COMPARISON BETWEEN ROLE OF ANTIBIOTIC THERAPY, INTRAVENOUS IMMUNOGLOBULINS AND EXCHANGE TRANSFUSION IN TREATMENT OF NEONATAL SEPSIS

Thesis
Submitted for fulfillment of the Master Degree in Pediatrics

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قالوا: سبحانك لَا عِلْمَ لَنَا إِلَّا مَا عَلَّمْتَنَا إِنَّكَ أَنتَ الْعَلِيمُ الْحَكِيمُ

صدق الله العظيم

(سورة البقرة الآية 23)
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Thanks to ALLAH who offered me the ability to perform this work.

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Dedication

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I would like to dedicate this theses to the soul of my beloved mother, I would like very much that she was with us now to hug me and bless me, and I pray that ALLAH have mercy on her and I wish from ALLAH to allow me to Join her in the next life.

No words can express my great feelings and my warm gratitude to my beloved husband Ahmed who never let me feel loaded, depressed or frustrated, but instead he always pushes me forward and removes every barrier in front of me to allow me achieve my goals and even more, he always dreams for me and tries to change circumstances around me to help me study and work, even he sometimes sacrifices his comfort and postpones his goals to help me as can as possible. All this without complaining or even makes me feel that. On the contrary, he makes great effort to change my mood, make me happy and give me hope whatever the circumstances. Finally I can’t dedicate this work to him, as simply no one can dedicate something to his real owner. Lastly I want to pray ALLAH to bless him, push him up to the highest rank and to help him achieve all his goals and even more, and to be the best doctor in the world. I can’t express my great thanks to him even throughout my whole life.

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<tr>
<td>AAP</td>
<td>American Academy of Pediatrics</td>
</tr>
<tr>
<td>APPs</td>
<td>antimicrobial proteins and peptides</td>
</tr>
<tr>
<td>ARR</td>
<td>Absolute risk reduction</td>
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<tr>
<td>BC</td>
<td>Blood culture</td>
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<tr>
<td>BCG</td>
<td>Bacillus Calmette and Guerin</td>
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<td>BPD</td>
<td>bronchopulmonary dysplasia</td>
</tr>
<tr>
<td>C3</td>
<td>Complement 3</td>
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<tr>
<td>CBC</td>
<td>Complete blood count</td>
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<tr>
<td>CD 64</td>
<td>Cluster of Differentiation 64</td>
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<tr>
<td>CDC</td>
<td>Centers for Disease Control and Prevention</td>
</tr>
<tr>
<td>cfu</td>
<td>colony-forming units</td>
</tr>
<tr>
<td>CoNS</td>
<td>Coagulase-negative staphylococci</td>
</tr>
<tr>
<td>CRP</td>
<td>C-reactive protein</td>
</tr>
<tr>
<td>CS</td>
<td>Caesarean section</td>
</tr>
<tr>
<td>CSF</td>
<td>Cerebrospinal fluid</td>
</tr>
<tr>
<td>CSFs</td>
<td>Colony stimulating factors</td>
</tr>
<tr>
<td>CVCs</td>
<td>central venous catheters</td>
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<tr>
<td>DFP32</td>
<td>diisopropylfluorphosphate no. 32</td>
</tr>
<tr>
<td>DIC</td>
<td>disseminated intravascular coagulation</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>ELBWs</td>
<td>Extremely low birth weight</td>
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<td>ELISA</td>
<td>enzyme-linked immunosorbent assay</td>
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<td>EOS</td>
<td>Early onset sepsis</td>
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<td>ET</td>
<td>Exchange transfusion</td>
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<tr>
<td>F</td>
<td>Fahrenheit</td>
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<td>g</td>
<td>Gram</td>
</tr>
<tr>
<td>GA</td>
<td>Gestational age</td>
</tr>
<tr>
<td>GBS</td>
<td>Group B streptococci</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>granulocyte/monocyte colony stimulating factor</td>
</tr>
<tr>
<td>HIE</td>
<td>Hypoxic ischemic encephalopathy</td>
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<tr>
<td>HIV</td>
<td>human immunodeficiency virus</td>
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<td>HR</td>
<td>Heart rate</td>
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<td>HRCi</td>
<td>heart rate characteristics index</td>
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<td>Hrs.</td>
<td>Hours</td>
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<td>HS</td>
<td>Highly significant</td>
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<tr>
<td>HSS</td>
<td>Hematological Scoring System</td>
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<td>ICH</td>
<td>Intracranial hemorrhage</td>
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<tr>
<td>IDM</td>
<td>Infant of diabetic mother</td>
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<td>Ig</td>
<td>Immunoglobulin</td>
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<td>IL</td>
<td>Interleukins</td>
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<td>IVIG</td>
<td>Intravenous immunoglobulin</td>
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<tr>
<td>kg</td>
<td>Kilogram</td>
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<td>LOS</td>
<td>Late onset sepsis</td>
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<td>LPs</td>
<td>Lumbar punctures</td>
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<td>MAS</td>
<td>Meconium aspiration syndrome</td>
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<tr>
<td>mg</td>
<td>Milligram</td>
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<tr>
<td>mL</td>
<td>Milliliter</td>
</tr>
<tr>
<td>mm</td>
<td>Millimeter</td>
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<tr>
<td>mm³</td>
<td>Cubic millimeter</td>
</tr>
<tr>
<td>MRSA</td>
<td>Methicillin-resistant Staphylococcus aureus</td>
</tr>
<tr>
<td>MV</td>
<td>Mechanical ventilation</td>
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<tr>
<td>NEC</td>
<td>Necrotizing enterocolitis</td>
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<tr>
<td>ng</td>
<td>Nanogram</td>
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<td>NICU</td>
<td>Neonatal intensive care unit</td>
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<tr>
<td>NNT</td>
<td>Number need to treat</td>
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<td>NS</td>
<td>Non-significant</td>
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<tr>
<td>NVD</td>
<td>Normal vaginal delivery</td>
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<td>P value</td>
<td>The probability value</td>
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<td>PCT</td>
<td>Procalcitonin</td>
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<td>PMN</td>
<td>Polymorphonuclear</td>
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<td>RBC</td>
<td>Red blood cell</td>
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<td>RBS</td>
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<td>RDS</td>
<td>Respiratory distress syndrome</td>
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<td>rpm</td>
<td>Round per minute</td>
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<td>RR</td>
<td>Respiratory rate</td>
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<td>RRR</td>
<td>Relative risk reduction</td>
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<td>S</td>
<td>Significant</td>
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<td>SBP</td>
<td>Systolic blood pressure</td>
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<td>Temp</td>
<td>Temperature</td>
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<tr>
<td>TNF</td>
<td>Tumor necrosis factor</td>
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<tr>
<td>TTN</td>
<td>Transient tachypnea of the newborn</td>
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<tr>
<td>UTI</td>
<td>Urinary tract infection</td>
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<td>VLBW</td>
<td>Very low birth weight</td>
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<tr>
<td>WBC</td>
<td>White blood cell</td>
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<td>WHO</td>
<td>World Health Organization</td>
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<td>µ-ESR</td>
<td>Micro-erythrocyte sedimentation rate</td>
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<td>µg.</td>
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<td>µL.</td>
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Introduction
Introduction

Neonatal sepsis or septicemia is a clinical syndrome characterized by systemic signs of circulatory compromise (e.g., Poor peripheral perfusion, pallor, hypotonia and poor responsiveness) caused by invasion of the bloodstream by bacteria in the first month of life (Thaver and Zaidi, 2009).

Sepsis is the commonest cause of neonatal mortality; it is responsible for about 30-50% of the total neonatal deaths in developing countries. It is estimated that up to 20% of neonates develop sepsis and approximately 1% die of sepsis related causes (Stoll, 1997).

Neonatal sepsis can broadly be classified in to early onset sepsis (<72 hours) and Late onset sepsis (>72 hours). Early onset sepsis (EOS) often presents as a fulminant, multi-system illness within 72 hours of delivery and is mainly due to bacteria acquired before and during delivery whereas late onset sepsis (LOS) is due to bacteria acquired after delivery (Nosocomial or community sources) and can present as either a fulminant or a smoldering infection (Sundaram et al., 2009).

Distal risk factors for neonatal sepsis include poverty and poor environmental conditions. Proximate factors include prolonged rupture of membranes, preterm labor, maternal pyrexia, unhygienic intrapartum and postnatal care, low birth weight, and prelacteal feeding of contaminated foods and fluids (Bahl et al., 2009).

Neonatal clinical sepsis syndrome identification is difficult as the clinical signs of neonatal septicemia can be very similar to those of other life-threatening diseases. However, recent studies in middle- and low-income countries have provided seven danger signs which can be used to identify infants with very severe disease including neonatal sepsis (Difficulty feeding, convulsions, movement only when stimulated, respiratory rate ˃60 breaths per minute, severe chest indrawing and axillary temperature ˃37.5°C or <35°C) (Edmond and Zaidi, 2010).

Automated blood culture systems have long been considered the gold standard for microbiological diagnosis. However, results of blood culture can be delayed by up to 48 hours (Benjamin et al., 2001).
Lumbar punctures (LPs) are commonly performed on newborns suspected of having sepsis. Over the last thirty years, numerous studies have evaluated the utility of lumbar punctures in identifying early onset neonatal sepsis (EOS) (Patrick et al., 2012).

Important hematological tests include microscopic examination of the blood for white cells (Total leucocyte count, differential, neutrophil count and immature neutrophil to total neutrophil ratio). Many of these indices are falsely low in a septic neonate. The most commonly used biological biomarker is C-reactive protein (CRP). Cytokines such as interleukin 6, interleukin 8, tumor necrosis factor-a, and procalcitonin have also been extensively studied (Verboon-Macielek et al., 2006).

As neonatal sepsis can be rapidly fatal if left untreated, highly effective antibiotic therapy must be used and delays in the provision of care must be minimized. Treatment must be effective against the causative pathogen, safe for the newborn, and feasible to deliver reliably in the hospital or community setting (Edmond and Zaidi, 2010).

Neonates should be nursed in a thermo-neutral environment taking care to avoid hypo/hyperthermia thereby reducing oxygen consumption. Aggressive nutritional support is needed to combat the catabolic state associated with sepsis (Carcillo et al., 2002).

Parenteral (intravenous or intramuscular) regimens for neonatal sepsis currently recommended by national pediatric associations are a combination of penicillin/ ampicillin and gentamicin, or third-generation cephalosporin (e.g., ceftriaxone or cefotaxime) for 10–14 days. These antibiotics are safe and retain efficacy when administered at extended intervals (e.g., Twice daily or daily dosing) (Darmstadt et al., 2009).

Polyclonal Intravenous immunoglobulin significantly reduces mortality and is a promising adjuvant in the treatment of sepsis and septic shock (Alejandria et al., 2002).

Double volume blood exchange transfusion has been used as a modality for managing sepsis for several decades. The earlier trials were uncontrolled and showed impressive improvements in neutrophil counts, immunoglobulin levels, recovery from sclerema and less mortality compared to historical experiences (Dalvi et al., 1991).
Aim of the work
Aim of the work

This study was conducted to evaluate the role of intravenous immunoglobulin and exchange transfusion as an adjunctive therapy in treatment of neonatal sepsis compared to antibiotic therapy alone, aiming to set up protocol for management of neonatal sepsis in neonatal intensive care unit (NICU) of Benha University Hospital.
Review of literature
Neonatal sepsis

i. Definition of neonatal sepsis

Neonatal sepsis or septicemia is a clinical syndrome characterized by systemic signs of circulatory compromise (e.g., Poor peripheral perfusion, pallor, hypotonia and poor responsiveness) caused by invasion of the blood stream by bacteria in the first month of life (Edmond and Zaidi, 2010).

ii. Incidence of neonatal sepsis

Neonatal sepsis is a common disorder affecting 1.1 to 2.7% of all neonates. Thus; bacterial infections remain the most common cause for mortality and morbidity in early human life (Stoll et al., 2011).

In 2013, the World Health Organization (WHO) estimates that the world’s neonatal mortality rate fall from 33 deaths per 1,000 live births in 1990 to 21 per 1,000 in 2012. In Egypt, neonatal mortality rate fell from 33 deaths per 1,000 live births in 1990 to 12 per 1,000 in 2012. The overall result was a reduction of global neonatal deaths from 4.6 million in 1990 to 2.9 million in 2012 (The UN Inter-agency Group for Child Mortality Estimation, 2013).

Neonatal septicemia remains one of the main causes of mortality and morbidity despite the progress in hygiene, introduction of new and potent antimicrobial agents for treatment and advanced measures for diagnosis. It is responsible for 30-50% of total neonatal deaths in developing countries. These neonatal deaths are attributed principally to infection, birth asphyxia and consequences of premature birth and low birth weight (Naher and Khamael, 2013).

There are wide disparities in neonatal care between high and low-income countries. In high-income countries the major concern is the increasing numbers of extremely premature neonates with high nosocomial infection rates due to multi resistant organisms in intensive care units. Health facility infections are also a major problem in low-
income countries, but the more pressing issues are the high proportion of home deliveries in unclean environments predisposing to sepsis and ensuring that all neonates have access to effective interventions from health care providers in the first days of life (Edmond and Zaidi, 2010).

iii. Classification and pathogenesis of neonatal sepsis

Neonatal sepsis can be classified into two subtypes depending upon whether the onset of symptoms is before 72 hrs. of life, early onset sepsis (EOS), or later, Late onset sepsis (LOS) (Paolucci et al., 2012).

EOS occurs in 1.5–2% of very low birth weight (VLBW) neonates, whereas up to 21% of VLBW neonates experience an episode of LOS (Srinivasana and Harris, 2012).

- Routes of infection:

Neonatal sepsis occurs via number of routes such as;

a. Intrauterine infection which occurs due to apparent or inapparent maternal bacteremia with transplacental transmission to the fetus. *Listeria monocytogenes* septicemia is an example of such infections. Furthermore a fetus may be infected by organisms from vagina invading the amniotic fluid through the cervix with or without intact membrane. The most common organisms found in the amniotic fluid and vagina are *Escherichia coli*, *Enterococcus faecalis*, *Staphylococcus aureus*, and *Group B beta hemolytic Streptococcus* is also occasionally present in the vaginal flora.

b. Intrapartum infection (Ascending Infection) which are acquired just before or during delivery with vertical transmission of the microorganisms from mother to neonate.

c. Postpartum infection (Nosocomial infection), since bacteria may be acquired from the delivery room or in the neonatal nursery via the main pathways, namely the respiratory and gastrointestinal tracts. After birth, the skin and umbilical cord become important alternative routes for the entrance of bacteria into the systematic circulation. The umbilical stump is a frequent site for cutaneous infection leading to septicemia (Naher and Khamael, 2013).
- **Types of neonatal sepsis:**

  1. **Early onset sepsis (EOS):**

     Early onset sepsis occurs within the first 72 hrs after birth by the vertical transfer of microorganisms existing in maternal passages. It is characterized by fulminant multiple organ damage. Symptoms of pneumonia may be revealed within the first week of life (Nemsadze, 2013).

     ➢ **Risk factors:**

     Early onset sepsis is caused by organisms present in the maternal genital tract. It can occur due to ascending infection following rupture of membranes or during the passage of the baby through infected birth canal and at the time of resuscitation resulting in intra-amniotic infection (Paolucci et al., 2012).

     Intra-amniotic infection is commonly referred to as “Chorioamnionitis,” intra-amniotic infection indicates infection of the amniotic fluid, membranes, placenta, and/or decidua. *Group B streptococci (GBS)* can also enter the amniotic fluid through occult tears (Polin, 2012).

     Chorioamnionitis is a major risk factor for neonatal sepsis. The diagnosis is typically based on the presence of maternal fever of >38°C (100.4°F) and at least two of the following criteria: Maternal leukocytosis (>15000 cells/mm³), maternal tachycardia (>100 beats/minute), fetal tachycardia (> 160 beats/minute), uterine tenderness, and/or foul odor of the amniotic fluid. These thresholds are associated with higher rates of neonatal and maternal morbidity (Riley et al., 2011).

     The major risk factors for chorioamnionitis include low parity, spontaneous labor, longer length of labor (Sum of 1st and 2nd stage of labor > 24 hrs.), rupture of membrane, multiple digital vaginal examinations (Single unclean or > 3 sterile vaginal examination(s) during labor, especially with ruptured membranes), meconium-stained amniotic fluid, internal fetal or uterine monitoring, and presence of genital tract microorganisms (e.g., *Mycoplasma hominis*) (Tita and Andrews, 2010).
Review of literature

Neonatal sepsis

Other maternal / perinatal conditions have been associated with an increased risk of EOS:

- Low birth weight (<2500 g) or prematurity.
- Rupture of membranes >18 hrs.
- Maternal colonization with GBS.
- Maternal urinary tract infection (UTI) raises the risk of prematurity and chorioamnionitis.
- Other variables include ethnicity (i.e., Black women are at higher risk of being colonized with GBS), low socioeconomic status, male sex and perinatal asphyxia (Apgar score <4 at 1 minute) (Stoll et al., 2011).

