

INTRODUCTION

Neonatal mortality contributes significantly to infant mortality rates in developing countries, various conditions are responsible for neonatal mortality among which is neonatal sepsis, which accounts for about 26% of neonatal mortality. About four millions neonatal deaths occur worldwide with reported highest rates in Sub-Sahara Africa (*Lawn et al., 2005*).

Neonatal sepsis can be categorized as early-onset or late-onset. Of newborns with early-onset sepsis, 85% present within 24 hours, 5% present at 24-48 hours, and a smaller percentage present within 48-72 hours. Onset is most rapid in premature neonates. Early-onset sepsis is associated with acquisition of microorganisms from the mother, transplacental infection or an ascending infection from the cervix which may be caused by organisms that colonize the mother's genitourinary tract. The neonate acquires the microorganisms as it passes through the colonized birth canal at delivery (*Klinger, 2009*).

Late-onset sepsis occurs at 4-90 days of life and is acquired from caregiving environment (*Hoogen et al., 2009*).

Several biomarkers have been proposed over the past years to manage critically ill newborns with acute inflammation and sepsis .The state of art in diagnosing and

monitoring neonatal sepsis, severe sepsis and septic shock consists of the measurement of plasma C-reactive protein (CRP) and procalcitonin (PCT) at the onset and course of the disease. CRP and PCT in combination are clinically significant in diagnosing and monitoring septic newborns. However, CRP and PCT have a very limited value for risk stratification and in predicting outcome. The availability of commercial methods for automated measurement of soluble CD14 subtype (presepsin) represent a challenge for evaluation of clinical practice and reliable markers of neonatal sepsis, specific for the very early diagnosis, the classification into class of severity and prediction of complications and deaths (***Michele Mussap et al., 2011***).

CD14 is present in macrophages, monocyte, granulocyte cells and their cell membranes and is said to be responsible for intracellular transduction of endotoxin signals. It's soluble fraction is present in blood and is thought to be produced in association with infections .It's called soluble CD14 subtype and its generic name is presepsin (***Shozushima et al., 2011***).

AIM OF THE WORK

The aim of the work was to assess the usefulness of human soluble CD14 as a biomarker of early-onset neonatal sepsis.



NEONATAL SEPSIS

Definition:

Neonatal sepsis, sepsis neonatorum and neonatal septicemia are terms refer to a constellation of clinical and laboratory findings associated with invasive infection during the first 30 days of life. Traditionally, the neonatal sepsis syndrome has been associated with bacteremia, but it may be caused by a variety of pathogens, including bacteria, viruses and fungi (*McMillan et al., 2006*). It encompasses various systemic infections of the newborn such as septicemia, meningitis, pneumonia, arthritis, osteomyelitis, and urinary tract infections. Superficial infections like conjunctivitis and oral thrush are not usually included under neonatal sepsis (*M. Jeeva Sankar et al., 2008*).

The term systemic inflammatory response syndrome (SIRS) is used to describe a clinical syndrome characterized by two or more of the following: (a) fever or hypothermia, (b) tachycardia, (c) tachypnea or hyperventilation, and (d) abnormal white blood cells or increase in immature forms. 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 (*Chiesa et al., 2004*).

When sepsis is accompanied by organ dysfunction, hypoperfusion, or hypotension, the sepsis is considered severe. Septic shock ensues when hypotension develops despite adequate fluid replacement. Finally, in the presence of altered organ function in an acutely ill patient, so severe that homeostasis can't be maintained without intervention, multiple-organ dysfunction syndrome is diagnosed.

The term systemic inflammatory response syndrome (SIRS) is most frequently used to describe this unique process of neonatal infection and the subsequent systemic response. Neonates with SIRS have a spectrum of clinical symptoms that represent progressive stages of the pathologic process (**Stoll**, 2008). National Neonatal Forum of India has defined neonatal sepsis as follows:

1. Probable (Clinical) Sepsis: In an infant having clinical picture suggestive of septicemia, if there is the presence of any one of the following criteria:
 - Existence of predisposing factors: maternal fever or foul smelling liquor or prolonged rupture of membranes (>24 hrs) or gastric polymorphs (>5 per high power field).
 - Positive septic screen--presence of two of the four parameters namely, TLC (< 5000/mm), band to total polymorphonuclear cells ratio of >0.2, absolute neutrophil count < 1800/cumm, C-reactive protein (CRP) >1mg/dl and micro ESR > 10 mm-first hour.

