INTRODUCTION

Neonatal sepsis is defined as 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 bloodstream, they may cause overwhelming infection without much localization (septicemia) or may be predominantly localized to the lung (pneumonia) or the meninges (meningitis) **(Paolucci et al., 2012).**

Neonatal sepsis can be classified into two sub-types depending upon whether the onset of symptoms is before 72 hours of life (early onset) or later (late onset). Early-onset infections are caused by organisms prevalent in the maternal genital tract or in the delivery area. Risk factors for early-onset sepsis include prematurity, low birth weight, premature and prolonged rupture of membranes, maternal fever, urological infection and chorioamnionitis. Late-onset sepsis (LOS) is caused by the organisms thriving in the external environment of the home or the hospital. The infection is often transmitted through the hands of the care-providers. The onset of symptoms is usually delayed beyond 72 hours after birth and the presentation is that of septicemia, pneumonia or meningitis. LOS is a common complication of prolonged admission to the NICU following preterm birth. Risk factors for LOS are invasive procedures such as resuscitation in delivery room, intubation, mechanical ventilation, central venous catheters, surgical procedures and staying in NICUs for prolonged period of time **(Stoll et al., 2002).**

Neonatal sepsis is associated with high morbidity and mortality rates if not treated promptly. Although ideally antibiotic therapy should be directed against the causative agent, results of cultures take around 48–72 h to be processed, and any delay in the initiation of treatment for sepsis may be catastrophic. Several acute-phase reactants such as C-reactive protein (CRP) have proven helpful in the diagnosis of an ongoing infection **(Khassawneh et al., 2007).**

Human adrenomedullin (ADM) is an immunomodulator with various described metabolic and vascular modulatory effects, and elevations in tissue levels of the peptide have been reported to occur as a response to disrupted blood circulation. It plays an important role in regulating blood volume; it also has potential antimicrobial effects, which have a protective effect against organ damage, particularly in the setting of sepsis **(Hinson et al., 2000).**

Its serum levels show rapid elevations during sepsis, followed by rapid clearance from the circulation, which makes it difficult to detect because of its half-life of 22 min. Pro-ADM a more stable precursor molecule to ADM, was reported to correlate well with other markers such as IL-6 and CRP as a predictor of prognosis in patients with sepsis. Elevations of pro-ADM have been reported in systemic inflammatory response syndrome (SIRS), sepsis, and septic shock in adults **(Eto.,2001).**

AIM OF THE WORK

This cross-sectional study aimed to:

1. Evaluate the value of pro-ADM measurement in neonatal sepsis and health neonates.

2. Evaluate the value of pro-ADM measurement in the prognosis of neonatal sepsis and its possible correlation with other traditional acute phase reactants as C-reactive protein level, TLC, Plt and I/T ratio.

3. Determine the diagnostic utilities [sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV)] of proadrenomedullin (pro-ADM) level for early detection of neonatal sepsis.

4. Define the optimal cutoff value for pro-ADM level using the receiver operating characteristics (ROC) curve so that it may be used as a reference with which future studies can be compared.

Chapter (*I*)

NEONATAL SEPSIS

Definitions:

Neonatal sepsis (NS), sepsis neonatorum or neonatal septicemia is defined as 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 (pneumonia) or the meninges (meningitis) **(Paolucci et al., 2012).**

Confirmed sepsis is diagnosed when either the blood culture and/or cerebrospinal fluid culture was positive. Suspected sepsis diagnosed in the absence of a positive culture when two or more of the following previously validated hematologic criteria were observed: absolute neutrophil count < 1000/μL or > 8000/μL; absolute band count > 1.500/μL; immature/total neutrophil ratio > 0.16; and platelet count < 150.000/μL **(Lee et al., 2012).**

Systemic inflammatory response syndrome (SIRS) is defined by the presence of at least two of the following four criteria, one of which must be abnormal temperature or leukocyte count:-

- Core temperature >38.5 or < 36.0°C.
- Tachycardia, defined as a mean heart rate >2 SD above normal for age; or for children <1 yr old, bradycardia, defined as a mean heart rate <10th percentile for age.
- Mean respiratory rate >2 SD above normal for age.
- Leukocyte count elevated or depressed for age or >10% immature neutrophils.