Several reasons make prematurity the single most important factor for neonatal sepsis: Innate immunological deficiencies, prolonged stays in the NICU, and notably, the higher use of indispensable but invasive medical interventions in these developmentally immature neonates (Marchant et al., 2013).

Premature neonates with a birth weight less than 1000 g (ELBW: Extremely low birth weight neonates) are particularly at risk with an inverse correlation between gestational age, birth weight and sepsis. Even late preterm neonates have a fourfold higher risk of sepsis than term neonates (Jaiswal et al., 2011).

GBS is not a risk factor if the mother has received adequate intrapartum therapy (Penicillin, ampicillin, or cefazolin for at least 4 hrs before delivery) or has a cesarean delivery with intact membranes in the absence of labor (Verani et al., 2010).

Regarding mode of delivery, it was observed that the incidence of sepsis was more in cases of babies born from caesarean section as compared to vaginal delivery. This finding is similar with a previous study which showed that baby born from caesarean section have a 1.89 times higher risk than non-caesarean to develop sepsis (Gandhi et al., 2013).
Causative organisms:

Perinatally acquired sepsis in the first 3 to 5 days of life is most often caused by group B Streptococci (Approximately 50%), followed by Escherichia coli (20%), coagulase negative or positive Staphylococcus (17%), other enteric gram negative organisms (7%), other Streptococci (3%), and various anaerobes (3%) (Gerdes, 2004).

Hemophilus influenza has also been reported. These infections are acquired mostly through the ascending infection route. While Listeria monocytogenes causes a septic or flulike syndrome in the mother, and passes transplacentally to the fetus (Gerdes, 2004).

Several studies have revealed increased rates of E coli infection among preterm neonates (Stoll et al., 2011).

However, there are many difficulties in interpreting etiological neonatal sepsis data, because many studies report selected populations of high risk neonates. Specimens from neonates in the first 24 hrs. of life are also seriously under represented, especially those from low birth weight babies and babies born outside health facilities (Tiskumara et al., 2009).

Intrapartum antibiotic prophylaxis against Streptococcus agalactiae has also led to a substantial change in the bacteria responsible for EOS; gram-negative bacilli and Staphylococcus spp. predominate in countries implementing this (Edmond and Zaidi, 2010).

2. Late onset sepsis (LOS):

LOS has varying definitions, with some groups including infections occurring 48 hrs. after birth, 72 hrs. after birth, or anytime 4 to 7 days after birth with or without clinical symptoms. On the other end, some clinicians define LOS up to 30 days of life, whereas others may include any infections occurring before discharge from the hospital (Vergnano et al., 2011).

LOS is caused by 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 hrs. after birth, and the presentation is that of septicemia, pneumonia, or meningitis (Paolucci et al., 2012).

The two most common presentations are catheter-associated bloodstream infections and ventilator-associated pneumonia (Polin and Randis, 2010).

➢ **Risk factors:**

The associated factors of late onset sepsis include low birth weight, low gestational age, mechanical ventilation, total parenteral nutrition and its duration, previous antimicrobial exposure, lack of breastfeeding, superficial infections (Pyoderma and umbilical sepsis), aspiration of feeds, disruption of skin integrity with needle pricks and the use of intravenous fluids or central venous catheter. These factors enhance the chances of entry of organisms into the blood stream of the neonates whose immune defenses are poor as compared to older children and adults. In addition, poor hand hygiene is associated with LOS (Paolucci et al., 2012).

Neonates of any gestational age are susceptible to late-onset sepsis (Figure 1). However, very low birth weight neonates (Those weighing less than 1,500 g) are particularly vulnerable because of the need for invasive monitoring, impaired host defense mechanisms, limited amounts of normal endogenous flora, reduced barrier function of neonatal skin and frequent exposure to broad-spectrum antibiotics (Polin and Randis, 2010).

Fig. 1: Factors that confer a greater risk for LOS in the neonate (Chu et al., 2012).

GA: Gestational age.
Causative organisms:

The most frequent microorganisms involved in LOS are Coagulase-negative staphylococci (CoNS), Enterobacteriaceae, including *Escherichia coli* and *Klebsiella pneumoniae*, and *Acinetobacter baumannii* (Paolucci et al., 2012).

*Methicillin-resistant Staphylococcus aureus* (MRSA) has recently emerged as another increasingly prevalent organism identified in LOS (Downey et al., 2010).

LOS due to Gram-negative organisms is associated with higher mortality. Increasing antibiotic resistance is also an increasing problem in gram negative bacteria causing LOS. *Escherichia coli* has been reported to be the most common Gram-negative rod causing LOS. Other Gram-negative organisms include *Klebsiella*, *Enterobacter*, and *Serratia*. Although less common, LOS caused by *Pseudomonas aeruginosa* carries the highest mortality risk among premature neonates, with reported rates of 45% to 74% (Lessa et al., 2009).

*Yeasts* account for 7% to 20% of LOS infections. Although less common than bacterial infections, blood stream infections due to yeasts carry significant mortality risk, and should be considered in ill neonates as a possible etiology for LOS. *Candida species*, most often *Candida albicans* and *Candida parapsilosis* are the most commonly encountered fungal organisms affecting premature neonates diagnosed with LOS (Chu et al., 2012).

There are also many other important neonatal infectious disease pathogens that are not associated with the sepsis syndrome including: *Treponema pallidum*, *rubella virus*, *herpes simplex virus*, *cytomegalovirus*, *toxoplasmosis*, *Clostridium tetani*, *Human immunodeficiency virus*, *hepatitis B virus*, and *Bordetella pertussis*. These infectious pathogens cause serious morbidities in young neonates and multifaceted disease syndromes including congenital anomalies, developmental disabilities, chronic liver disease, neonatal tetanus, and apnea. They are also important causes of morbidity and mortality in older age groups (Menezes, et al., 2009).
iv. Neonatal immunity and pathophysiology of neonatal sepsis

Neonates have a functionally immature immune system. They have extremely low immunoglobulin (Ig) levels except for IgG to specific maternal antigens transferred passively across the placenta during the last trimester of pregnancy. T cell function is relatively unimpaired but complement activity is half that of healthy adults. Neonates have a low neutrophil storage pool, and their existing neutrophils have impaired capacity to migrate from the blood to sites of infection (Levy, 2007).

Deficiencies of both innate and adaptive immunity contribute to the impaired neonatal host defense. A domination of native immune cells, functional impairments, and lower leukocyte subset numbers contribute further to an increased susceptibility, although basic functions, such as recognition and phagocytosis of bacteria, are already developed in the same proportion as in adults (Gille et al., 2012).

These immunological problems are reflected in the clinical presentation of neonatal sepsis. Neonates have a rapid and fulminant progression of septicaemic disease, nonspecific clinical signs of infection, and difficult to interpret laboratory results including hematological and immunological biomarkers of infection and inflammation. Low birth weight (Preterm and small for gestational age) neonates have even poorer functional immunity, and are especially at risk of sepsis. However, neonates do have well-functioning cationic membrane active antimicrobial proteins and peptides (APPs) which have microbicidal properties. These APPs can be found in the vernix caseosa covering the skin at birth, and in the neonatal gastrointestinal and respiratory tracts (Edmond and Zaidi, 2010).

v. Clinical signs of neonatal sepsis

The clinical manifestations are cardiovascular dysfunction, increasing imbalance between oxygen transport and supplies, altered mechanisms of metabolism, and thus leading to multiple organ failure and possible death (Umlauf et al., 2013).
Signs and symptoms of sepsis include:

➤ Nonspecific clinical signs: (3P-Signs)
  • Poor breathing.
  • Poor sucking.
  • Poor looking.

➤ Symptoms of respiratory disorders:
  • Tachypnea (>60 per minute).
  • Chest retraction.
  • Grunting while breathing.
  • Inflating the nostrils (Nose wings).
  • Apnea/bradypnea (<30 per minute).
  • Hypoxia.
  • Irregular breathing.

➤ Symptoms of gastrointestinal and neurologic Disorders:
  - Gastrointestinal:
    • Loss of appetite.
    • Vomiting and diarrhea
    • Abdominal distension
    • Splenomegaly.
  - Neurologic:
    • Convulsions.
    • Hypotonia and hypodynamia.
    • Lethargy.

➤ Symptoms of cardiovascular and skin disorders:
  - Cardiovascular:
    • Hypotension.
    • Metabolic acidosis.
    • Tachycardia.
  - Skin:
    • Pale or marble with petechiae or purple.
    • Mottling.
    • Cold or wet.
    • Cyanosis.
    • Jaundice (Resch E and Resch B, 2013).
There is no evidence that the signs are different in preterm and term neonates. Late clinical signs are indicative of severe septicemia: Sclerema, shock, features of disseminated intravascular coagulation, pulmonary hemorrhage and collapse (Rosenberg et al., 2009).

Neonatal clinical sepsis syndrome identification is difficult as the clinical signs of neonatal septicemia can be very similar to those of other life-threatening diseases such as necrotizing enterocolitis, hyaline membrane disease, and perinatal asphyxia. However, recent studies in middle- and low-income countries have provided seven danger signs which can be used to identify neonates with very severe disease including neonatal sepsis:

- History of difficulty of feeding.
- History of convulsions.
- Movement only when stimulated.
- Respiratory rate ≥60 breaths per minute.
- Severe chest indrawing.
- Axillary temperature ≥37.5°C.
- Axillary temperature ≤35.5°C (Edmond and Zaidi, 2010).

These signs provide high sensitivity and moderate specificity for detecting serious illness in neonates in low-resource settings and have now been incorporated into the new neonatal World Health Organization (WHO) Integrated Management of Childhood Illness guidelines (Edmond and Zaidi, 2010).

Neonatal sepsis is known to be associated with a decrease in normal heart rate variability, as well as transient heart rate decelerations, even before clinical manifestations of sepsis become evident. This is thought to be due to impaired autonomic regulation and abnormal vagal firing associated with the systemic inflammatory response. Studies have suggested that the heart rate characteristics index (HRCi), a composite of statistical measures obtained from continuous heart rate monitoring, could be used as a noninvasive and early predictor of impending sepsis (Srinivasana and Harris, 2012).
vi. Diagnosis of neonatal sepsis

Clinicians are frustrated by the limitations in the diagnosis of neonatal sepsis and they would benefit from reliable tests in diagnosing sepsis early in its course. Currently, no single test fulfills the criteria of an ideal diagnostic test. In neonatology, tests which use hematological indices remain in widespread use, despite the continuing concerns about their reliability in diagnosing neonatal sepsis. These concerns largely stem from the demonstrated marked variations in the predictive accuracy of the hematological parameters (Sucilathangam et al., 2012).

The nonspecific nature of clinical signs in neonates probably leads to frequent overuse of broad spectrum antibiotics with the potential to select for resistant bacteria and fungi, especially in preterm neonates. Therefore, there is a great need for better rapid diagnostic tests to differentiate neonates with sepsis from those who are sick from other causes (Marchant et al., 2013).

Diagnostic tools of neonatal sepsis include:

1. Sepsis screen

All neonates suspected to have sepsis should have a septic screen to corroborate the diagnosis. However, the decision to start antibiotics need not be conditional to the sepsis screen result, if there is a strong clinical suspicion of sepsis. The various components of the septic screen include total leukocyte count, absolute neutrophil count, immature to total neutrophil ratio, micro-erythrocyte sedimentation rate and C reactive protein (table 1) (Sankar et al., 2008).

Components:

<table>
<thead>
<tr>
<th>component</th>
<th>Abnormal values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total leucocyte count</td>
<td>&lt;5000 mm$^3$</td>
</tr>
<tr>
<td>Absolute neutrophil count</td>
<td>Low count as per Manroe’s chart for term and Mouzinho’s chart for VLBW neonates</td>
</tr>
<tr>
<td>Immature to total neutrophil ratio</td>
<td>&gt;0.2</td>
</tr>
<tr>
<td>Micro-erythrocyte sedimentation rate</td>
<td>&gt;15 mm in 1st hour</td>
</tr>
<tr>
<td>C reactive protein</td>
<td>&gt;1 mg/dl</td>
</tr>
</tbody>
</table>

*VLBW: very low birth weight*
- **Immature to total neutrophil ratio (ITR):** Value above 27% in term neonates is considered positive. For preterm, it is considered to be 20%. ITR is defined as:

\[
\text{ITR} = \frac{\text{Immature neutrophils (Band forms, metamyelocytes & myelocytes)}}{\text{Mature + immature neutrophils}}
\]

(Polin, 2012).

- **C reactive protein (CRP):** Quantitative CRP assayed by nephelometry is superior to CRP by The enzyme-linked immunosorbent assay (ELISA) and semi-quantitative CRP by a latex agglutination kit (Polin, 2012).

- **Micro-erythrocyte sedimentation rate (μ-ESR):** Value (In mm in first hour) of more than 3+ age in days in the first week of life or more than 10 thereafter is considered positive (Polin, 2012).

- **Role of sepsis screen:**
  - **Role of sepsis screen in early onset sepsis (EOS):**
    
    There is no rationale for performing a “Sepsis screen” in suspected EOS. The negative predictive value of various sepsis screen parameters is too low to confidently rule out EOS (Polin, 2012).

    Scores obtained in the first several hours after birth have been shown to have poorer sensitivity and negative predictive value than scores obtained at 24 hrs. of age (Polin, 2012).

  - **Role of sepsis screen in late onset sepsis (LOS):**
    
    The neonate must be categorized into those with low probability of sepsis or high probability of sepsis. The rule of thumb is “Low probability” represents situations where the clinician would be willing to withhold antibiotics if the sepsis screen is negative. Those assessed to have a low probability of sepsis (e.g. Single episode of apnea or vomiting, but otherwise well) should undergo a sepsis screen. The purpose of the sepsis screen is to rule out sepsis rather than to rule in sepsis (Kudawla et al., 2008).

    Neonates assessed to have a high clinical probability of sepsis (For which the clinician is convinced that antibiotics must be started) may not be subjected to a sepsis screen, because a negative screen would not alter the decision to start antibiotics (Malik et al., 2003).
Interpretation:

Presence of two abnormal parameters in a screen is associated with a sensitivity of 93-100%, specificity of 83%, positive and negative predictive values of 27% and 100% respectively in detecting sepsis. Hence, if two or more parameters are abnormal, it should be considered as a positive screen and the neonate should be started on antibiotics. If the screen is negative but clinical suspicion persists, it should be repeated within 12 hrs. If the screen is still negative, sepsis can be excluded with reasonable certainty. For early onset sepsis, documentation of polymorphs in the neonatal gastric aspirate at birth could serve as a marker of chorioamnionitis and it may be taken as one parameter of sepsis screen (Sankar et al., 2008).

2. Hematological scoring system (HSS)

The HSS is a simple, quick and cost effective tool which can be used as a screening test for early diagnosis of neonatal sepsis (Narasimha et al., 2011).

The HSS assigns a score of one for each of the seven criteria found to be significantly associated with sepsis (Table 2) with one exception. An abnormal total polymorphonuclear leucocyte (PMN) count is assigned a score of 2 instead of 1, if no mature polymorphs are seen on the peripheral smear to compensate for the low immature to mature neutrophil ratio (Rodwell et al., 1988).

Components:

Hematologic scoring system of Rodwell et al. in 1988 includes the following:

a) White blood cell (WBC) and platelet count.
b) White blood cell differential count.
c) Nucleated red blood cell count (To correct WBC count).
d) Assessment of neutrophil morphology for degenerative changes. (Rodwell et al., 1988).

Immature neutrophils include promyelocyte, myelocyte, metamyelocytes and band form. Degenerative changes in neutrophils
include vacuolization, toxic granulations, and Dohle bodies. Interpretation is shown in (Table 3). Minimum score is 0 and maximum score is 8 (Rodwell et al., 1988).

**Table 2: Hematological scoring system (Narasimha et al., 2011).**

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Abnormality</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total WBC count</td>
<td>≤ 5,000 / µl</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>≥ 25,000 at birth</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>≥ 30,000 12-24 hrs.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>≥ 21,000 day 2 onward</td>
<td></td>
</tr>
<tr>
<td>Total PMN count</td>
<td>No mature PMN seen</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Increased / decreased</td>
<td></td>
</tr>
<tr>
<td>Immature PMN count</td>
<td>Increased</td>
<td>1</td>
</tr>
<tr>
<td>I:T PMN ratio</td>
<td>Increased</td>
<td>1</td>
</tr>
<tr>
<td>I:M PMN ratio</td>
<td>≥ 0.3</td>
<td>1</td>
</tr>
<tr>
<td>degenerative changes in PMN</td>
<td>Toxic granules / cytoplasmic vacuoles</td>
<td>1</td>
</tr>
<tr>
<td>platelet count</td>
<td>≤ 150,000 / µl</td>
<td>1</td>
</tr>
</tbody>
</table>

The normal values are

- Total PMN count: 1800-5400
- Immature PMN count: 600
- Immature: Total PMN ratio: 0.120
- Immature: Mature PMN ratio: ≥ 0.3

**Table 3: Interpretation of HSS (Narasimha et al., 2011).**

<table>
<thead>
<tr>
<th>Score</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 2</td>
<td>Sepsis is unlikely</td>
</tr>
<tr>
<td>3 or 4</td>
<td>Sepsis is possible</td>
</tr>
<tr>
<td>≥ 5</td>
<td>Sepsis or infection is very likely</td>
</tr>
</tbody>
</table>

- **Total white blood cell counts:**
  Total white blood cell counts have little value in the diagnosis of early onset sepsis and have a poor positive predictive accuracy. Many investigators have analyzed subcomponents of the white blood cell count (Neutrophil indices: Absolute neutrophil count, absolute band count, and immature to total neutrophil (ITR)) to identify infected neonates. Like most diagnostic tests for neonatal sepsis, neutrophil indices have proven most useful for excluding neonates without infection rather than identifying infected neonates (Polin, 2012).
- **The absolute neutrophil count:**

The absolute neutrophil count varies considerably in the immediate neonatal period and normal reference ranges are available from Manroe’s charts (Figure 2). The lower limit for normal total neutrophil counts in the neonate begins at 1800/mm$^3$, rises to 7200/mm$^3$ at 12 hrs. of age and then declines and persists at 1800/mm$^3$ after 72 hrs. of age. For VLBW neonates, the reference ranges are available from Mouzinho’s charts (Figure 3) (Sankar et al., 2008).