- Radiological evidence of pneumonia.
2. Culture Positive Sepsis: In an infant having clinical picture suggestive of septicemia, pneumonia or meningitis, if there is presence of either of the following:
- Isolation of pathogens from blood or CSF or urine or abscess.
 - Pathological evidence of sepsis on autopsy.

(NNPD, 2005)

Epidemiology:

The World Health Organization (WHO) estimates that 1 million deaths per year (10% of all under-five mortality) are due to neonatal sepsis and that 42% of these deaths occur in the first week of life (*Lawn et al., 2005*).

Most of these deaths take place in the developing world where mortality from sepsis may be as high as 85%, in the developed world neonatal mortality from sepsis has remained around 20% for nearly three decades (*Haque, 2010*).

In the preterm infant, inflammatory mediators associated with neonatal sepsis may contribute to brain injury and poor neurodevelopmental outcomes (*Anderson-Berry et al., 2010*).

The mortality rate in neonatal sepsis may be as high as 50% for infants who are not treated. Infection is a major cause of fatality during the first month of life, contributing to 13-15% of all neonatal deaths. Neonatal meningitis, a serious

morbidity of neonatal sepsis, occurs in 2-4 cases per 10,000 live births and significantly contributes to the mortality rate in neonatal sepsis; it is responsible for 4% of all neonatal deaths (*Arnon and Litmanovitz, 2008*).

Black infants have an increased incidence of GBS disease and late-onset sepsis. This is observed even after controlling for risk factors of low birth weight and decreased maternal age (*Anderson- Berry et al., 2010*).

Mode of infection:

1. 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 and Remington, 2001*).

Intrauterine infection is a result of maternal infection with a variety of agents (cytomegalovirus [CMV], *Treponema pallidum*, *Toxoplasma gondii*, rubella virus, varicella virus, parvovirus B 19) 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, stillbirths, acute or delayed disease in the neonatal period or asymptomatic persistent infection with sequelae later in life (*Stoll, 2008*).

2. 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., 2004*).

Fetal infection can result from aspiration of infected amniotic fluid leading to stillbirth, premature delivery or neonatal sepsis. Initial colonization of the neonate usually takes place after rupture of the maternal membranes. In most cases, the infant is colonized with the micro flora of the birth canal during delivery. However, particularly if the rupture of membranes lasts longer than 18 hours, vaginal bacteria may ascend and in some cases, produce inflammation of the fetal membranes, umbilical cord and placenta (*Chiesa et al., 2004*).

3. Postnatal infection:

It occurs in the delivery room or the newborn nursery via respiratory tract, gastrointestinal tract, and umbilical stump, infected circumcision or cutaneous wound. These infections may be transmitted through: Umbilical or peripheral venous catheters, equipments of resuscitation, inhalation therapy, total parenteral nutrition (TPN), or exchange transfusion. Direct transmission of organisms may occur through the hands of nursery or other adult personnel when hand washing techniques are inadequate (*Gomella et al., 2004*).

Classification:

Neonatal sepsis is categorized as early or late onset. Early onset sepsis occurs at <72 hours. Eighty-five percent of newborns with early-onset infection present within 24 hours, 5% present at 24-48 hours, and the rest present within 48-72 hours. Onset is most rapid in premature neonates. In severe cases the neonate may be symptomatic in utero (fetal tachycardia, poor beat to beat variability).

Early onset sepsis is associated with acquisition of microorganisms from the mother. Transplacental infection or an ascending infection from the cervix may be caused by organisms that colonize in the mother's genitourinary tract, with acquisition of the microbe by passage through a colonized birth canal at delivery. The microorganisms most commonly

associated with early-onset infection include group B Streptococcus (GBS), Escherichia coli, coagulase-negative Staphylococcus, Haemophilus influenza type B.

Late-onset sepsis occurs at > 72 hours of life and is acquired from the care giving environment. The infant's skin, respiratory tract, conjunctivae, gastrointestinal tract (GIT), and umbilicus may become colonized from the environment, leading to the possibility of late-onset sepsis from invasive microorganisms. Vectors for such colonization may include vascular or urinary catheters, other indwelling lines, or contact from caregivers with bacterial colonization.

Organisms that have been implicated in causing late-onset sepsis syndrome include coagulase-negative staphylococcus aureus, Escherichia coli (E.coli), Klebsiella, Pseudomonas, Enterobacter, Candida, GBS, Serratia, Acinetobacter, and anaerobes (*Van den Hoogen et al., 2009*).

Pneumonia is more common in early onset sepsis, whereas meningitis and bacteremia are more common in late-onset sepsis (*Anderson - Berry et al., 2010*).

Pathogenesis of early onset neonatal sepsis:

Includes:

- A. Risk factors (maternal and obstetric factors, neonatal factors and virulence factors of the causative organism)
- B. Causative agents.

Risk factors for early-onset sepsis (EOS):

1. Maternal and obstetric risk factors:

a. Prolonged premature rupture of membranes (PROM):

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 an untreated infection of the urinary tract or birth canal and is also associated with previous preterm delivery, uterine bleeding in pregnancy, and heavy cigarette smoking during pregnancy (*Bell et al., 2004*).

Rupture of membranes without other complications for more than 24 hours prior to delivery is associated with a 1% increase in the incidence of neonatal sepsis; however, when chorioamnionitis accompanies the rupture of membranes, the incidence of neonatal infection is quadrupled. Many prepartum and intrapartum obstetric complications have been associated with increased risk of infection in the newborn, the most significant of which are prolonged rupture of fetal membranes. In one study of 963 pregnancies complicated by premature rupture of membranes, the incidence of early sepsis increased from 2% among infants born within 23 hours of membrane rupture to 7% and 11% among those delivered 24 to 47 hours and 48 to 71 hours after rupture, respectively. The risk was

highest for the premature, LBW infants (*MacDonald et al., 2005*).

Once the membranes have ruptured for > 18 hours, the risk of sepsis in the neonate increases approximately 10-folds over the baseline to a rate of 1% for proven and 2% for suspected sepsis (*Gerdes, 2004*).

A recent multicenter study demonstrated that clinical chorioamnionitis and maternal colonization with GBS are the most important predictors of subsequent neonatal infection following PROM (*Anderson-Berry et al., 2010*).

b. Chorioamnionitis and maternal fever:

The generally accepted definition for chorioamnionitis 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 leucocytosis ($\text{TLC} > 18,000/\text{mm}^3$). Rate of neonatal infection increases significantly in the presence of chorioamnionitis which is diagnosed by amniotic fluid analysis or histologically. The relationship between chorioamnionitis and other risk variables is strong (*Gotoff, 2000*).

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.

Once a diagnosis of clinical chorioamnionitis has been established, delivery is indicated to remove the infected fetus and placenta from the uterus and reduce the risk of sepsis to the fetus and mother. However, the optimal time frame to deliver the fetus is unknown. It appears from several studies that a diagnosis-to-delivery interval of up to 12 hours is not associated with increased neonatal morbidity. However, the risks to the fetus, if a delay beyond 12 hours occurs, are unknown (*Gerdes, 2004*).

c. Maternal colonization with group B streptococci (GBS):

The most common etiology of neonatal bacterial sepsis is GBS. Nine serotypes exist, and each is related to the polysaccharide capsule of the organism. Types I, II, and III are commonly associated with neonatal GBS infection. The type III strain has been shown to be most highly associated with central nervous system (CNS) involvement in early-onset infection, whereas types I and V have been associated with early-onset disease without CNS involvement (*Anderson-Berry et al., 2010*).

Approximately 30% of women have asymptomatic GBS colonization during pregnancy. GBS is responsible for approximately 50,000 maternal infections per year in women,

but only 2 neonates per 1000 live births are infected. Women with heavy GBS colonization and culture results which are chronically positive for GBS have the highest risk of perinatal transmission. Also, heavy colonization at 23-26 weeks of gestation is associated with prematurity. Colonization at delivery is associated with neonatal infection (*McKenna and Iams, 2004*).

d. Untreated urinary tract infection (UTI):

UTI of any cause raises the risk of sepsis in the neonate due to raising the risk of prematurity and chorioamnionitis (*Gerdes, 2004*).

e. Procedures disturbing the integrity of uterine contents:

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

f. Traumatic or septic delivery:

Infection of skin abrasions after use of obstetric forceps or infection of cephalohematoma following fetal monitoring procedures increases the risk of infection in the neonates; another risk factor is uterine inertia with high forceps extraction (*Chiesa et al., 2004*).