SIRS plus infection equals sepsis **(Pavare et al., 2009).**

The fetal inflammatory response syndrome (FIRS), considered to be the fetal counter part of SIRS, is frequently present in neonates delivered as a result of spontaneous preterm labor. Intrauterine infection is one of the most important mechanisms of disease in preterm birth. Indeed, FIRS is an independent risk factor for perinatal morbidity/mortality and has also been associated with infection-related neonatal complications, bronchopulmonary dysplasia, and impaired neurological outcomes including cerebral palsy. Moreover, fetal microbial invasion or other insults may result in a systemic fetal inflammatory response that can progress toward multiple organ dysfunctions, including the hematopoietic system, the adrenals, heart, kidneys, thymus and skin. Currently, FIRS is defined by an elevated umbilical cord plasma interleukin (IL)-6 concentration and/or the presence of funisitis (neutrophils in the wall of the umbilical cord vessels and/or Wharton's jelly). Another approach to diagnose FIRS is to measure C-reactive protein (CRP) concentration in umbilical cord blood, which has been shown to be elevated in patients with funisitis and congenital neonatal sepsis **(Madsen-Bouterse et al., 2010).**

Severe sepsis is defined as sepsis complicated by organ dysfunction. Septic shock is defined as tachycardia with signs of decreased perfusion including decreased peripheral pulses, altered alertness, and cool extremities or reduced urinary output. Hypotension occurs later than in adults and is a sign of late and decompensated shock in children **(Jawad et al., 2012).**

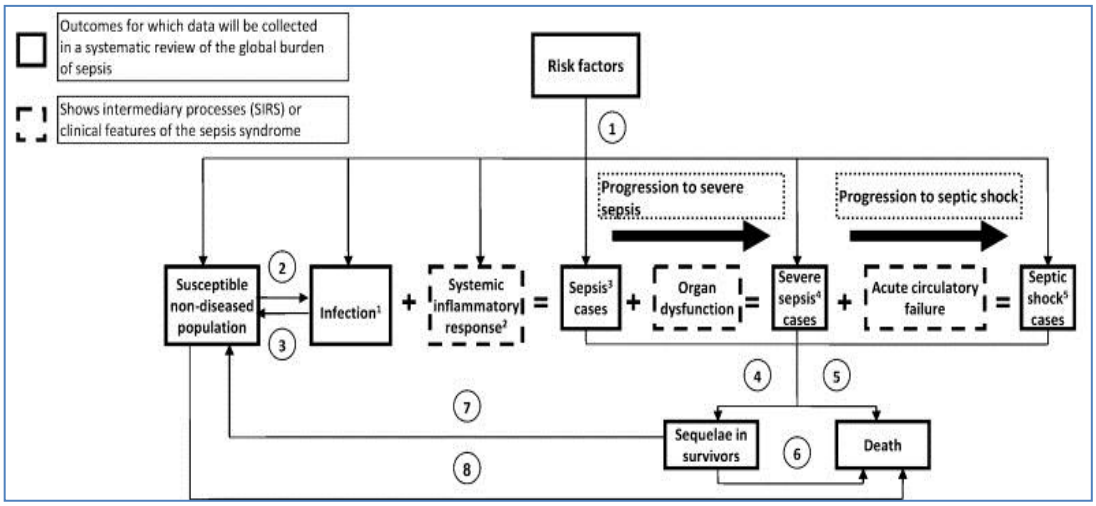

Fig. (1): Natural history of sepsis diagram

1) Potentially modifiable risk factors that increase the probability of infection, SIRS and sepsis in a non-diseased population or severe sepsis and septic shock in septic patients; 2) Incidence of sepsis ; 3) Remission; 4) Sepsis-complication; 5) Case-fatality; 6) Complication-fatality; 7) Individuals with sequelae who are exposed to the risk factor(s) and are susceptible to acquire infection, SIRS, sepsis, severe sepsis or septic shock again; 8) General mortality **(Jawad et al., 2012).**

Classification:

Neonatal sepsis can be classified into two sub-types depending upon whether the onset of symptoms is before 72 hours of life (early onset) or later (late onset).

Early-onset sepsis (EOS):

Neonatal sepsis may 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 remains a common and serious problem for neonates, especially preterm infants. Group B streptococcus (GBS) is the most common etiologic agent, while Escherichia coli is the most common cause of mortality **(Stefanovic, *2011*).**

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 the mother's genitourinary (GU) tract; the neonate acquires the microorganisms as it passes through the colonized birth canal at delivery. The microorganisms most commonly associated with early-onset infection include the following:

Group B Streptococcus (GBS)
Escherichia coli
Coagulase-negative Staphylococcus
Haemophilus influenzae
Listeria monocytogenes