**Fig. 2: Manroe’s chart** (Manroe et al., 1979).

**Fig. 3: Mouzinho’s chart** (Mouzinho et al., 1994).

Neutropenia may be a better marker for neonatal sepsis and has better specificity than an elevated neutrophil count, because few conditions besides sepsis (Maternal pregnancy-induced hypertension, asphyxia and hemolytic disease) depress the neutrophil count of neonates. The definitions for neutropenia vary with gestational age, type of delivery, site of sampling, and altitude. In late preterm and term neonates, the definition for neutropenia most commonly used is that
suggested by Manroe et al (<1800/mm³ at birth and <7800/mm³ at 12–14 hrs. of age) (Polin, 2012).

- **The absolute immature neutrophil count:**

The absolute immature neutrophil count follows a similar pattern to the absolute neutrophil count and peaks at approximately 12 hrs. of life. The number of immature neutrophils increases from a maximal value of 1100 cells/mm³ at birth to 1500 cells/mm³ at 12 hrs. of age. Absolute immature counts have a poor sensitivity and positive predictive accuracy for early onset sepsis (Polin, 2012).

- **The ITR:**

  The ITR has the best sensitivity of any of the neutrophil indices. The ITR is <0.22 in 96% of healthy preterm neonates born at <32 weeks’ gestational age. Unlike the absolute neutrophil count and the absolute band count, maximum normal values for the ITR occur at birth (0.16) and decline with increasing postnatal age to a minimum value of (0.12). A single determination of the ITR has a poor positive predictive accuracy (Approximately 25%) but a very high negative predictive accuracy (99%). The ITR may be elevated in 25% to 50% of uninfected neonates (Polin, 2012).

  Analysis found that an abnormal immature to total neutrophil ratio followed by an abnormal immature to mature neutrophil ratio were the most sensitive indicators in identifying neonates with sepsis (Narasimha et al., 2011).

  The timing of the white blood cell count is critical. Counts obtained 6 to 12 hrs. after birth are more likely to be abnormal than are counts obtained at birth, because alterations in the numbers and ratios of mature and immature neutrophils require an established inflammatory response. Therefore, once the decision is made to start antimicrobial therapy soon after birth, it is worth waiting 6 to 12 hrs. before ordering a white blood cell count and differential count (Newman et al., 2010).

- **Platelet counts:**

  Despite the frequency of low platelet counts in infected neonates, they are nonspecific, insensitive, and late indicators of sepsis. Moreover, platelet counts are not useful to follow clinical response to antimicrobial
agents, because they often remain depressed for days to weeks after a sepsis episode (Polin, 2012).

Thrombocytopenia was frequently associated with sepsis and indicated poor prognosis. This is thought to be due to increased platelet destruction, sequestration secondary to infections, failure in platelet production due to reduced megakaryocytes or damaging effects of endotoxin (Makkar et al., 2013).

➢ Conclusion:

Considering all four parameters i.e.: Sensitivity, specificity, positive predictive value and negative predictive value, immature to mature PMN ratio and degenerative changes were the most reliable tests for diagnosing sepsis. An abnormal immature to mature PMN ratio was highly sensitive in identifying sepsis. Degenerative changes in neutrophils were not found to be a very sensitive indicator of sepsis. Thrombocytopenia was consistently associated with poor prognosis. The higher the score; the greater was the likelihood of sepsis. A score of ≤2 suggests that sepsis was unlikely (Narasimha et al., 2011).

HSS can improve the diagnostic accuracy of complete blood count. It can be employed as a screening test for diagnosing sepsis. But it is important to simplify and standardize the interpretation of this global test (Rodwell et al., 1988).

HSS is still used as a screening test for diagnosing sepsis and to differentiate infected neonates from the non-infected ones. Furthermore, the sensitivity and the specificity of the test are also high, with certainty of sepsis increasing with the score (Makkar et al., 2013).

3. Blood culture

➢ Pearls and pitfalls:

Blood culture (BC) is the traditional gold standard for diagnosis; however, false negative results are common. Bacteremia is often of low density and intermittent and the blood samples obtained are frequently small in volume. Additionally, neonates and/or mothers may have
received antibiotics prior to the blood culture, further reducing the diagnostic yield of the test (Srinivasana and Harrisa, 2012).

BC also suffers from the disadvantages of low sensitivity and reporting delay of 24–72 hrs. (Paolucci et al., 2012).

The sensitivity of blood culture for identifying sepsis is only 50% to 80% at best (Gerdes, 2004).

Contamination rates may also be very high due to the technical difficulties of sterile venipuncture in small babies. There may also be misinterpretation of the role of coagulase-negative staphylococci (e.g., S. epidermidis), as these organisms are both normal skin flora and pathogenic organisms in preterm and neonates with indwelling blood vessel catheters (Edmond and Zaidi, 2010).

The diagnostic capabilities of blood culture systems have improved over the last decade with the advent of automated continuous blood culture monitoring systems. Although these systems can save time, subcultures are required for specific biochemical or other assays, ultimately needed for pathogen identification (Paolucci et al., 2012).

Important procedures to improve the sensitivity and specificity of blood cultures include proper skin disinfection before collection, culturing early in the septic episode, taking an appropriate volume of blood per culture, and, if collecting through an existing intravenous device, ensuring that a peripheral culture is also collected and, where practical, more than one bottle per episode. This is not always feasible in a very tiny neonate (Paolucci et al., 2012).

➢ **Volume of blood culture:**

The volume of blood inoculated in the culture bottle significantly influences the blood culture positivity. A single blood culture in a sufficient volume is required for all neonates with suspected sepsis. Data suggest that 1.0 ml of blood should be the minimum volume drawn for culture when a single pediatric blood culture bottle is used. Dividing the specimen in half and inoculating aerobic and anaerobic bottles is likely to decrease the sensitivity (Polin, 2012).
Bacteremias with high concentrations of organisms require less blood to be sampled than low density bacteremias. If organisms were present at densities of <4 colony-forming units (cfu)/ml, blood volumes of 0.5ml or less had a significantly diminished chance of detecting bacteremia. This finding did not differ between organisms (Paolucci et al., 2012).

Furthermore, up to 25% of neonates with sepsis have low colony count bacteremia (≤4 cfu/ml), and two-thirds of neonates younger than 2 months of age have colony counts <10 cfu/ml (Polin, 2012).

- **Number of blood cultures:**

  A single positive blood culture in an asymptomatic neonate may be a false positive and, depending on the organism, may have little clinical significance (Vergnano et al., 2011).

  There is limited information to guide the practitioner on the optimal number of blood cultures that should be obtained. Some data have suggested that multiple site blood cultures may improve pathogen detection if bacteremia is intermittent, if there is a low density of bacteria present in the circulation, if there is an over dilution of the small volume of blood obtained during a culture with the blood culture broth, or if there is an inhibitory effect of the intrapartum antibiotic therapy administered to mothers (Paolucci et al., 2012).

  Indeed, several authors have suggested that multiple-site blood cultures may be more efficacious. There are no neonatal data; as usual practice is to take only one blood culture before starting antibiotic treatment. This decreased sampling is attributed to the small circulating blood volumes of neonates, the potential for increased transfusion requirements, the technical difficulties posed, and the possible rapid deterioration of neonates in the setting of sepsis. This study strongly indicated that a single site blood culture with blood volume of ≥1mL should be sufficient to document “true” gram positive, gram negative or fungal sepsis in neonates (Paolucci et al., 2012).

  However, given the low virulence of CoNS, and its ubiquity in the environment, it is frequently difficult to distinguish true infection from
specimen contamination. The diagnosis of a true CoNS bacteremia relies on 2 cultures obtained from different sites within 24 hrs. growing the same organism with the same sensitivities, although this is rarely done in practice (Chu et al., 2012).

Given the low likelihood of anaerobic infections, many experts recommend deferring routine anaerobic culture in favor of using a larger aliquot of blood to inoculate a single aerobic culture bottle. Inoculating the full milliliter into a single aerobic bottle will have a higher yield for most patients (Plantino et al., 2013).

- **Timing of blood collection:**

  Practically, the optimal time to culture for bacteremia is “As early as possible” in the course of a septic episode and the interval between repeated blood cultures does not appear to be important. Wherever feasible, BC should be obtained before initiation of antibiotic therapy (Paolucci et al., 2012).

- **Interpretation of blood culture:**

  Time to positivity of neonatal blood cultures was significantly shorter for gram negative organisms. Authors suggest shortening the total incubation time of neonatal blood cultures to a maximum of 3 days. According to results showed, authors propose to narrow the antimicrobial spectrum to solely target gram positive bacteria when the culture is still negative after 48 hrs. and to cease antimicrobial therapy when the culture is still negative after 72 hrs. in clinically well neonates (Paolucci et al., 2012).

  Interpreting positive results depends on clinical presentation, how the culture was taken, the organisms grown, and the time taken for the blood culture to become positive. Some organisms, such as Neisseria meningitides and Candida albicans, are nearly always significant, even in the context of a well-looking child. Cultures positive with potential pathogens that may also be contaminants are far more difficult to interpret, the most common of which is CoNS. These results must always be interpreted in the specific clinical context in which they are seen. Whereas CoNS grown from a previously well child presenting from the
community are almost always a contaminant, CoNS growing three days later from the same child after being in hospital with an indwelling intravascular device may well be significant (Paolucci et al., 2012).

Cultures drawn through indwelling intravenous devices are more likely to be contaminated with CoNS colonizing the lumen of the device, which may not be causing systemic infection (Paolucci et al., 2012).

Use of umbilical venous catheters, indwelling arterial lines or capillary blood samples for culture increase the risk of contamination. If catheter-associated sepsis is suspected, a culture should be obtained through the catheter as well as through a peripheral vein (Hasana et al., 2008).

4. Lumbar puncture (LP)

➢ Timing and indications:

The decision to perform a lumbar puncture in a neonate with suspected early onset sepsis remains controversial. In the high-risk, healthy-appearing neonate, data suggest that the likelihood of meningitis is extremely low (Polin, 2012).

In the neonate with clinical signs that are thought to be attributable to a noninfectious condition, such as respiratory distress syndrome, the likelihood of meningitis is also low (Polin, 2012).

However, in bacteremic neonates, the incidence of meningitis may be as high as 23% (Polin, 2012).

In 2002, the American Academy of Pediatrics (AAP) and the Centers for Disease Control and Prevention (CDC) released guidelines stated that LPs should be reserved for neonates with “Signs” of a systemic infection and should not be performed on neonates without “Signs”. Despite this, “Signs” of a systemic infection can be subtle such as poor feeding, irritability or a high pitched cry, leading to some variability in the diagnosis. In addition, it is unclear how broadly these guidelines have been adopted (Patrick et al., 2012).
Blood culture alone cannot be used to decide who needs a lumbar puncture, because blood cultures can be negative in up to 38% of neonates with meningitis (Polin, 2012).

Although the number of neonates with a positive cerebrospinal fluid (CSF) culture and a negative blood culture was low, these cases are a reminder that meningitis will be missed in some neonates if LP is not part of the sepsis workup. Moreover; antibiotic therapy may change with early onset meningitis caused by gram negative organisms (Stoll et al., 2011).

It should also be noted that in the scenario of a positive blood culture, the importance of performing LP may be dependent on the organism identified, given that isolates such as group B Streptococcus or Candida portend such poor outcomes if spread to the CSF (Chu et al., 2012).

The lumbar puncture should be performed in any neonate with a positive blood culture, neonates whose clinical course or laboratory data strongly suggest bacterial sepsis and neonates who initially worsen with antimicrobial therapy. For any neonate who is critically ill and likely to have cardiovascular or respiratory compromise from the procedure, the lumbar puncture can be deferred until the neonate is more stable (Polin, 2012).

**Precautions of LP:**

The cellular and biochemical abnormalities in the CSF with bacterial meningitis persist for up to 3 days. gram positive bacteria clear in 36 hrs. of appropriate therapy whereas gram negative bacteria may take up to 5 days. If the LP is traumatic, the CSF should be sent for gram stain and culture. The concentration of glucose is not significantly altered by a traumatic lumbar puncture. Therefore a low CSF glucose in the setting of a traumatic LP is abnormal. Nothing much is gained by using the various formulas for adjusting the WBC count in a traumatic CSF, based on the red blood cell (RBC) counts. Adjustment merely results in a loss of sensitivity with marginal gain in specificity (Polin, 2012).
Ideally, the WBC count must be performed within 30 minutes of drawing the sample. It must be noted that CSF, WBC and glucose rapidly fall with time, giving spurious results (Rajesh et al., 2010).

Lumbar punctures are invasive procedures and have been noted to be more difficult to perform in neonates than in other populations and to cause neonatal distress. In this population, complications such as “bloody taps” and contaminated specimens are common, and therefore limiting a neonate's exposure to an LP may be desirable (Patrick et al., 2012).

➢ Interpretation:

The mean number of white blood cells in uninfected preterm or term neonates was consistently <10 cells/mm (Shah et al., 2011).

The median number of white blood cells in neonates who were born at greater than 34 weeks’ gestation and had bacterial meningitis was higher than those born at less than 34 weeks’ gestation and had meningitis, also neonates with meningitis attributable to gram negative pathogens typically have higher CSF white blood cell counts than do neonates with meningitis attributable to gram positive pathogens (Polin, 2012).

Protein concentrations in uninfected, term neonates are <100 mg/dl, Preterm neonates have CSF protein concentrations that vary inversely with gestational age. In the normoglycemic neonate, glucose concentrations in CSF are similar to those in older neonates and children (70%–80% of a simultaneously obtained blood specimen). A low glucose concentration is the CSF variable with the greatest specificity for the diagnosis of meningitis. Protein concentrations are higher and glucose concentrations are lower in term than in preterm neonates with meningitis. However, meningitis occurs in neonates with normal CSF values, and some of these neonates have high bacterial inoculation (Kestenbaum et al., 2010).

5. Urine analysis and culture

The signs of urinary tract infection (UTI) in neonates are nonspecific and varied. The common clinical signs in cases of UTI in
neonates are: Failure to thrive (50%), fever (39%), vomiting (37%), diarrhea (25%), cyanosis (23%), jaundice (18%), irritability & lethargy (17%) (Tamim et al., 2003).

A urine culture should not be part of the sepsis workup in a neonate with suspected early onset sepsis. Unlike urinary tract infections in older neonates (Which are usually ascending infections), urinary tract infections in neonates are attributable to seeding of the kidney during an episode of bacteremia (Polin, 2012).

Routine urine microscopy has poor correlation with culture and must not be relied upon for diagnosing UTI. More accurate microscopic analysis of uncentrifuged urine can be performed with a hemocytometer and reporting cells per cubic millimeter. With hemocytometer, a cutoff of \( \geq 10 \) WBCs/mL has a sensitivity of 82% and specificity 94% (Lin et al., 2000).

Urine culture must always be performed on a sample obtained by a supra-pubic puncture or by a fresh bladder catheter. In neonates, use of ultrasound guidance simplifies supra-pubic aspiration and improves the diagnostic yield of obtaining a urine specimen from 60% to almost 97% (Buys et al., 1994).

6. **Gastric aspirates**

The fetus swallows 500 to 1000 mL of amniotic fluid each day. Therefore, if there are white blood cells present in amniotic fluid, they will be present in gastric aspirate specimens at birth. However, these cells represent the maternal response to inflammation and have a poor correlation with neonatal sepsis. Gram stains of gastric aspirates to identify bacteria are of limited value and are not routinely recommended (Polin, 2012).

7. **Body surface culture**

Bacterial cultures of the axilla, groin, and the external ear canal have a poor positive predictive accuracy. They are expensive and add little to the evaluation of a neonate with possible bacterial sepsis (Polin, 2012).
8. Tracheal aspirate

Cultures and gram stains of tracheal aspirate specimens may be of value if obtained immediately after endotracheal tube placement. Once a neonate has been intubated for several days, tracheal aspirates are of no value in the evaluation of sepsis (Polin, 2012).

9. Acute phase reactants

A wide variety of acute phase reactants have been evaluated in neonates with suspected bacterial sepsis. However, only C-reactive protein (CRP) and procalcitonin concentrations have been investigated in sufficiently large studies (Vouloumanou et al., 2011).

➢ C-reactive protein (CRP):

Since the end of the 1980s, CRP has been routinely investigated and used for the diagnosis of neonatal sepsis. CRP is an acute phase protein synthesized by the liver within 6-8 hrs. in response to inflammatory cytokines, peaks at 24-48 hrs., and then diminishes rapidly after elimination of the source. Elevated levels of CRP are present during bacterial, viral and other infections, and in noninfectious inflammatory diseases and malignancies (Payasli et al., 2013).

In variable studies using CRP ≥1 mg/dl as the cut-off value, the range of reported statistical outcomes is as follows: Sensitivity (70% to 93%); specificity (41% to 98%); positive predictive accuracy (6% to 83%); and negative predictive accuracy (97% to 99%). On the other hand, quantitative CRP values, particularly when repeated, are highly specific and have good sensitivity (Payasli et al., 2013).

The increase in the serum levels of CRP is rather slow during the first 12-24 hrs. of infection, and this may cause false negative results. In addition, increase in CRP levels in non-infected clinical conditions (Trauma, surgical intervention, recent vaccination, prolonged rupture of membranes, fetal distress, perinatal asphyxia, intraventricular hemorrhage, meconium aspiration, burns and malignancies) can cause a false positive test. In spite of the reduced early sensitivity, CRP still
remains the preferred index in most neonatal intensive care units (Payaslı et al., 2013).

If CRP remains negative in repeating determinations over a period of 2-3 days, it allows early cessation of antimicrobial therapy in clinically healthy neonates (Umlauf et al., 2013).

There is a steady increase in the CRP levels in the infected neonates. However, in the non-infected neonates, the levels showed a slight increase at 24 hrs. but started to fall by 48 hrs. (Prashant et al., 2013).

Some authors have suggested that serial determinations may be useful for identification of neonates who do not have a bacterial infection as well: Two consecutive CRP values <10 mg/l carry a 99% negative predictive value in accurately identifying not infected neonates. At 48 hrs. after onset of symptoms with at least two normal CRP values and negative blood cultures, infection can be ruled out and antibiotics can be stopped (Resch E and Resch B, 2013).

After 3 days of life, CRP is the best single test in early detection of neonatal septicemia. Serial serum CRP estimation confirms the diagnosis; monitor the course of infection and the efficacy of antibiotic treatment. In episodes of neonatal septicemia, where, antibacterial treatment fails, CRP levels are moderately elevated, the day prior to treatment start and increase continuously thereafter. Whereas, success of treatment is generally accompanied by a decline in CRP within four days (Kumar, 2013).

A large number of methods are available for the determination of CRP in the serum. Although, electroimmunoprecipitation assay, immunometric assay and laser nephelometry are sensitive and quantitative methods for the estimation of CRP. The facilities for such specialized investigations are not available at all centers. In their absence, latex agglutination method is quiet sensitive, quick and semi quantitative method for CRP determination (Kumar, 2013).
Procalcitonin (PCT):

Procalcitonin (PCT) is a 116-amino acid protein, a precursor of calcitonin which is produced by the thyroid. In sepsis, macrophages and the monocytic cells of the liver are involved in the synthesis of PCT (Sucilathangam et al., 2012).

Procalcitonin concentrations increase within 2 hrs. of an infectious episode, peak at 12 hrs. and normalize within 2 to 3 days in healthy adult volunteers. A physiologic increase in procalcitonin concentration occurs within the first 24 hrs. of birth, and an increase in serum concentrations can occur with noninfectious conditions (e.g., Respiratory distress syndrome). Procalcitonin concentration has a modestly better sensitivity than does CRP concentration but is less specific (Benitz, 2010).

In a recent meta-analysis the sensitivity and specificity of PCT in the diagnosis of early onset sepsis were 76% and 76%. Though the sensitivity during the early stages of sepsis may be superior to CRP, the significant rapid variations of basal levels after birth, the increase after non-infectious conditions such as asphyxia, maternal pre-eclampsia, and intracranial hemorrhage, and the need for several different cut-off values with changing neonatal age, have limited its diffusion as an early marker in comparison to CRP (Resch E and Resch B, 2013).

There is evidence from studies that significant reductions in use of antimicrobial agents can be achieved in patients whose treatment is guided by procalcitonin concentration (Hayashi and Paterson, 2011).

The procalcitonin concentration was elevated in culture positive neonates and decreased with appropriate antibiotic therapy. In some cases of culture positive neonates other sepsis screening tests were negative but the level of procalcitonin was elevated. These findings support the usefulness of the PCT to establish an early diagnosis of neonatal sepsis (Zahedpasha et al., 2009).

PCT is highly specific for bacterial infection and it helps differentiating it from viral infection. It correlates well with the progression and the severity of the infection. PCT helps in early diagnosis of the sepsis on the day of the admission itself, before the blood culture
report is ready (Usually after 3-5 days). PCT helps in avoiding antibiotic therapy where it is not required and thereby reducing the cost and the occurrence of bacterial resistance. PCT can also be employed for the prognosis of sepsis (Sucilathangam et al., 2012).

The sensitivity of PCT for detecting sepsis (More than 0.5 ng /ml) was 92.8%, its specificity 75.0%, its positive predictive value was 59.0% and its negative predictive value was 96.0 %. and the sensitivity of CRP for predicting sepsis (More than 6 mg/l) was 50.0%, its specificity was 69.4%, its positive predictive value was 38.8% and its negative predictive value was 78.1% (Sucilathangam et al., 2012).

However, when PCT is used together with CRP, a negative PCT test result may help in “ruling out”, while a raised CRP result helps in “ruling in”, the possibility of sepsis, particularly of the late onset type (Sucilathangam et al., 2012).

➢ Cytokines:

Cytokines such as interleukins (IL6 & IL8) and tumor necrosis factor (TNF-a) rise quickly after infection even in neonates, and are more sensitive to low concentrations of pathogens than CRP. However, cord and postnatal blood cytokine concentrations can be depressed in the presence of pregnancy-induced hypertension and can rise after induced vaginal or urgent cesarean delivery, delivery room intubation, muscular damage, and inflammation from other causes. Simultaneous measurement of multiple biomarkers may improve both sensitivity and specificity (Edmond and Zaidi, 2010).

IL-6 detects sepsis at an early stage of infection with a maximum of serum levels as early as 1-2 hrs. after inflammation reaction has started, potentially even prior to onset of clinical symptoms. The diagnostic window is short, due to the instability of IL-6, resulting in a short half life time of <20 minutes. In contrast, CRP has its optimum sensitivity and specificity during the window of 24–48 hrs. after onset of symptoms. Thus, a diagnostic gap of several hours might occur (Umlauf et al., 2013).
The combined measurement of a cytokine (IL6 or IL8) and CRP is currently considered as the most reliable method with the highest sensitivity and specificity for early diagnosis of both EOS and LOS (Benitz, 2010).

10. New techniques to diagnosing neonatal sepsis

Abnormal hematological counts, acute-phase reactants, and inflammatory cytokines are neither sensitive nor specific, especially at the onset of illness. Further, microbiological culture results are not usually available until at least 48-72 hrs. after the specimen reaches the laboratory, and high false-negative rates of culture results may occur. Thus rapid diagnostic tests that differentiate infected from non-infected neonates, have the potential to make a significant impact on neonatal care (Payaslı et al., 2013).

- **Antigen detection techniques**

  Antigen detection techniques allow rapid detection and identification of microorganisms without culturing. The most commonly used commercially available test is the latex agglutination assay, which is based on specific agglutination by bacterial cell wall antigens of antibody coated latex particles. However, these tests can only detect specific organisms such as *Streptococcus agalactiae* and are associated with high false positive and negative rates (Edmond and Zaidi, 2010).

- **The polymerase chain reaction (PCR)**

  PCR has been widely used in biomedical research laboratories for pathogen identification in neonatal sepsis and in some clinical hospital laboratories. The high sensitivity of PCR allows detection of bacterial DNA even when concentrations are low (Edmond and Zaidi, 2010).

  PCR can be used to distinguish bacterial septicaemic disease from other causes of neonatal illness such as asphyxia or complications of prematurity. However, it has been used with varying success in the analysis of whole blood for neonatal sepsis; specificity is generally high but sensitivity can be as low as 40% (Jordan and Durso, 2005).
Our experience of the molecular diagnosis of neonatal sepsis is based on the employment of a commercial multiplex real time PCR. This test detects more than 25 bacterial and fungal species in a single reaction (Paolucci et al., 2012).

The biggest problem with real time PCR testing is that the specimen must be collected with a sterile venipuncture, which may be difficult in young neonates. Neonatal capillary heel prick specimens are easier to collect but highly contaminated by skin flora. There is also high potential for contamination of enrichment media, reagents, or the sample during collection and processing (Edmond and Zaidi, 2010).

Other problems include low sensitivity due to competition from human DNA in whole blood, especially if white cell counts are high. Also, bacterial organisms require lysis before their DNA can be available for analysis, and gram positive organisms are difficult to lyse because of their resilient cell wall. Real time PCR technologies are also expensive and currently can be used only by highly trained staff (Edmond and Zaidi, 2010).

➢ Micro technologies

Microfluidics has provided the greatest recent contribution to the diagnosis of neonatal sepsis. Microfluidics is the study of the behavior, precise control, and manipulation of fluids geometrically constrained to submillimetre (Nanolitre or picolitre) channels (Edmond and Zaidi, 2010).

In the future, new diagnostic technologies involving microfluidics may considerably reduce the amount of blood volumes required for diagnosis. At present, the relatively high cost of this technique limits its routine use in the clinical setting (Marchant et al., 2013).

This technique has been used in the identification of the specific sepsis pathogen in bacterial meningitis, acute viral respiratory tract infections and neonatal sepsis. It is used also in the detection of their antimicrobial resistance and virulence genes in research settings (Andrade et al., 2008).
However, they are not yet in clinical use nor licensed by regulatory authorities (Edmond and Zaidi, 2010).

➢ Flow cytometry

Flow cytometry is a confidential diagnostic tool. Advanced flow cytometry is undeniably the best tool for analyzing signaling processes, proliferation and differentiation, cell to cell interactions, surface markers, intracellular molecules and proteins secreted by cells (Venet et al., 2011).

Flow cytometry allows easy measurement of a various number of parameters in the pathway of neonatal sepsis. Nevertheless, none of these parameters is able to be the one parameter in diagnosis of EOS. So far, the combination of CRP and IL6 remains the diagnostic resource of choice in detection of EOS and LOS. Solely CD64 seems to have the potential to complement the existing combination of CRP and a cytokine to increase sensitivity up to 100%. Larger trials to define standard measurement protocols and reference values are highly desirable (Umlauf et al., 2013).

➢ Polymorphonuclear elastase (PMN elastase)

PMN elastase is a serine protease stored in the azurophilic granules of neutrophils and secreted by neutrophils during Inflammation (Payaslı et al., 2013).

Various published studies have shown PMN elastase to be a useful marker of early infection in the neonate. Septic neonates showed significantly increased PMN elastase levels at the time of recognition of infection (Payaslı et al., 2013).

The sensitivity and specificity of serum PMN elastase in the early diagnosis of neonatal sepsis were 76% and 81% respectively. Findings suggest that PMN elastase is an almost perfect marker and more sensitive and specific than CRP in the diagnosis of neonatal sepsis. However, lack of correction for reference ranges for neonatal PMN elastase values may influence the outcome of PMN elastase as a marker for bacterial infection. In addition, methodological difficulties in detecting PMN elastase and the absence of their routine usage in all centers have limited its use in daily practice (Payaslı et al., 2013).
vii. Prevention of neonatal sepsis

1. Before delivery

➢ Maternal nutrition:

Many older studies have demonstrated that improving maternal health and nutrition before delivery is directly associated with improved neonatal health outcomes (Edmond and Zaidi, 2010).

Randomized controlled trials (RCTs) of maternal protein-calorie and multiple micronutrient and supplementation have demonstrated significant improvements in rates of prematurity and birth weight and variable impact on mortality; but no studies have examined their impact on rates of neonatal sepsis (Haider and Bhutta, 2012).

➢ Maternal immunization:

Maternal immunization is an important method of providing neonates with appropriate antibodies as soon as they are born. Examples of successful interventions include maternal tetanus toxoid and influenza immunization (Zaman et al., 2008).

Studies of maternal immunization with Streptococcus agalactiae (S. agalactiae) type III conjugate vaccine have demonstrated excellent placental transfer and persistence of protective levels in 2 months old neonates. A recent modeling study estimated that vaccination with S. agalactiae vaccine would prevent 4% of preterm births and 60%–70% of neonatal S. agalactiae infections (Edmond and Zaidi, 2010).

Encouraging results are also emerging from studies of maternal immunization with pneumococcal polysaccharide and conjugate vaccines (Edmond and Zaidi, 2010).

The vaccines all have excellent safety profiles. However, barriers to maternal immunization include: Liability issues for vaccine manufacturers in developed countries, education of the public and health care providers regarding the benefits of maternal immunization, and poor ascertainment of data from low-income countries (Healy and Baker, 2007).
Intrapartum intravenous antimicrobial agents for the prevention of group B Streptococci (GBS) infections:

The only intervention proven to decrease the incidence of early onset neonatal sepsis (EOS) is maternal treatment with intrapartum intravenous antimicrobial agents for the prevention of GBS infections (Verani et al., 2010).

Intrapartum antibiotic prophylaxis has been highly effective in reducing both EOS and maternal sepsis in developed countries (Ohlsson and Shah, 2013).

Prevention of E. coli sepsis, especially among preterm neonates, remains a challenge. Despite the data shown on increasing incidence for E. coli EOS, no prevention or screening programs are possible during pregnancy and at delivery. Sepsis caused by E. coli can be only diagnosed on a clinical basis and with the support of blood culture (Paolucci et al., 2012).

- Choice

Adequate prophylaxis is defined as penicillin (The preferred agent), ampicillin, or cefazolin given for ≥4 hrs. before delivery. Erythromycin is no longer recommended for prophylaxis because of high resistance rates. In parturients who have a nonserious penicillin allergy, cefazolin is the drug of choice. For parturients with a history of serious penicillin allergy (Anaphylaxis, angioedema, respiratory compromise or urticaria), clindamycin is an acceptable alternative agent, but only if the woman’s rectovaginal GBS screening isolate has been tested and documented to be susceptible. If the clindamycin susceptibility is unknown or the GBS isolate is resistant to clindamycin, vancomycin is an alternative agent for prophylaxis. However, neither clindamycin nor vancomycin has been evaluated for efficacy in preventing early onset GBS sepsis in neonates (Verani et al., 2010).

- Duration

The origin of this four hour duration for Intrapartum GBS antibiotic prophylaxis is unclear. In the presence of at least one risk factor such as premature delivery <37 week gestation, rupture of membranes >6 hrs., or
maternal fever of ≥37.5°C, intrapartum antibiotic prophylaxis of <4 hrs. results in higher rates of vertical transmission of neonatal GBS colonization (Turrentine et al., 2013).

Yet even in the presence of maternal risk factors, if Intrapartum antibiotic was given at least 2 hrs. before delivery, the effectiveness in preventing early onset GBS disease was demonstrated. However, the majority of neonates exposed to GBS at birth are delivered to colonized mothers without additional risk factors (Turrentine et al., 2013).

- **Indications**

Intrapartum antimicrobial agents are indicated for the following situations:

1. Positive antenatal cultures or molecular test at admission for GBS (Except for women who have a cesarean delivery without labor or membrane rupture).
2. Unknown maternal colonization status with gestation <37 weeks, rupture of membranes >18 hrs. or temperature >100.4°F (>38°C).
3. GBS bacteriuria during the current pregnancy.
4. Previous neonate with invasive GBS disease (Verani et al., 2010).

- **Obstacles**

Missed opportunities for prevention of GBS were identified, including failure to screen all women who deliver at term, failure to provide antibiotics to all colonized women or to those who delivered preterm with unknown colonization status, and false-negative GBS screens among some women who deliver neonates with GBS infection. Negative GBS screens among women who deliver neonates with GBS are particularly troubling and may be attributable to insufficient sampling, delay in processing, suboptimal laboratory techniques, recent antibiotic use, or colonization after screening was performed. Accurate rapid GBS diagnostic tests at the time a woman presents in labor would help reduce the number of early onset GBS cases. Of note, the recently updated Centers for Disease Control and Prevention (CDC) GBS prevention guidelines recommend chemoprophylaxis for women with risk factors at delivery, despite negative screening cultures (Stoll et al., 2011).
2. During labour and delivery

There is strong evidence that clean delivery practices and hand washing during delivery reduces rates of neonatal sepsis in both home and health facility settings. The reasons for lack of successful scale up of hand washing interventions into policy, programs, and behavior change are less clear (Curtis et al., 2009).

New studies indicate that maternal antisepsis interventions such as vaginal chlorhexidine during labor may have a significant impact on rates of neonatal mortality and sepsis in developing countries. However, other studies from high-income countries have demonstrated little effect on rates of human immunodeficiency virus (HIV) or neonatal infections (McClure et al., 2007).

3. After delivery

In the hospital setting, the mainstay of prevention against neonatal sepsis includes strict hand washing practices; careful aseptic procedures in the management of intravenous lines, skin care, judicious use of antibiotics, promoting early enteral (As opposed to parenteral) nutrition, preferably using breast milk (i.e., To enhance the neonate’s own gastrointestinal immune defenses), and minimizing invasive interventions (e.g., Prompt removal of central venous catheters (CVCs) & reducing mechanical ventilation). Hand washing is a widely accepted and cost effective measure to decrease the occurrence of nosocomial infections including coagulase negative staphylococci (CoNS); yet universal compliance is difficult to achieve (Marchant et al., 2013).

There is also strong evidence that hand washing by health care providers after delivery can reduce neonatal sepsis and infection rates, especially in hospitals. There is less evidence for the importance of rigorous hand washing and use of antiseptics in mothers of their own neonates (Rhee et al., 2008).

In high-income settings, studies have not shown an advantage of antibiotics or antiseptics over simply keeping the umbilical cord clean. However, umbilical stump chlorhexidine cleansing has recently been shown to substantially reduce neonatal deaths (Mullany et al., 2006(A)).
There is emerging evidence that neonatal skin antisepsis preparations such as sunflower seed oil provides cheap, safe, and effective protection against nosocomial infections in hospitalized preterm neonates. Application of chlorhexidine to neonatal skin has also been shown to be effective in reducing neonatal sepsis (Mullany et al., 2006(B)).

The number of central lines experienced by the neonate from birth, rather than the duration of insertion, was an important predictor of CoNS sepsis. Some authors have proposed the use of prophylactic antibiotics immediately before and for 12 hrs. after removal of a CVC in preterm neonates (Marchant et al., 2013).

Clinical trials of vancomycin added to parenteral nutrition solutions have demonstrated a decrease in the incidence of CoNS sepsis in preterm neonates, without reduction in mortality or duration of hospital stay. Others have proposed using antibiotic-coated devices for CVC. However, these measures carry a risk of increasing antimicrobial resistance and have not been universally adopted. Antimicrobial “Locks”, that is, leaving a microbicidal substance within the catheters in between administration of other drugs represents another proposed solution to decrease bacterial colonization. Antiseptics (e.g., Alcohol & taurolidine), anticoagulants (e.g., Heparin & EDTA), and antibiotics (e.g., Vancomycin & rifampicin) have all been studied (Marchant et al., 2013).

However, clinical experience with these methods is very limited in very low birth weight (VLBW) neonates. In the absence of more definitive evidence, the standard of care is to use strict hand hygiene and skin antisepsis protocols prior to, during, and after catheter insertion (Marchant et al., 2013).

Neonatal immunization has long been considered an important method of reducing neonatal infections. However, response varies according to the antigen. Bacillus Calmette and Guerin (BCG), polio, and hepatitis B vaccines are highly immunogenic when given at birth. However, maternal antibodies interfere with a neonate’s response to measles vaccine when administered less than six months. Protein antigen vaccines (e.g., Pertussis and tetanus toxoid) given at birth have been
shown to produce poor responses compared to the same antigen given at two months of age and are associated with later tolerance. Studies also indicate that *S. agalactiae* and *Streptococcus pneumoniae* vaccines are both likely to be ineffective when given in the neonatal period (Edmond and Zaidi, 2010).

Breastmilk contains secretory immunoglobulin A (IgA), lysozymes, white blood cells, and lactoferrin and has been shown to encourage the growth of healthy lactobacilli and reduce the growth of *E. coli* and other gram negative pathogenic bacteria (Levy, 2007).

RCTs that focused on increasing early initiation and exclusive breastfeeding rates demonstrated significant reductions in diarrhea and acute respiratory infections in neonates. Other observational studies have demonstrated impact on infection specific mortality rates during the neonatal period (Mullany et al., 2008).

Neonatal micronutrient supplementation trials have focused on vitamin A supplementation. Older studies have shown significant reductions in respiratory disease in low birth weight neonates after the administration of parenteral vitamin A. More recently, trials of neonate vitamin A supplementation have shown encouraging reductions in neonatal mortality (Gogia and Sachdev, 2009).

In high-income countries, clinical trials of immune stimulants such as granulocyte/monocyte colony stimulating factor (GM-CSF) to enhance the quantity and quality of neonatal neutrophils and monocytes appear promising but have not yet shown a significant clinical benefit (Buhimschi et al., 2007).

The benefits of intravenous infusion of immunoglobulin G (IgG) in the prevention and treatment of neonatal sepsis in premature neonates were demonstrated in a trial of more than 3400 neonates. The investigators randomized neonates receiving antibiotics for suspected or proven infection to either receiving two infusions of polyvalent IgG or matching placebo infusions 48 hrs. apart. This study found no differences between groups in death or major disability at age 2 years; no significant differences in rates of EOS or late onset neonatal sepsis (LOS) were reported (Brocklehurst et al., 2011).
Antifungal prophylaxis has also been studied for prevention of *Candida* infections. Although topical nystatin and prophylactic fluconazole appear to provide significant reduction in *Candida* LOS, this strategy should be balanced against the low reported rates of invasive candidiasis in VLBW neonates (Downey et al., 2010).

viii. Treatment of neonatal sepsis

1. General supportive care

Success in diagnosis and treatment of neonatal sepsis is only partially due to the use of appropriate antibiotics. Clinical monitoring of asymptomatic at-risk neonates is paramount in the well-baby nursery, so that the early signs and symptoms of sepsis can be recognized and action taken. Once symptomatic neonates with sepsis should be treated in an intensive-care nursery, with full cardiopulmonary monitoring and availability of ventilatory support. Cardiac output and perfusion are maintained with volume infusions and pressor agents, as needed. Anemia, thrombocytopenia, and disseminated intravascular coagulation are treated with appropriate transfusions. Aggressive nutritional support is needed to combat the catabolic state associated with sepsis (Gerdes, 2004).

- Management of septic shock in neonates:

Fluid resuscitation with isotonic boluses (20 ml/kg over 15 minutes each) to a maximum volume of (60 mL/ kg) may be accomplished. In addition, hypoglycemia and hypocalcemia should be corrected. Hypocalcemia must be treated with slow intravenous administration of calcium gluconate at a dose of 2 ml/kg. If shock persists, central venous and arterial access should be obtained and vasoactive agents should be started, with dopamine as a first line agent. If after the first hour circulation is not restored with further pressor support, a possibility of adrenal insufficiency should be considered and hydrocortisone therapy should be initiated (Carcillo and Fields, 2002).

2. Antimicrobial therapy

As neonatal sepsis can be rapidly fatal if left untreated, highly effective antibiotic therapy must be used and delay in the provision of
care must be minimized. Treatment must be effective against the causative pathogen, safe for the neonate, and feasible to deliver reliably in the hospital or community setting (Edmond and Zaidi, 2010).

The trend which was being applied for neonates who were suspected to have neonatal sepsis lead to unnecessary and increased antibiotic consumption, a higher incidence of the side effects due to their use, increased resistance to the antibiotics, a long hospitalization, the separation of the neonates from their mothers and increased health costs (Sucilathangam et al., 2012).

The goal of antibiotic use before the identification of the infectious microbe, or empirical antibiotic therapy, is to eradicate harmful organisms as early in the clinical course as possible. However, the lifesaving potential of antibiotic use in neonates at high risk for infection must be balanced against the possible negative consequences of widespread use in low risk neonates. Antibiotic therapy can alter the neonatal micro biome, potentially making the neonate more susceptible to opportunistic infection (Chu et al., 2012).

The reasons for the rise in antimicrobial resistance are manifold and complex, but it is true that excessive and indiscriminate uses of antibiotics increase the risk of selection and may spread the antimicrobial resistance among organisms. In neonatal intensive care units (NICUs), particularly in developing countries multidrug antibiotic resistance is an emerging crisis. Moreover, the spectrum of organisms that cause neonatal infections changes from time to time and varies from region to region. Continuous surveillance for antibiotic susceptibility patterns, rational use of antibiotics and the strategy of antibiotic cycling can provide some answers to it (Shah and Desai, 2013).

➢ Indications of empirical antibiotics:

Available diagnostic testing is not helpful in deciding which neonate requires empirical antimicrobial therapy but can assist with the decision to discontinue treatment (Polin, 2012).

Most neonates with early onset sepsis (EOS) exhibit abnormal signs in the first 24 hrs. of life. Approximately 1% of neonates will
appear healthy at birth and then develop signs of infection after a variable time period (Polin, 2012).

Every critically ill neonate should be evaluated and receive empirical broad spectrum antimicrobial therapy after cultures, even when there are no obvious risk factors for sepsis. The greatest difficulty faced by clinicians is distinguishing neonates with early signs of sepsis from neonates with noninfectious conditions with relatively mild findings (e.g., Tachypnea with or without an oxygen requirement). In this situation, data are insufficient to guide management (Polin, 2012).

In more mature neonates without risk factors for infection who clinically improve over the first 6 hrs. of life (e.g., Need for oxygen is decreasing and respiratory distress is resolving), it is reasonable to withhold antimicrobial therapy and monitor the neonates closely. The 6 hour window should not be considered absolute; however, most neonates without infection demonstrate some improvement over that time period. Any worsening of the neonate’s condition should prompt starting antimicrobial agents after cultures have been obtained (Polin, 2012).

EOS does occur in neonates who appear healthy at birth. Therefore, some clinicians use diagnostic tests with a high negative predictive accuracy as reassurance that infection is not present (Allowing them to withhold antimicrobial agents). The decision of whether to treat a high risk neonate depends on the risk factors present, the frequency of observations, and gestational age. The threshold for initiating antimicrobial treatment generally decreases with increasing numbers of risk factors for infection and greater degrees of prematurity (Polin, 2012).

In fall of 2010, the Centers for Disease Control and Prevention (CDC) released updated guidelines for the diagnostic evaluation of neonates at risk of group B streptococcus (GBS) EOS. The guidelines clarify that only a limited evaluation (Blood culture and empirical antibiotics) should be completed for a neonate without signs of sepsis whose mother is diagnosed with chorioamnionitis. Additionally, it recommends observation for term neonates that are well-appearing without prolonged rupture of membranes (Even if their mothers met criteria for intrapartum antibiotics and they were not received), potentially limiting antibiotic use in this group (Verani et al., 2010).
Once treatment is indicated, a blood culture should be performed before treatment. A chest radiograph should be ordered if there is respiratory distress. A lumbar puncture should be performed if there are symptoms referable to meningitis, or if symptomatic sepsis is the leading diagnosis. Serial sepsis screens using white blood cell count (WBC), Immature to total neutrophil ratio (ITR) and C reactive protein (CRP) are recommended, not necessarily to decide on initiation of treatment, but aids to discontinue treatment if the screens are negative (Gerdes, 2004).

Despite all difficulties and limitations, it is obligatory to establish the required evidence so that use/no use of prophylactic antibiotics in neonates can be justified. At the moment, it remains an unanswered question that needs due debate in order to generate a well-accepted generalized opinion (Mushtaq et al., 2012).

- Duration of empirical antibiotics:

The duration of therapy differs for gram positive and gram negative sepsis. A ten day antibiotic course seems to be reasonable for Gram-positive sepsis; longer courses may be necessary for gram negative sepsis and sepsis with organ involvement (2–6 weeks) (Stronati and Borghesi, 2012).

Uncomplicated meningitis attributable to GBS is treated for a minimum of 14 days. Other focal infections secondary to GBS (e.g., 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 (Nizet and Klein, 2010).

The duration of antimicrobial therapy in neonates 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-amniotic infection or premature rupture of membrane. In those instances, postnatal blood cultures may be sterile (False negative). When considering the duration of therapy in neonates with negative blood cultures, the decision should include consideration of the clinical course as well as the risks associated with longer courses of
antimicrobial agents. The average duration of treatment in neonates with negative blood cultures was 5 ± 3 days (Polin, 2012).

Recent data suggest an association between prolonged empirical treatment of preterm neonates (≥5 days) with broad spectrum antibiotics and higher risks of late onset sepsis, necrotizing enterocolitis, and mortality. To reduce these risks, antimicrobial therapy should be discontinued at 48 hrs. in clinical situations in which the probability of sepsis is low (Polin, 2012).

It is also important to avoid treating colonization (Positive endotracheal cultures without evidence of pneumonia) and prophylactic antibiotic use for invasive devices (Patel et al., 2009).

➢ Choice of empirical antibiotics:

Rational choice of antibiotics for the neonate with presumed infection requires review of antibiotic susceptibility of the predominant organisms that cause disease at the local level (Stoll et al., 2011).

The initial choice of drugs for empirical treatment is dependent on knowledge of the probable pathogens based on the perinatal history, including any maternal symptoms, cultures, or instrumentation. For instance, if a mother was known to have a gentamicin-resistant gram negative urinary tract infection (UTI), one would choose an antibiotic appropriate to that organism. Likewise, if a mother had a history of recent instrumentation such as amniocentesis, one would consider the possibility of coagulase negative Staphylococcus. If there are no mitigating issues in the history, then the septic neonate is likely to have one of the common pathogens (Gerdes, 2004).

The first microbial colonization in a neonate occurs during the passage through the birth canal and therefore the empiric treatment for EOS is based on the most common organisms detected at birth and that of late onset sepsis depends on presenting clinical signs (Mushtaq et al., 2012).

- Choice in early onset sepsis (EOS):

Antibiotic combinations should be used for empirical treatment. Ampicillin plus gentamicin is still the best antibiotic combination for EOS (Palazzi et al., 2006).
Ampicillin is effective against enterococci, some gram negative bacteria (E. coli, Proteus and Klebsiella), GBS and Listeria monocytogenes. Aminoglycosides widen the antimicrobial spectrum, being effective against some ampicillin-resistant Enterobacteriaceae (Some strains of E. coli, Proteus and Klebsiella) and some ampicillin-resistant Enterococci. Gentamicin is the most frequently used aminoglycoside in term and preterm neonates, and may be administered in a single daily dose. It is important to note the synergistic effect of ampicillin and gentamicin on several organisms (Stronati and Borghesi, 2012).

Aminoglycosides may be associated with important adverse effects and they require frequent monitoring of blood levels. Preterm neonates have immature organs and therefore may not tolerate some antibiotics as well as term neonates. Further to these significant disadvantages, the majority of treated babies do not have proven sepsis (Gordon and Jeffery, 2005).

Third generation cephalosporins are effective against the majority of bacterial pathogens and achieve high bactericidal concentrations in the cerebrospinal fluid (CSF), but they should not be used in the absence of proven bacterial sepsis to reduce the emergence of resistant organisms and fungal infections (Stronati and Borghesi, 2012).

Ceftriaxone is contraindicated in neonates because it is highly protein bound and may displace bilirubin, leading to a risk of kernicterus (Polin, 2012).

- Choice in late onset sepsis (LOS):
  Antistaphylococcal penicillin (Oxacillin, nafcillin or flucloxacillin) plus aminoglycoside is an effective combination for late onset sepsis (LOS) (Isaacs, 2006).

  When a staphylococcal infection by a methicillin-resistant staphylococcus is suspected or proven, vancomycin or teicoplanin should be used in combination with an aminoglycoside. Teicoplanin has fewer side effects (Oto- and nephrotoxicity) and a longer half-life than vancomycin, but the emergence of resistant organisms has been described less frequently with vancomycin administration (Stronati and Borghesi, 2012).
The presence of a central venous catheter (CVC) or other indwelling foreign material is highly associated with persistence of infection despite appropriate antibiotic therapy, because of biofilm formation. In vivo antibiotic action is also antagonized by the neutralization of pharmaceuticals like vancomycin by the polysaccharides of coagulase negative staphylococcus (CoNS) biofilms. In such cases, it may be imperative to remove the CVC (Marchant et al., 2013).

It has been recommended against empirical vancomycin therapy to prevent the emergence and spread of vancomycin-resistant strains (Chu et al., 2012).

The use of vancomycin should be initiated if, in the judgment of the clinician, the neonate is critically ill and the postulated infecting organism may be a methicillin-resistant strain of a gram positive organism (Chu et al., 2012).

Carbapenems may be an option for severe infections by multi-resistant organisms. Carbapenems have a very large antimicrobial spectrum (Almost all gram negative and gram positive pathogens) which is resistant to known betalactamases. Meropenem is used because of a greater effectiveness against Hemophilus influenza, Enterobacteriaceae and Pseudomonas. The incidence of seizures is lower than with imipenem and cilastatin (Stronati and Borghesi, 2012).

The potential for significant life-threatening toxicity among neonates associated with chloramphenicol makes it the least preferred empiric parenteral therapy (Darmstadt et al., 2009(A)).

Oral antibiotic therapy must be considered in settings where referral is not possible and there are no health care providers trained to give parenteral antibiotics. The incremental benefit of injectable over oral antibiotics is not known, and oral antibiotic therapy is better than no antibiotic therapy at all (Darmstadt et al., 2009(B)).

Oral cotrimoxazole in treatment of serious neonatal bacterial infections resulted in high resistance rates and side effects such as neonatal jaundice also have been reported (Thaver and Zaidi, 2009).
Oral amoxicillin is highly efficacious against Streptococcus species and some gram negative bacilli and has an excellent safety record. However, it has no anti-Staphylococcus coverage and resistance is emerging in gram negative bacilli such as E. coli. New, better-absorbed oral antibiotics are also being considered. The new second generation cephalosporins (e.g., Cefadroxil and cefuroxime) have an excellent safety profile, a spectrum of activity similar to cotrimoxazole, and may be more effective. Ciprofloxacin also is increasingly accepted as safe in neonates and warrants further investigations for treatment of infections in neonates. However, the current cost of these agents and potential for exacerbating antimicrobial resistance may limit widespread use in developing countries (Darmstadt et al., 2009(B)).

Once an organism is identified and sensitivities are determined, therapy should be changed to the most specific, safest, and least expensive drug or drugs to which the organism is sensitive. Monitoring of serum levels is required for neonates receiving full courses of aminoglycosides or vancomycin (Gerdes, 2004).

Follow up:

With most infections, positive culture sites should be recultured after 48 hrs. of treatment. The WBC count and ITR may increase dramatically as the neonate responds to treatment, and should begin to normalize by 72 hrs. CRP is a useful adjunct to monitor the effectiveness of treatment; neonates whose CRP concentrations do not gradually decrease after 48 to 72 hrs. of therapy may not be responding properly. Neonates who do not respond well may have infection with a resistant organism, a focal or metastatic focus infection, a viral illness, or a noninfectious process. The goal of treatment should be to have an asymptomatic neonate with negative repeated cultures and normal WBC counts and CRP, all occurring with at least 3 days of antibiotic treatment remaining. Finally, when the neonate is discharged from care, appropriate follow-up arrangements should be made to ensure continued progress (Gerdes, 2004).

Treatment of meningitis:

The choice of antibiotics is the main tool for the treatment of meningitis, to treat all potential pathogens, a combination of three antibiotics
should be considered for initial empirical antibiotic therapy (Usually ampicillin with a third generation cephalosporin and an aminoglycoside). Once culture results are available, antibiotic therapy can be modified and one antibiotic can be withdrawn on the basis of the isolated organism and its sensitivity pattern (Stronati and Borghesi, 2012).

For multi resistant organisms, meropenem together with an aminoglycoside may be a reasonable choice (Stronati and Borghesi, 2012).

Antibiotic therapy should be started as soon as possible and continued for at least 21 days. The effectiveness of the initial empirical antibiotic therapy should be proven by a negative CSF culture, performed 24–48 hrs. after the onset of symptoms and a lumbar puncture for CSF culture before the withdrawal of the antibiotic treatment (Stronati and Borghesi, 2012).

If the CSF is not sterile at 24–48 hrs., a focal infection of the brain (Cerebral abscess, subdural empyema and obstructive ventriculitis) should be suspected and excluded by imaging techniques (Stronati and Borghesi, 2012).

Herpetic encephalitis should be always considered in a neonate with suspected meningitis. Acyclovir (20–30 mg/kg every 8 hrs.) should be started when gram staining of the CSF is negative and should be withdrawn when the herpetic infection has been excluded (Stronati and Borghesi, 2012).

No consensus has yet been reached on the effectiveness of corticosteroid therapy with dexamethasone to improve the prognosis of neonates with meningitis, and no randomized clinical trials have been performed (Stronati and Borghesi, 2012).

➢ **Treatment of fungal infections:**

The most important neonatal risk factors for nosocomial candidiasis are prolonged administration of broad spectrum antibiotics (Courses longer than 21 days), extreme prematurity (Gestational age at birth < 28 weeks) because of impaired humoral and cellular immunity, and the presence of venous catheters (Especially if prolonged) (Stronati and Borghesi, 2012).
Additionally, total parenteral nutrition, intravenous lipid solutions, administration of H2-blockers, endotracheal intubation, disseminated intravascular coagulation and prolonged hospital stay were also significant risk factors for invasive Candidiasis. All types of catheters (Vascular, vesical, peritoneal catheters, ventriculoperitoneal, thoracic drains & endotracheal tubes) favor adhesion, growth and penetration of microorganisms (Bendel, 2005).

Delay in starting enteral nutrition, steroid therapy, hyperglycemia, neutropenia, and abdominal or cardiac surgery are additional risk factors (Bendel, 2005).

The first line antifungal drug for the treatment of fungal sepsis is amphotericin B, which is generally well tolerated by the neonate. Fluconazole is the most studiedazole in the neonatal age range. Its use for the prevention of fungal infections in high risk neonates has been evaluated by several studies, but amphotericin B is the first choice for treatment of an infection (Stronati and Borghesi, 2012).

The duration of antifungal treatment should be decided on the basis of clinical and microbiological factors: the fungal species, the presence of a central venous catheter, which should always be removed in neonates with fungal infections, the number of positive cultures, CSF cell count and chemistry, imaging. In neonates with systemic fungal infections, intravenous treatment should be prolonged for at least 2–4 weeks after the last negative blood culture. Longer courses may be needed for meningitis and end organ dissemination (Stronati and Borghesi, 2012).

Starting empirical antifungal treatment before the availability of culture results has not been the subject of prospective studies. Some high risk populations, such as extreme low birth weight (ELBW) neonates with sepsis and risk factors for fungal infections may be helped by empirical treatment while awaiting culture results, especially if antibiotics are not apparently effective (Stronati and Borghesi, 2012).

3. Adjunctive therapies

In the healthy state there is a balance or homeostasis within the immune system ensured by mutual interaction between its components.
Severe bacterial infection induces the patho-physiological conditions leading to: a) Generalized inflammatory response (Hyper inflammation). b) Immune paralysis. c) Anti-inflammatory response (Haque, 2006).

Immune paralysis causes dysregulation of other systems leading to multi-organ dysfunction and failure. Thus, the aim of treatment in severe infection is to kill the pathogens with antibiotics, control the hemodynamic impairment and organ dysfunction. Along with this providing immunotherapy to restore immune homeostasis is proving to be an important causal approach to modulate and affect the inflammatory process (Haque, 2006).

In a study of very low birth weight (VLBW)/preterm neonates who survive at least one episode of proven sepsis in the neonatal period have 30-80% increased rate of neurodevelopmental impairment and a 30-100% increased rate of poor head growth (An indirect reflection of poor neurological development) at 18-22 months despite the use of powerful antibiotics. Thus, clinicians and researchers have sought adjunctive therapies to use alongside standard supportive and antibiotic treatment due to their very broad and potent antibacterial and anti-inflammatory activity (Melvan et al., 2010).

Immunotherapy in augmenting the immature immune system has been extensively studied, but no definitive standards of care have been derived from these studies. WBC transfusions, intravenous immunoglobulin (IVIG) infusions, and treatment with colony stimulating factors such as granulocyte and granulocyte macrophage colony stimulating factor have not been shown to definitely improve outcome in neonates with sepsis (Gerdes, 2004).

**A. Exchange transfusion (ET)**

It will be discussed later

**B. Intravenous immunoglobulin (IVIG)**

It will be discussed later
C. Granulocyte transfusion

Neonates, particularly preterm neonates, have defective humoral and phagocytic immunity, predisposing to increased incidence of bacterial and fungal infections. Neonatal neutrophil granulocytes exhibit both quantitative and qualitative abnormalities (Tarnow-Mordi et al., 2010).

In neutropenic neonates with sepsis, transfusion of granulocytes could potentially reduce mortality and morbidity, but there is insufficient evidence on safety and efficacy to justify routine use (Tarnow-Mordi et al., 2010).

Granulocytes, predominantly neutrophils, are prepared in a concentrated form for transfusion to reduce the volume of the transfusion (Tarnow-Mordi et al., 2010).

However, it can take several hours to prepare granulocyte concentrates. In addition, there are potential severe complications, notably fluid overload, transmission of blood borne infection, graft versus host disease caused by mature lymphocytes in the transfusion, pulmonary complications secondary to leukocyte aggregation and sequestration, and sensitization to donor erythrocyte and leukocyte antigens (Tarnow-Mordi et al., 2010).

D. Colony stimulating factors (CSFs)

The discovery and synthesis of hemopoietic CSFs created new opportunities for sepsis prevention and treatment. Granulocyte macrophage colony stimulating factor (GM-CSF) and granulocyte colony stimulating factor (G-CSF) are naturally occurring cytokines that stimulate the production and antibacterial function of neutrophils and monocytes (Tarnow-Mordi et al., 2010).

G-CSF is important for differentiation, mobilization, and proliferation of bone marrow white cell precursors. A dose of 5–10 μg/kg/day subcutaneously or intravenously for 3–10 days may improve neutrophil counts in neutropenic neonates and reduce mortality in neonates with sepsis associated with severe neutropenia. Administration
should be stopped for neutrophil counts greater than 20000/μl. (Stronati and Borghesi, 2012).

There are limited data suggesting that CSFs treatment may reduce mortality when systemic infection is accompanied by severe neutropenia. The evidence is too weak to support the prophylactic use of G-CSF or GM-CSF, and CSFs are not recommended to treat established infection without further evidence from adequately powered trials (Tarnow-Mordi et al., 2010).

For the planned 2 year outcome evaluation, no developmental advantage was evident for the prophylaxis group and rates of disability, developmental score profiles and economic outcomes were very similar between the two groups, leading to the conclusion that GM-CSF is ineffective in improving outcomes to discharge or to 2 years of age (Marlow et al., 2013).

**E. Activated protein C (APC)**

Endotoxins or other microbiologic products produced by infecting organisms activate host immunologic defense systems. Excessive host response may lead to apoptosis, organ failure, disseminated intravascular coagulation (DIC) and death (Tarnow-Mordi et al., 2010).

An endothelial cell glycoprotein, thrombomodulin, combines with thrombin and converts protein C to its activated form. Activated protein C promotes fibrinolysis and has anti-inflammatory action by blocking production of tumor necrosis factor (TNF). APC has a short half-life and in sepsis, conversion to the activated form is reduced and the circulating pool of protein C is rapidly depleted leading to an increase in inflammation, intravascular coagulation, and multiorgan failure (Tarnow-Mordi et al., 2010).

Clinical trials demonstrating safety and effectiveness of recombinant human APC are lacking. Septic neonates are at high risk of bleeding, and preterm neonates are at risk of intraventricular hemorrhage. Recombinant human APC increases the risk of bleeding and should be used only in the context of clinical trials (Tarnow-Mordi et al., 2010).
F. Pentoxifylline

Pentoxifylline improves endothelial cell function and reduces excessive coagulation in sepsis. No adverse effects caused by Pentoxifylline or other outcomes of interest were reported. These results are promising, but need to be confirmed in larger studies (Tarnow-Mordi et al., 2010).

G. Reduction of oxidative stress: Selenium and melatonin

Sick preterm neonates are exposed to many sources of oxygen radicals, from high concentrations of inspired oxygen, frequent alteration of blood flow to major organs, and inflammation with accumulation of neutrophils and macrophages. Low blood selenium concentrations in preterm neonates have been suggested as a potential risk factor for sepsis, chronic neonatal lung disease, and retinopathy of prematurity (Tarnow-Mordi et al., 2010).

Supplementation with selenium was not associated with improved survival or with reduction in neonatal chronic lung disease or retinopathy of prematurity. The role of selenium supplementation is unclear, although it might be considered in areas where soil selenium levels are extremely low (Tarnow-Mordi et al., 2010).

Melatonin reduces oxidative stress from toxic free radicals in animals and there are theoretical reasons that it might be helpful in sepsis (Gitto et al., 2009).

H. Glutamine

Endogenous biosynthesis of glutamine may be insufficient for tissue needs in states of metabolic stress. It has been postulated that glutamine supplementation may benefit preterm neonates, particularly very low birth weight neonates (Tubman et al., 2008).

Glutamine supplementation had no statistically significant effect on other neonatal morbidities including invasive infection, necrotizing enterocolitis (NEC), or neurodevelopment at 18 months. The available data do not support glutamine supplementation to prevent sepsis in preterm neonates (Tarnow-Mordi et al., 2010).
I. Lactoferrin

Lactoferrin is a glycoprotein found mainly in human colostrum. It forms part of the innate immune response to infection and has broad spectrum antimicrobial activity against bacteria, fungi, viruses, and protozoa. Acid proteolysis of lactoferrin yields peptides called “Lactoferricins” with enhanced antimicrobial activity. Lactoferrin inhibits the growth of *Streptococcus epidermidis* and *Candida albicans* in vitro, and lactoferrin and lactoferricins are highly effective against antibiotic-resistant Klebsiella species and *Staphylococcus aureus* in vitro (Mohan and Abrams, 2009).

J. Probiotics

Probiotic bacteria are defined as live microbial supplements that colonize the gut and provide health benefits to the host. In babies born after normal birth at term, the gut is colonized with probiotic organisms from the mother, such as lactobacilli and bifidobacteria, which upregulate local and systemic immunity, increase anti-inflammatory cytokines, decrease the permeability of the gut to bacteria and toxins, and suppress pathogens associated with NEC (Delcenserie et al., 2008).

Current probiotic products are safe and effective in reducing all-cause mortality and NEC by over half, with no increase in sepsis (Deshpande et al., 2007).

A commentary from the European Society of Pediatric, Gastroenterology, Hepatology and Nutrition Committee on Nutrition did not recommend probiotics for preterm neonates, but was submitted for publication before the most recent evidence became available (Samanta et al., 2009).

Probiotics are different from conventional drugs; there are many types and optimum production, transport, dosage, and contraindications are unclear. There are also theoretical concerns that probiotics could enhance transfer of antibiotic resistance genes (Lin et al., 2008).

K. Breast milk

Lack of breast milk may be the commonest immunodeficiency of infancy. Early human milk is rich in a variety of immune, nonimmune,
and anti-inflammatory components that may accelerate intestinal maturation, resistance to infection and epithelial recovery from infection (Hanson, 2007).

These and other mechanisms may explain the strong inverse associations between breast milk and gastroenteritis, septicemia, meningitis, and NEC in low birth weight or preterm neonates (Cotten et al., 2009).

i. Prognosis

Systemic infection remains a major cause of mortality and morbidity in the neonatal period. Of particular concern is the association between the inflammatory response and later risk of developmental delay and neurocognitive impairment, possibly mediated by damage to the periventricular white matter in the perinatal and neonatal periods (Volpe, 2009).

Neurologic sequelae following neonatal meningitis include learning and language defects, motor disorders, seizures, hearing and visual defects, and behavioral disturbances (Stronati and Borghesi, 2012).

It is estimated that each episode of sepsis prolongs the duration of a neonate’s hospital stay by about 2 weeks, resulting in an incremental cost. The duration of hospital stay increased by four to seven days in all VLBW with a nosocomial infection (Marchant et al., 2013).

In developed countries, advances in medical care have enabled a greater proportion of premature neonates to survive with an increased risk of infection. However, because the greatest burden of neonatal sepsis falls on developing countries, the global economic impact is difficult to estimate. Together, prematurity and neonatal infections account for the greatest burden of neonatal deaths overall. The limited access to medical resources combined with geographical comorbidities (e.g., Severe malnutrition) can lead to mortality from neonatal sepsis remaining unacceptably high in developing countries (Marchant et al., 2013).

Multiple studies show that neonatal sepsis has major long term neurodevelopmental consequences in survivors, particularly in preterm
neonates. In modern intensive care, about half of extremely preterm neonates born at 24 weeks’ gestation and the majority of neonates over 25 weeks’ gestation generally survive. The risk of such morbidity in extremely premature neonates is inversely proportional to their gestational age. In VLBW neonates, neonatal sepsis dramatically increases the long term risk of motor, cognitive, neurosensory and visual impairments. The risk of adverse neurodevelopmental outcome in VLBW neonates with sepsis is further increased with other comorbidities such as bronchopulmonary dysplasia. This increased risk of neurodevelopmental impairment in preterm neonates with sepsis has several reasons, including a high risk of meningitis; heightened adverse effect of sepsis-associated cardiovascular instability during a vulnerable period for the developing brain; and increased neurotoxic effects of inflammatory mediators. Surprisingly, the risk of adverse neurodevelopmental outcome in VLBW neonates surviving from neonatal sepsis does not appear to depend on the infecting organism, although in some studies extremely premature neonates who experienced sepsis had a greater risk of a hearing impairment when the infection involved gram negative, fungal, or combined infections (Marchant et al., 2013).
Exchange transfusion (ET)

➢ Rationale of ET:

While ET has been traditionally used to treat neonates with hyperbilirubinemia, the indications for the use of this procedure have expanded. Several reports have described the use of ET in the treatment of severe neonatal septicemia, as when generalized sclerema develops in septicaemic neonates treated exclusively with antibiotics and supportive measures; the clinical course is almost invariably fatal (Christensen et al., 1984).

Incomplete development of the host defense system of the neonate is largely responsible for the high mortality in neonatal sepsis. Immunoglobulin A (IgA), IgM and complement (C3) are not transferred transplacentally. Transfer of IgG occurs progressively, particularly after 20 weeks gestation and levels are low in premature neonates. Several workers have attempted to compensate for these defects by performing ET (Mathur et al., 1993).

ET using fresh, whole, adult blood may reduce the severity of sepsis. The volume to be transfused is 160–180 mL/kg (About twice the neonatal blood volume). Benefits depend on the removal of endotoxins, cytokines, and molecules that increase the permeability of vascular endothelium. Other advantages are attributed to the presence of complement factors, antibodies and coagulation factors in the transfused blood, and on improved lung and tissue perfusion and oxygen delivery because of the shift in the oxygen dissociation curve with the transfusion of adult hemoglobin. However, there is still little evidence of its effectiveness in reducing morbidity and mortality, and its use should be limited to patients with severe sepsis with septic shock and disseminated intravascular coagulation (Stronati and Borghesi, 2012).

Neonates were selected to receive ET if they had evidence of systemic bacterial infection, were neutropenic according to the criteria of Manroe et al. and had either a markedly diminished number of post mitotic neutrophils (Polymorph nuclear leucocyte (PMN), band neutrophils and metamyelocytes) on a bone marrow aspirate (<6%) or a
Review of literature

Exchange transfusion

blood immature/total neutrophil ratio of > 0.80 (Which in other studies has correlated with depleted marrow postmitotic neutrophils) (Christensen et al., 1984).

It may correct hypovolemia and also improve hemostatic mechanisms, and may also provide substances which enhance the humoral or cellular defense mechanisms. The absolute numbers of neutrophils and eosinophils in the peripheral blood have been shown to rise considerably during the week after exchange transfusion (Xanthou et al., 1974).

A rise in serum opsonic activity has been found in neonates after exchange transfusion and also an increase in the activity of lymphocytes (Christensen et al., 1984).

It was suggested that GBS septicemia resembles endotoxin shock. Exchange transfusion showed to be effective in the treatment of endotoxin shock, producing an immediate improvement of the cardiac output, arterial blood pressure, urine output, acid base balance and improvement in pulmonary perfusion and ventilation on low birth weight neonates (Christensen et al., 1984).

Results

Double volume ET has been used as a modality for managing sepsis for several decades. The earlier trials were uncontrolled and showed impressive improvements in neutrophil counts, immunoglobulin levels, recovery from sclerema and less mortality compared to historical experiences (Christensen et al., 1984).

Mathur et al. conducted a study on septicaemic neonates, there was a non-significant reduction in mortality, but there were significant improvements in total leucocyte count, absolute neutrophil count and neutrophil functions in the ET group (Mathur et al., 1993).

On sclerematous neonates, a significant reduction in mortality and a significant improvement in immunoglobulin levels with ET were found (Gunes et al., 2006).
In a non-randomized, controlled trial, ET was compared with IVIG and controls. There was a non-significant reduction in mortality both in the ET and IVIG groups compared to controls and a significant rise in IgM levels in the ET group (Gunes et al., 2006).

In contrast to the increase in blood neutrophil counts immediately after the exchange transfusion which was observed, other reports describe a diminution in circulating neutrophils (Christensen et al., 1984).

➤ Factors modifying the results

When granulocytes, labeled with diisopropylfluorphosphate no. 32 (DFP32), were subjected to storage at 1-6°C for 24 hrs. and then reinfused into autologous recipients, the neutrophils were removed from the circulation as rapidly as they were infused, suggesting that the stored neutrophils had been seriously damaged. Therefore, when an exchange transfusion is performed using stored blood. It is likely that the patient’s own circulating neutrophils will be removed and replaced with nonfunctional donor neutrophils. If the patient has a sufficient number of neutrophils within the neutrophil storage pool, those cells will likely be quickly liberated into the blood, increasing the blood neutrophil count back to pre-exchange transfusion values (Christensen et al., 1984).

Adults and older children maintain a very large neutrophil storage pool, generally equaling 12-14 times the number of neutrophils in the blood. Exchange transfusion with stored blood in these subjects would only remove 1/12 to 1/14 of their total available neutrophils (Christensen et al., 1984).

However, neonates, particularly those born prematurely, may have a storage pool of only 2-3 times the blood neutrophil pool and therefore an exchange transfusion with stored blood would likely remove one-half to one-third of all their available neutrophils (Christensen et al., 1984).

Furthermore, in adults with bacterial infection, the neutrophil storage pool generally does not diminish significantly, but in infected neonates the neutrophil reserve sometimes becomes completely depleted, a condition which is associated with very high mortality (Christensen et al., 1984).
Therefore, if stored blood is used, an exchange transfusion will likely remove most of the few neutrophils a septic patient has remaining. Given the critical importance of the neutrophil in bacterial killing, the perpetuation of neutrophil deficiency by exchange transfusion with stored blood may, in some cases, be counterproductive. In contrast, if fresh whole blood is used in exchange transfusions, septic, neutropenic neonates actually received large quantities of viable donor neutrophils (Christensen et al., 1984).

If ET is used in the management of neonatal sepsis, special attention must be given to the immunological properties of such blood. The transfusion of blood with inadequate opsonic activity may prove to be detrimental. A surprising finding was that half the samples of donor blood exhibited an abnormal opsonic capacity of polymorphonuclear leucocytes. The most likely cause is that blood had been stored and then been incorrectly handled (Pelet, 1979).

The neutrophil content of the donor blood can be increased by 18-55%, if the donor performs 2 min of vigorous physical exercise 4-5 min prior to blood donation. If a double-volume exchange transfusion is employed, the recipient can be expected to receive about as many neutrophils as are generally provided in an apheresis-collected leukocyte transfusion (Christensen et al., 1984).

Fresh whole blood is more universally available than leukocyte concentrates, is less expensive to process, and can generally be obtained much more quickly. Clearly, however, further studies must be accomplished in order to demonstrate any putative effect of this procedure on survival (Christensen et al., 1984).

Thus ET is an inexpensive and simple mode of immunotherapy in neonatal sepsis, particularly useful for the developing world, as ET with fresh whole blood in septicaemic neonates with sclerema improves survival, particularly in the more premature group, and significantly increases IgG, IgA and enhances IgM levels (Gunes et al., 2006).
Intravenous immunoglobulin (IVIG)

Sepsis is a pathogen initiated but a cytokine-mediated condition in which immune homeostasis is disturbed. Since so many factors are involved in the cascade and process of sepsis, there cannot be a magic bullet for treatment, so to improve the outcome from neonatal sepsis we will need not only to kill the pathogen but also to modulate the immune system to regain immune homeostasis. Based on the evidence presented above immunomodulation with IVIG in the management of neonatal sepsis is worthy of serious consideration (Haque, 2006).

IVIG has been used to treat and prevent neonatal sepsis since 1980’s but its use still remains controversial (Haque, 2006).

There is a rational to use IVIG in either adjunctive treatment of neonatal sepsis or in the prevention by low immunoglobulin levels associated with the immature innate immune system of preterm neonates. Studies so far revealed a benefit for IgM enriched IVIG in the use as adjunctive sepsis treatment by an overall significantly reduced mortality rate. Prophylactic use of IVIG resulted in marginally reduced rates of nosocomial infections (Resch E and Resch B, 2013).

IVIG preparations contain antibodies that help the body to neutralize bacterial toxins. There are two types of preparations. These are polyclonal immunoglobulins that contain several antibodies directed at endotoxin and inflammatory mediators, and monoclonal immunoglobulins which target a specific inflammatory mediator or antigen. IVIG are blood products, specifically pooled sera derived from human donor blood (Alejandria et al., 2013).

➢ Pathophysiology & Mechanism of action of IVIG

Similar to most immunoglobins, the transplacental transport of IgG from the mother to fetus begins around 32 weeks of gestational age and increases until term. IgM is not transplacentally transferred. Premature neonates born prior to 32 weeks gestation have profound IgG deficiencies. The major function of IgG in host defense is to opsonize bacteria and neutralize viruses. Levels of postnatal IgG are often low due
Review of literature

Intravenous immunoglobulin

to insufficient production by the immature neonatal immune system and catabolism of maternal IgG. Opsonic activity is also type-specific; therefore humoral immunity transferred to the neonate will be insufficient if the mother does not have immunity to the specific pathogen (Melvan et al., 2010).

Administration of IVIG probably exerts its major effect on neonatal host defenses by providing opsonic antibody against neonatal pathogens that enhance phagocytosis and killing of bacteria by neutrophils. IVIG may also neutralize toxins, immunomodulate T cells and macrophages, especially cytokine synthesis, and affect B-cell function and the complement system (Jenson and Pollock, 1997).

IVIG once infused is immediately and completely bioavailable in the recipient’s circulation. It is distributed relatively rapidly between plasma and extra-vascular fluid. Infused IVIG has a half-life of about 7 to 14 days in the neonate (Haque, 2004).

Though the method by which it does this is not clearly understood but possible mechanisms of action include:

- Offering broad spectrum of antibodies.
- Neutralization and clearance of endotoxins, exotoxins and superantigens.
- Help killing the organism by increasing opsonisation.
- Increase macrophage surveillance.
- Increase number and activity of PMN’s and improve chemotaxis.
- Increase in opsonisation and phagocytosis.
- Inhibition of release of pro-inflammatory cytokines and stimulation of the release of their antagonists.
- Complement mediated killing of pathogens.
- Scavenging of activated complement factor C3b and C4b.
- Regain immunological balance and reduction in bacterial cell adherence.
- Modulation of pro and anti-inflammatory cytokines (Haque, 2006).
Manufacturing process

Immunotherapy was a common method of treatment of infectious diseases in the preantibiotic era with serotherapy being a popular approach to serious infections by use of antisera from large animals. This administration unfortunately was associated with the risk of anaphylaxis and serum sickness. Further on, immunoglobulins obtained from pooled human plasma were used, but antibodies provided by these preparations always represented those common to the donor population, and intravenous injection of early human IgG preparations was complicated by severe allergic reactions. The next step was the purification of human immunoglobulins, so multiple formulations of safe, pooled, human immunoglobulins for the intravenous use were generated (Resch E and Resch B, 2013).

IVIG is not a generic drug. Commercial IVIG preparations differ in their antibody titer profile depending on the donor pool used. Most manufacturers use a donor pool between 1000 to 100,000 donors. The greater the donor pool the greater antibody spectrum is likely. Preparations differ with regard to their method of manufacturing, for example in the methods used for purification, virus inactivation and viral elimination. Thus each IVIG preparation differs from another even in the same product (Haque, 2006).

World Health Organization (WHO) in 1982 has set minimum standards required of commercially available IVIG preparations. They include:

- The minimal plasma donor pool of at least 1000.
- The preparation must contain more than 90% of intact IgG and a small amount of IgA as possible.
- The product should be free from fragments and aggregates and be modified as little as possible biochemically.
- The product should also contain all subclasses in naturally occurring proportions and its antibody spectrum should be known (Haque, 2006).
Composition of IVIG

IVIG preparations with high concentrations of antibodies to bacteria that are commonly isolated from neonates in specific local settings or geographical areas may be more effective in reducing adverse outcomes (Ohlsson and Lacy, 2010).

However, the use of antistaphylococcal immunoglobulins to prevent staphylococcal infection in VLBW neonates has recently been reviewed and is currently not recommended (Shah and Kaufman, 2009).

Another commercially available product has been enriched with IgM to give the preparation a more physiological composition. It is thought that the pentameric structure of IgM provides superior opsonic and neutralizing ability against bacterial toxins (Haque, 2006).

Therapeutic schedule of IVIG

All the published studies using IVIG vary in the dosage and therapeutic schedule used. There is no consensus on the optimal dosage or duration of IVIG therapy in neonatal sepsis. It is not clear whether one should attain a certain plasma level of immunoglobulin for prolonged period or to spike the immune system with intermittent bolus therapy (Haque, 2006).

In neonates an IgG level of 400 mg/dl and an IgM level above 20 mg/dl is sufficient. Higher IgG levels have been found to inhibit the functions of the immune system, inhibiting maturation and function of dendritic cells, blocking the reticuloendothelial system and the proliferation of T lymphocytes whilst accentuating the production of pro-inflammatory cytokines (Haque, 2006).

A dose of 500 mg/kg/day for 1–5 days to a maximum total dose of 2–2.5 g/kg has been suggested (Stronati and Borghesi, 2012).

It is intuitive that prophylactic administration of IVIG for low birth weight premature neonates should be shortly after birth, and the consensus of these studies indicates that it is well tolerated and, if used, should be administered within the first 12 to 24 hrs. of life. Administration of IVIG for sepsis should follow shortly after the diagnosis or suspicion of sepsis (Jenson and Pollock, 1997).
Side effects

Over the years IVIG has been proven to have a very good safety profile with adverse reaction rates well below 5%. In the neonate minor self-limiting hemolysis has been reported (Haque, 2006).

The IVIG preparation was well-tolerated by all neonates, and no adverse events were observed by monitoring blood gas analysis, clinical examination, monitoring of respiration, pulse and body temperature. Follow-up at an average age of 2.5 years showed no evidence of harmful effects of IVIG treatment in the neonatal period (Resch E and Resch B, 2013).

Use of IVIG in the prevention and treatment of neonatal infection

Multiple studies have evaluated the efficacy of IVIG as prophylaxis or treatment for infection in neonates. A meta-analysis showed a reduction in mortality for neonates with subsequently proven infection treated with IVIG, but studies reporting benefit included few very premature neonates. A second meta-analysis showed a very modest reduction in the development of LOS following IVIG prophylaxis in preterm neonates (Wynn et al., 2010).

- Prevention of infection
  - Benefits
    The benefit of passive immunization by prophylactic administration of IVIG for prevention of bacterial infections has been established for patients with primary agammaglobulinemia and with symptomatic human immunodeficiency virus (HIV) infection (Jenson and Pollock, 1997).

Routine administration of IVIG for other immunocompromised hosts has not consistently been shown to clearly decrease the incidence of bacterial infections (Jenson and Pollock, 1997).

Exogenous immunoglobulin given at birth to premature low birth weight neonates may be beneficial for prevention of EOS and for LOS (Jenson and Pollock, 1997).
Administered as a prophylactic agent to low-birth weight preterm neonates immediately after birth revealed significantly higher specific IgG in blood sera compared to controls with an effect even lasting ten days after the last infusion. These results suggest that specific IgG titers might be well indicative of its opsonic activity and might protect against bacteremia (Resch E and Resch B, 2013).

- **Results**

The earliest studies using IVIG as prophylactic therapy in neonates failed to demonstrate effectiveness in prevention of bacterial infections probably because of the low doses of immune globulin necessitated by the intramuscular route of administration (Jenson and Pollock, 1997).

A number of systemic reviews have shown significant reduction in frequency of infections, mortality and morbidity. Others showed no reduction in mortality from infection or reduction in the incidence of necrotizing enterocolitis (NEC), bronchopulmonary dysplasia (BPD), or length of stay. No adverse effects of IVIG were reported from any of the studies (Tarnow-Mordi et al., 2010).

Others found that IVIG administration resulted in 3% reduction in sepsis and a 4% reduction in one or more episode of serious infection. As the incidence of infection was low (In developed countries) this reduction in the rate of sepsis did not justify the routine use of IVIG prophylaxis, so the decision to its use should depend on the costs and values assigned to the clinical outcomes (Haque, 2006).

- **Treatment of infection**

Though IVIG was first used in neonatal sepsis over a quarter of a century, many clinicians still view IVIG therapy as either experimental or not ‘evidence-based’! (Haque, 2006).

Similar to the prevention studies, treatment studies have also been subjected to a number of meta-analyses and systemic reviews which have all shown significant reduction in mortality from infection in neonatal sepsis (Haque, 2006).
The use of polyvalent IVIG was not associated with significant differences in the risk of major complications or other adverse outcomes in neonates with suspected or proven sepsis (Tarnow-Mordi et al., 2010).

Another systematic review found that adjuvant use of IVIG in the treatment of neonatal sepsis showed that there is no significant difference in the mortality rate or the length of hospital stay (Franco et al., 2012).

The benefit of IVIG in decreasing the acute mortality associated with neonatal sepsis is demonstrable. Overall mortality could not be evaluated during the entire hospitalization because the contribution of numerous confounding factors and comorbidities could not be excluded (Jenson and Pollock, 1997).

There was no difference in mortality rate between the IVIG, ET and control groups (Gunes et al., 2006).

- **Monoclonal (IVIG G) vs. polyclonal (IVIG GMA)**

In adults and children, there is a strong trend in favor of IVIG GMA over IgG preparations with a 34% and 15% reduction of mortality, respectively, compared to an even higher 50% and 37% relative reduction of mortality in neonates, respectively (Resch E and Resch B, 2013).

In neonates and especially preterm neonates, therapy with polyclonal IVIG should be understood much more as a substitutional therapy than as an adjunctive therapy (Resch E and Resch B, 2013).

Polyclonal IVIG containing pentameric IgM has been postulated to confer superior toxin neutralization and bacterial agglutination and to reduce mortality in gram-negative septic shock in all age groups. However, two RCTs in neonates did not show a difference in mortality (Norrby-Teglund et al., 2006).

A Systematic review found that sepsis-related mortality was significantly reduced only in patients who received polyclonal IVIG compared with treatment with monoclonal antibodies (Gunes et al., 2006).
Patients & methods
Patients & methods

This interventional study was conducted to neonates born at or referred to Benha University Hospital and admitted to the neonatal intensive care unit (NICU) from November 2012 to May 2013.

Patients

Thirty neonates with sepsis were enrolled consecutively in the study, out of these thirty neonates, 12 were preterm (40%) and 18 were term (60%) and regarding sex, there were 14 males (46.6%) & 16 females (53.4%). Sepsis was diagnosed using the hematological scoring system (HSS) and C reactive protein (CRP). Patients had the following inclusion and exclusion criteria:

- **Inclusion criteria:**
  1- Patients aged from 1 day to 1 month.
  2- Gestational age from 28 to 42 weeks.
  3- C-reactive protein ≥ 10 mg/l.
  4- The hematological scoring system (HSS) ≥ 5.

- **Exclusion criteria:**
  1- Age more than 1 month
  2- Two C-reactive protein (CRP) measurements 24 hrs. apart that are <10 mg/l.
  3- The hematological scoring system (HSS) <5.

The study was approved by the hospital’s ethics committee and informed consent was obtained from parents before inclusion.

All neonates enrolled in this study received antibiotic therapy and other supportive measures then were divided into three groups:

**Group I:** (No=10) Includes neonates who received intravenous immunoglobulin (IVIG) in addition to previous measures. In this group, the mean birth weight was 2,400 kg, the mean gestational age was 36.3 weeks (4 preterm & 6 term) and sex distribution was (5 males & 5 females). Five patients had respiratory complications and two had neurologic...
Patients & methods

deterioration. All required increased oxygen environment including assisted ventilation in all cases. Hypotension and shock were treated with volume expanders. Transfusions of packed RBCs and fresh frozen plasma were required in all cases.

Group II: (No=10) Includes neonates who received exchange transfusion (ET) with fresh, whole blood in addition to previous measures. In this group, the mean birth weight was 2,700 kg, the mean gestational age was 36.1 weeks (4 preterm & 6 term) and sex distribution was (5 males & 5 females). Eight patients had respiratory complications and one had neurologic deterioration. All required increased oxygen environment including assisted ventilation in all cases. Hypotension and shock were treated with volume expanders. Transfusions of packed RBCs and fresh frozen plasma were required in all cases.

Group III: (No=10) Includes neonates who received only antibiotic therapy and other supportive measures. In this group, the mean birth weight was 2,300 kg, the mean gestational age was 35.8 weeks (4 preterm & 6 term) and sex distribution was (4 males & 6 females). Five patients had respiratory complications and one had neurologic deterioration. All required increased oxygen environment including assisted ventilation in six cases. Hypotension and shock were treated with volume expanders. Transfusions of packed RBCs and fresh frozen plasma were required in five cases.

Methods

All neonates incorporated in this study were subjected to the following:

1- Careful history taking stressing on: History of difficulty of feeding or poor suckling, history of apnea or dyspnea, history of loss of appetite, vomiting or diarrhea, history of convulsions, history of pallor, cyanosis or jaundice and history of hypo- or hyperthermia.

2- Full clinical examination stressing on: general look, vital signs (Respiratory rate, heart rate, temperature and blood pressure), head and skin examination (Pallor, jaundice, cyanosis, mottling and
Patients & methods

hypo- or hyperthermia), chest examination (Signs of respiratory distress), heart examination (Tachycardia and hypotension), abdominal examination (Abdominal distention and splenomegaly) and neurological examination (Hypotonia and lethargy).

3- The following investigations were done: Complete blood count (CBC), C-reactive protein (CRP) and blood culture. Both CBC and CRP were done three times: At time of diagnosis of sepsis, 48hrs. and 1 week after treatment, while blood culture was only done once at time of diagnosis.

Complete blood count (CBC)

Skin was rubbed with antiseptic and 1 cm of blood was taken by a lancet to puncture the skin and make it bleed, blood was collected in a test tube containing 20 mcg of EDETA and analyzed as soon as possible using Sysmex xf 500 cell counter for red blood cell count, hemoglobin level, hematocrit value, white blood cell (WBC) count (Total and differential) and platelet count. Results of CBC were interpreted using Hematological scoring system (table 4, 5).

**Table (4): Hematological Scoring System**

<table>
<thead>
<tr>
<th>criteria</th>
<th>abnormality</th>
<th>score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total WBC count</td>
<td>( \leq 5,000 ) / ( \mu l )</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>( \geq 25,000 ) at birth</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>( \geq 30,000 ) 12-24 hrs.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>( \geq 21,000 ) day 2 onward</td>
<td></td>
</tr>
<tr>
<td>Total Polymorphonuclear cell (PMN) count</td>
<td>No mature PMN seen</td>
<td>2</td>
</tr>
<tr>
<td>Immature PMN count</td>
<td>Increased / decreased</td>
<td></td>
</tr>
<tr>
<td>I:T PMN ratio</td>
<td>Increased</td>
<td>1</td>
</tr>
<tr>
<td>I:M PMN ratio</td>
<td>( \geq 0.3 )</td>
<td>1</td>
</tr>
<tr>
<td>Degenerative changes in PMN</td>
<td>Toxic granules / cytoplasmic vacuoles</td>
<td>1</td>
</tr>
<tr>
<td>Platelet count</td>
<td>( \leq 150,000 ) / ( \mu l )</td>
<td>1</td>
</tr>
</tbody>
</table>

The normal values are
- Total PMN count: 1800-5400
- Immature PMN count: 600
- Immature: Total PMN ratio: 0.120
- Immature: Mature PMN ratio: \( \geq 0.3 \)
Table (5): Interpretation of HSS

<table>
<thead>
<tr>
<th>Score</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 2</td>
<td>Sepsis is unlikely</td>
</tr>
<tr>
<td>3 or 4</td>
<td>Sepsis is possible</td>
</tr>
<tr>
<td>≥ 5</td>
<td>Sepsis or infection is very likely</td>
</tr>
</tbody>
</table>

Quantitative CRP

Skin was rubbed with antiseptic and 3 cm of blood was taken by a lancet to puncture the skin and make it bleed, blood was collected in a plain test tube, left to clot, then centrifuged for 10 minutes at 1500 rpm, serum was separated and analyzed using Turbox plus. Results were considered positive above 6 mg/l.

The blood culture

Under complete aseptic condition, 3-5 cm of blood was taken by a lancet to puncture the skin and make it bleed, blood was collected in a Bactec peds plus/F culture vial, then cultured on Bactec medium and results were detected by Bactec 9050 after 3-5 days.

Results were different between groups, where in group I blood culture yielded gram positive cocci in three neonates, gram negative bacilli in one, candida in three and the rest of patients were blood culture negative. In group II blood culture yielded gram positive cocci in three neonates, gram negative bacilli in two, candida in one and the rest of patients were blood culture negative. In group III blood culture yielded gram positive cocci in three neonates, gram negative bacilli in two, candida in one and the rest of patients were blood culture negative.

The three groups were compared regarding: Gestational age, birth weight, sex, mode of delivery, provisional diagnosis and complications.

Treatment was given according to the following protocol: All ill patients whatever the presentation and the postnatal age were admitted to NICU where blood samples were taken for blood culture, CBC and CRP and empirical antibiotics were given. If HSS and CRP were compatible with sepsis, the result of blood culture was awaited for three days and...
then antibiotics were changed according to the result of culture and sensitivity. If blood culture yielded no growth, clinical condition was assessed. If there is clinical improvement, same antibiotics were continued. If not, antibiotics were changed according to clinical suspicion, then after two days, the patient was reassessed. If there is clinical improvement, same antibiotics were continued. If not, IVIG was given, but if there was marked clinical deterioration, exchange transfusion (ET) was done.

Antibiotics were given to all patients: Ampicillin, 100 to 300 mg/kg/day, gentamicin, 5 to 7.5 mg/kg/day as empirical antibiotic therapy in early onset sepsis and meronam, 20 to 40 mg/kg/day, vancomycin, 30 to 50 mg/kg/day as empirical antibiotic therapy in late onset sepsis. Later Antibiotics were changed according to culture and sensitivity results.

Intravenous immunoglobulin (IVIG) was given once in a dose of 400 mg/kg bodyweight through a peripheral intravenous catheter, avoiding mixing with other solutions or drugs. The IVIG used was Human Normal Immunoglobulin G (GREEN CROSS CORP, Korea). It was infused according to the manufacturer’s instructions over a period of 4 to 6 hours.

A double volume ET (170 ml/kg) with citrated, fresh (<72 hours old) cross matched adult whole blood was done once. The donor blood was tested for hepatitis B surface antigen (HBsAg), hepatitis C virus antibodies (HCV Abs), human immunodeficiency virus (HIV) and syphilis. None of the patients in the antibiotic group required exchange transfusion for jaundice.
Calculations and statistics

Data were analyzed using statistical package for social science (SPSS 16) software package under Windows 8 enterprise edition 2013 operating system & statistics package BS3 on android 4.4 ice cream sandwich operating system. Graphic presentation of data was done by using EXCEL 2007 and SPSS 16 software package software.

- The incidence was defined as number of cases per 100 cases
- Description of quantitative variables as mean, SD for normally distributed data and median value for abnormally distributed data.
- Description of qualitative variables as number & percentage.
- Student t test was used to assess the statistical significance of the difference between two population means in study involving independent samples.
- One way Anova test for statistical comparison of quantitative data which is normal distributed, independent & more than two groups
- Chi-square test was used to compare qualitative variable between independent group's samples.
- Mann-Whitney U test (Z value) for two independent samples of abnormal data distribution.
- Kruskal – Walls test for comparison of more than two independent groups of abnormally distributed data.
- Level of significance
  - Probability level (P-value) ≥ 0.05 = Non significant (NS).
  - P-value < 0.05 = Significant.
  - P-value < 0.01 = highly significant.
  - P-value < 0.001 = very highly significant.
  - P-value of 0.00 = absolute significant
Results
Results

Table 6: Percentage of admission diagnosis in the study group.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No (No= 30)</th>
<th>% (100.0)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiratory distress syndrome (RDS)</td>
<td>13</td>
<td>43.4</td>
</tr>
<tr>
<td>Hypoxic ischemic encephalopathy (HIE)</td>
<td>8</td>
<td>26.6</td>
</tr>
<tr>
<td>Meconium aspiration syndrome (MAS)</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>Transient tachypnea of the newborn (TTN)</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>Congenital pneumonia</td>
<td>1</td>
<td>3.3</td>
</tr>
<tr>
<td>Infant of diabetic mother (IDM)</td>
<td>1</td>
<td>3.3</td>
</tr>
<tr>
<td>Intracranial hemorrhage (ICH)</td>
<td>1</td>
<td>3.3</td>
</tr>
</tbody>
</table>

This table shows that RDS was the most common admission diagnosis in the study group followed by HIE.

Fig. 4: This histogram shows different diagnosis at admission in the study groups.
**Table 7:** Comparison between the study groups regarding birth weight (in kg) and gestational age (in weeks).

<table>
<thead>
<tr>
<th>Item</th>
<th>IVIG (No=10)</th>
<th>ET (No=10)</th>
<th>Antibiotic (No=10)</th>
<th>p</th>
<th>significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>2.4±0.47</td>
<td>2.7±0.92</td>
<td>2.3±0.65</td>
<td>&gt;0.05</td>
<td>NS</td>
</tr>
<tr>
<td>GA (Weeks)</td>
<td>36.3±2.8</td>
<td>36.1±2.9</td>
<td>35.8±3.2</td>
<td>&gt;0.05</td>
<td>NS</td>
</tr>
</tbody>
</table>

- IVIG: Intravenous immunoglobulins
- ET: Exchange transfusion
- GA: Gestational age
- NS: Non significant

This table shows no significant difference between the three groups regarding birth weight and gestational age.

**Fig. 5:** This bar chart shows comparison between the study groups regarding birth weight (in kg).

**Fig. 6:** This bar chart shows comparison between the study groups regarding gestational age (in weeks).
This table shows no significant difference between the three groups regarding sex.

Table 8: Comparison between the study groups regarding sex.

<table>
<thead>
<tr>
<th>Item</th>
<th>IVIG (No=10)</th>
<th>ET (No=10)</th>
<th>Antibiotic (No=10)</th>
<th>p</th>
<th>significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>%</td>
<td>No</td>
<td>%</td>
<td>No</td>
</tr>
<tr>
<td>Male</td>
<td>5</td>
<td>50</td>
<td>5</td>
<td>50</td>
<td>4</td>
</tr>
<tr>
<td>Female</td>
<td>5</td>
<td>50</td>
<td>5</td>
<td>50</td>
<td>6</td>
</tr>
</tbody>
</table>

Fig. 7: This bar chart shows comparison between the study groups regarding sex.
**Results**

**Table 9:** Comparison between the study groups regarding mode of delivery.

<table>
<thead>
<tr>
<th>Item</th>
<th>IVIG (No=10)</th>
<th>ET (No=10)</th>
<th>Antibiotic (No=10)</th>
<th>p</th>
<th>significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>%</td>
<td>No</td>
<td>%</td>
<td>No</td>
</tr>
<tr>
<td>NVD</td>
<td>1</td>
<td>10</td>
<td>3</td>
<td>30</td>
<td>5</td>
</tr>
<tr>
<td>CS</td>
<td>9</td>
<td>90</td>
<td>7</td>
<td>70</td>
<td>5</td>
</tr>
</tbody>
</table>

- NVD: Normal vaginal delivery
- CS: Caesarean section

This table shows no significant difference between the three groups regarding mode of delivery.

**Fig. 8:** This bar chart shows comparison between the study groups regarding mode of delivery.
**Results**

*Table 10: Comparison between the study groups regarding admission diagnosis.*

<table>
<thead>
<tr>
<th>Item</th>
<th>IVIG (No=10)</th>
<th>ET (No=10)</th>
<th>Antibiotic (No=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>%</td>
<td>No</td>
</tr>
<tr>
<td>RDS</td>
<td>5</td>
<td>50</td>
<td>4</td>
</tr>
<tr>
<td>MAS</td>
<td>1</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>HIE</td>
<td>3</td>
<td>30</td>
<td>2</td>
</tr>
<tr>
<td>TTN</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>IDM</td>
<td>1</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Congenital pneumonia</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>ICH</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

This table shows that RDS was the most common admission diagnosis followed by HIE in the three groups.

*Fig. 9: This bar chart shows comparison between the study groups regarding admission diagnosis.*
Results

Table 11: Comparison between the study groups regarding the need for mechanical ventilation (MV) & blood transfusion during hospital stay.

<table>
<thead>
<tr>
<th>Item</th>
<th>IVIG (No=10)</th>
<th>ET (No=10)</th>
<th>Antibiotic (No=10)</th>
<th>p</th>
<th>significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>MV</td>
<td>No</td>
<td>%</td>
<td>No</td>
<td>%</td>
<td>No</td>
</tr>
<tr>
<td>Blood transfusion</td>
<td>10</td>
<td>100</td>
<td>10</td>
<td>100</td>
<td>6</td>
</tr>
</tbody>
</table>

- S: significant

This table shows significant difference between the three groups regarding the need for MV & blood transfusion where it was found in all cases of IVIG & ET unlike cases of antibiotic therapy where only 60% of cases required MV and only 50% of cases required blood transfusion.

Fig. 10: This bar chart shows comparison between the study groups regarding the need for mechanical ventilation (MV) & blood transfusion during hospital stay.
## Results

**Table 12:** Comparison between the study groups regarding vital data at time of sepsis diagnosis.

<table>
<thead>
<tr>
<th>Vital data</th>
<th>IVIG (No=10)</th>
<th>ET (No=10)</th>
<th>Antibiotic (No=10)</th>
<th>p</th>
<th>significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR</td>
<td>143.4±13.2</td>
<td>151.3±10.6</td>
<td>133.6±9.6</td>
<td>&lt;0.01</td>
<td>HS</td>
</tr>
<tr>
<td>RR</td>
<td>57.4±15.8</td>
<td>64.8±8</td>
<td>53±8.4</td>
<td>&gt;0.05</td>
<td>NS</td>
</tr>
<tr>
<td>Temp.</td>
<td>36.7±0.48</td>
<td>36.8±0.24</td>
<td>36.9±0.211</td>
<td>&gt;0.05</td>
<td>NS</td>
</tr>
<tr>
<td>SBP</td>
<td>80.1±8.3</td>
<td>79.8±10.3</td>
<td>74.6±10.8</td>
<td>&gt;0.05</td>
<td>NS</td>
</tr>
</tbody>
</table>

- **HR:** Heart rate  
- **RR:** