunoglobulin (IVIG) provides immunoglobulin G (IgG) that can bind to cell surface receptors, provide opsonic activity, activate complement, promote antibody-dependent cytotoxicity and improve neutrophilic chemo-luminescence. Theoretically, infectious morbidity and mortality could be reduced by the administration of IVIG (Ohlsson and Lacy, 2015).

The undisputable results of a trial, which enrolled 3493 infants, and meta-analyses (n = 3973) showed no reduction in mortality during hospital stay, or death or major disability at two years of age in infants with suspected or proven infection. Although based on a small sample size (n = 266), this update provides additional evidence that IgM-enriched IVIG does not significantly reduce mortality during hospital stay in infants with suspected infection. Routine administration of IVIG or IgM-enriched IVIG to prevent mortality in infants with suspected or proven neonatal infection is not recommended (Ohlsson and Lacy, 2015).

Granulocyte Transfusion

Granulocyte transfusion has been shown to be suitable for infants with significant depletion of the storage neutrophil pool however, the documentation of storage pool depletion requires a bone marrow aspiration, and the granulocyte transfusion must be administered quickly to be beneficial. The number of potential adverse effects, such as graft versus host reaction, transmission of cytomegalovirus (CMV) or hepatitis B, and pulmonary leukocyte sequestration, is considerable. Therefore, this therapy in neonatal sepsis remains experimental (Stoll *et al.*, 2012).

Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF)

It is often used in neonatal sepsis, however a recent study found that, while GM-CSF corrects neutropenia if present, it has no effect on reducing sepsis or improving survival (Carr *et al.*, 2009).

Double Volume Exchange Transfusion (DVET)

DVET removes bacterial toxins and pro-inflammatory cytokines from the blood and replaces with fresh and immunologically replete blood thereby leading to improvement in tissue perfusion and oxygenation. DVET showed a trend towards reduction in mortality of 21% in comparison to ST in severely septic neonates of >1000 g birth weight. A significant improvement was observed in the cardiovascular and hematological organ functions following DVET. DVET was associated with a significant improvement in IgA, IgG, IgM, complement 3 and metabolic acidosis in comparison to the standard therapy. Thus, DVET is a safe procedure in severely septic neonates (Aradhya *et al.*, 2016).

Prognosis of Neonatal Sepsis

Bacterial sepsis and meningitis continue to be major causes of morbidity and mortality in the newborn. This is despite improvements in antimicrobial therapy, advances in neonatal life support measures, and the prompt recognition of the perinatal risk factors for infection. Sepsis neonatorum can be devastating and surviving infants can have significant neurologic sequel as a consequence of central nervous system involvement, septic shock, or hypoxemia secondary to severe parenchymal lung disease or persistent pulmonary hypertension (Guerina *et al.*, 2012).

Stoll (2012) stated that inflammatory lesions were found in about 25% of newborn infant autopsies, only second in frequency to hyaline membrane disease. The mortality rate for sepsis varies from 10-40%, and for meningitis about 15-50% with significant neurologic sequelae as hydrocephalus, mental retardation, blindness, hearing loss, motor disability and abnormal speech pattern. These can occur in up to 30-50% of survivors. Babies with septicemia often have multiple additional problems as they may develop broncho-pulmonary dysplasia secondary to hyperventilation for their persistent pulmonary hypertension (PPH). In addition, they may also have intracranial hemorrhage or periventricular leukomalacia (Rennie and Robertson, 2013).

Despite careful hygiene and powerful broad spectrum antibiotic treatment, neonatal septicemia remains an unsolved problem associated with a high mortality (Guiral *et al.*, 2012).

CONCLUSION

It can be concluded that the incidence of neonatal sepsis varies among the different geographic areas. The sepsis has been classified as early-onset sepsis and the late-onset sepsis. Neonatal sepsis includes maternal risk factors and neonatal risk factors. Males have an approximately two-fold higher incidence of sepsis than females. Systemic antibiotics potentiate the overgrowth of certain organisms. Corticosteroid treatment increases the risk of invasive candidiasis among

premature infants. Causative organisms are bacterial, fungal, viral, and protozoal organism's infections. Mode of infection is prenatal, natal, and postnatal infections. Identification of infection may be made by isolating the etiologic agent from a sterile body fluid (blood, CSF, and urine), or by demonstrating endotoxin or bacterial antigen in a body fluid. Investigations are including isolation of the microorganism, blood culture, urine culture, tracheal aspirate culture, isolation of organisms from CSF, complete blood cell count, cerebrospinal fluid analysis, C-Reactive protein, and tissue release of procalcitonin. This review highlighted the incidence of neonatal sepsis, classification, risk factors, causative organisms, pathophysiology, prognosis, clinical manifestations, complications, systemic examination, and treatment.

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