Trends in the epidemiology of early-onset sepsis show a decreasing incidence of *GBS* disease. This can be attributed to the implementation of a prenatal screening and treatment protocol for *GBS* **(Jones and Heath.2014).**

In a study conducted in 2009 involving 4696 women, prenatal cultures showed a *GBS* colonization rate of 24.5%, with a positive culture rate of 18.8% at the time of labor. As many as 10% of prenatally culture-negative women were found to have positive cultures at the time of labor. With intrapartum antibiotic prophylaxis rates of 93.3%, 0.36 of 1000 infants developed early-onset *GBS* disease **(Matsubara et al., 2013).**

Late-onset sepsis (LOS):

- The onset of symptoms is usually delayed beyond 72 hours after birth and the presentation is that of septicemia, pneumonia or meningitis.
- Caused by the organisms present in the external environment of the home or the hospital. The infection is often transmitted through the hands of the care-providers.
- Risk factors for LOS are invasive procedures such as resuscitation in delivery room, intubation, mechanical ventilation, central venous

catheters, surgical procedures and staying in NICUs for prolonged period of time. The use of broad spectrum antibiotics is risk factor for fungal NS.

- Organisms that are associated with LOS are *Staphylococcus aureus, CoNS, E. coli, Klebsiella, Pseudomonas, Enterobacter, Candida, GBS, Serratia, Acinobacter and anaerobes*. Trends in LOS show an increase in CoNS sepsis. LOS has a higher case fatality rate when gram negative bacteria are involved **(Stefanovic, *2011*).**

Epidemiology:

The reported incidence of NS varies from 7.1 to38 per 1000 live births in Asia, from 6.5 to 23 per 1000live births in Africa, and from 3.5 to 8.9 per 1000 live births in South America and the Caribbean. By comparison, rates reported in the United States and Australasia range from 1.5to 3.5 per 1000 for EOS sepsis and up to 6 per 1000 live births for LOS sepsis, a total of 6–9 per 1000 for NS **(Vergnano et al., 2005).**

The World Health Organization (WHO) estimated that regarding neonatal sepsis:

- Neonatal Mortality rate (per 1000 live births) worldwide is 26 in 2008.
- Neonatal Mortality rate (per 1000 live births) at African Region is 40 in 2008.
- Neonatal Mortality rate (per 1000 live births) in Egypt is 13 in 2008.
- Distribution of causes of death among children aged <5 years worldwide showed that neonatal mortality was 6% in 2008.
- Distribution of causes of death among children aged <5 years at African Region showed that neonatal mortality was 5% in 2008.
- Distribution of causes of death among children aged <5 years in Egypt showed that neonatal mortality was 1% in 2008 **(WHO -World health statistics, 2008).**

Mode of infection:

1. Prenatal infection: 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. Certain bacteria, particularly *Treponemapallidum* and *Listeria monocytogenes*, can reach the fetus through the maternal blood stream despite placental protective mechanisms, causing trans-placental infection. This process is uncommon, but it leads to either congenital infection not unlike infections caused by certain viruses or *Toxoplasma* or to stillbirth resulting from over whelming infection **(Chiesa et al., 2004).**

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 **(Badrawi et al., 2001)**. The commonest causative organisms are *Group B Streptococci (GBS), gram-negative enteric organisms*, *Staphylococcus Aureus* and *Streptococcus Fecalis* **(*McGraw,* 2012)**.

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 **(Fig.2)**.

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, 2012)**.

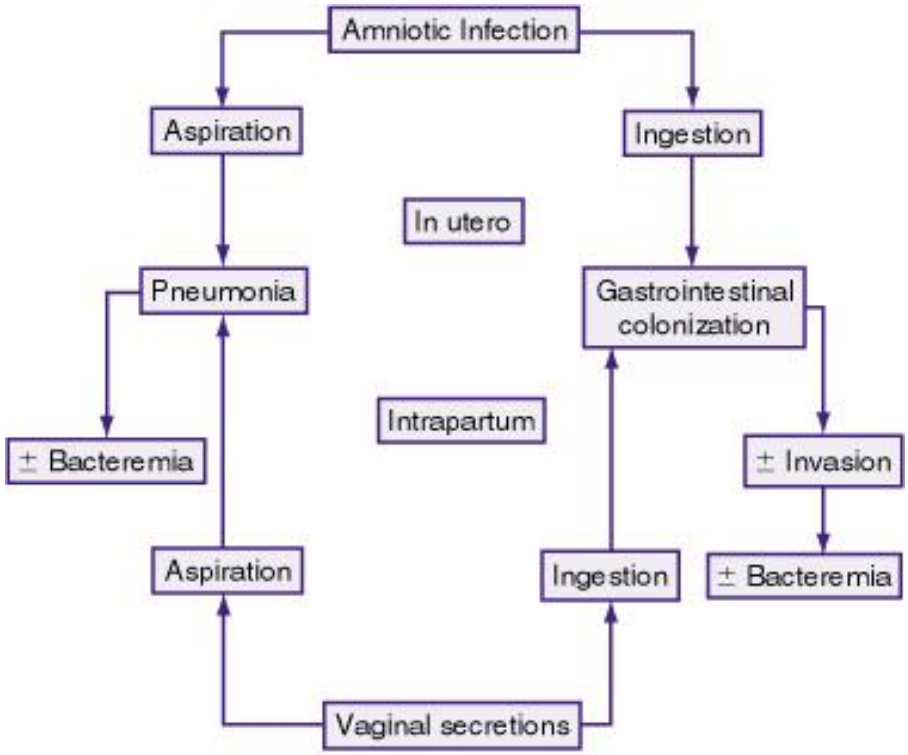

Fig. (2): Pathways of ascending or intrapartum infection **(Stoll, 2012).**

3. Postnatal infection: Bacteria can be introduced after birth from the environment surrounding the baby, either in the nursery or at home. Sophisticated equipments for respiratory and nutritional support combined with invasive techniques provide life support to the ill infant. Arterial and venous umbilical catheters, central venous catheters, peripheral arterial and venous cannulas, urinary indwelling catheters, hyper alimentation infusions, and assisted ventilation provide enormous opportunities for relatively non virulent pathogens to establish infection and to invade the host **(Chiesa et al., 2004).**

Pathogenesis of early onset neonatal sepsis

A) Risk factors for early-onset sepsis (EOS):

a. Maternal and obstetric risk factors:

1. *Racial group and socioeconomic status:* 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 **(Satar and Ozlü, 2012).**
2. *Prolonged premature rupture of membranes (PROM):* Premature rupture of membranes (PROM) refers to the loss of integrity of membranes >18 hours before onset of labor, with resulting leakage of amniotic fluid and establishment of communication between the amniotic cavity and the end cervical canal and vagina. PROM occurs in approximately 5–10 % of all pregnancies, of which approximately 80 % occur at term (term PROM).PROM occurs when intrauterine pressure overcomes membrane resistance. This happens as a result of weakening of membrane either congenital or acquired (smoking and vitamin C deficiency), or because of damaging factors, either mechanical (amniocentesis or amnioscopy) or physical–chemical damage by infection *(Trichomonas, GBS, bacterial vaginosis, etc.).* Failure of mechanical support such as cervical dilatation can lead to PROM, favoring bacterial contamination as well **(Shah and Doshi, 2012).** At term gestation, less than 1% of women with intact membranes will have organisms cultured from amniotic fluid. The rate can be higher if the integrity of the amniotic cavity is compromised by procedures before birth (eg, placement of a cerclage or amniocentesis) **(Polin, 2012).**There is a general agreement that the term and near term pregnant patients with PROM should be delivered to avoid infection to both mother and the infant as the dangers of infection goes on increasing with prolonged latent period **(Poornima and Dharma, 2012).**

3. *Chorioamnionitis and maternal fever:* Clinical chorioamnionitis was defined as a body temperature ≥ 37.8℃ on 2 occasions at least 4 hours apart, and > 2 of the following criteria: uterine tenderness; malodorous vaginal discharge; maternal leukocytosis (>15.000/μL); maternal tachycardia (>100 beats/min); and fetal tachycardia (>160 beats/min). Histologic chorioamnionitis was diagnosed in the presence of acute inflammatory changes in any of the tissue samples (amnion, chorion-decidua, umbilical cord, and chorionic plate) **(Lee et al., 2012).**
4. *Maternal colonization with group B streptococci (GBS):* Maternal intrapartum *GBS* colonization is the primary risk factor for early-onset disease in infants. Pregnant women with *GBS* colonization are 25 times more likely than pregnant women with negative prenatal cultures to deliver infants with early-onset *GBS* disease. In the absence of any intervention, an estimated 1% to 2% of infants born to colonized mothers develop early-onset *GBS* infections. Approximately 10% to 30% of pregnant women are colonized with *GBS* in the vagina or rectum. *GBS* colonization during pregnancy can be transient, intermittent, or persistent **(Jones and Heath, 2014).**

   The gastrointestinal tract serves as the primary reservoir for GBS and is the likely source of vaginal colonization. Heavy colonization, defined as culture of *GBS* from direct plating rather than from selective broth only, is associated with higher risk for early-onset disease. *GBS* identified in clean-catch urine specimens during any trimester is considered a surrogate for heavy maternal colonization and also is associated with a higher risk for early-onset *GBS* disease. Previous delivery of an infant with invasive *GBS* disease is a risk factor for early-onset disease in subsequent deliveries. Neonatal infection occurs primarily when *GBS* ascends from the vagina to the amniotic fluid after onset of labor or rupture of membranes, although *GBS* also can invade through intact membranes. *GBS* can be aspirated into the fetal lungs, which in turn can

lead to bacteremia. Infants also can become infected with *GBS* during passage through the birth canal **(Verani et al., 2010).**

5. *Disturbed integrity of uterine contents:* Procedures disturbing the integrity of the uterine contents, such as amniocentesis, cervical cerclage, trans - cervical 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).**
6. *Asymptomatic bacteriuria:* It has been associated with premature birth, LBW, PROM, maternal peripartum infection, and septic or traumatic delivery **(Satar and Ozlü, 2012).**
7. *Traumatic or septic delivery:* In developing countries, unsafe birthing practices are common, with only 35% of births in some of the least developed countries being attended by a skilled birth attendant, often resulting in unhygienic practices such as delivery onto a unsterile floor, unsterile cord cutting **(Waters et al., 2011).**

b) Neonatal risk factors:

1. *Prematurity and low birth weight (LBW):* Infant birth weight is inversely related to risk of EOS. The increased risk of EOS in preterm infants is also related to complications of labor and delivery and immaturity of innate and adaptive immunity **(Polin, 2012).**

2. *Apgar score:* Apgar score less than 6 at 5 minutes is one of independent risk factors for culture-proven sepsis **(Zakariya et al., 2012).**

3. *Sex***:** 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)**.

4. *Hypothermia:* It is 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 **(Satar and Ozlü, 2012).**

5. **Metabolic disorders:** It may predispose to infection. Infants with galactosemia have increased susceptibility to sepsis caused by gram-negative enteric bacilli, in particular *E. coli* **(Satar and Ozlü, 2012).**

B) Risk factors for late-onset sepsis (LOS):

1. Resuscitation:

Resuscitation at birth, particularly if it involves endotracheal intubation, insertion of an umbilical vessel catheter, or both, is associated with an increased risk of NS. Explanations include the presence of infection at the time of birth or acquisition of infection during the invasive procedures associated with resuscitation **(Stoll, 2004).**

2. Broad-spectrum antibiotics:

Recent data suggest an association between prolonged empirical treatment of preterm (PT) infants (≥5 days) with broad-spectrum antibiotics and higher risks of LOS, necrotizing enterocolitis (NEC), and mortality. To reduce these risks, antimicrobial therapy should be discontinued at 48 hours in clinical situations in which the probability of sepsis is low **(Abdel Ghany et al., 2012) (Polin, 2012).**

3. Invasive procedures and nutritional history:

LOS is often associated with use of indwelling vascular catheters or central lines. It was described the rise in incidence as coincident with change in skin disinfection usage and general use of 3rd generation cephalosporins to which the *CoNS* were resistant. *CoNS* are among the most common causes of blood culture contaminants, since they are the most common microorganisms found colonizing the skin and mucous

membranes of neonates. The virulence factor of *CoNS* may well be a biofilm formation, which aids colonization of not only intravascular devices but also tissues, and may also allow the organism to persist in hospital environments. Thus, *CoNS* strains must be tested for biofilm formation or its genetic markers and if proven so, the offending central lines must be removed **(Yilmaz et al., 2010).**

The gastrointestinal tract (GIT) is an important source of potential pathogens causing nosocomial sepsis as the immature intestinal epithelium can permit translocation of bacteria and yeast. Delayed enteral feeding, frequent use of antibiotic therapy, and altered acquisition of normal digestive micro flora are important contributing factors for the increased risk of NEC in preterm infants and sepsis is often a complication of NEC **(Nair and Soraisham, 2013).**

4. Neonatal intensive care unit (NICU) length of stay:

Less mature infants also require longer periods of mechanical ventilation, central venous access, and hospitalizations, placing them at higher risk for nosocomial infections and LOS **(Hornik et al., 2012).**

5. Nursery design and staffing:

The most common source of postnatal infections in hospitalized newborns is hand contamination of health care personnel **(Stoll, 2012).**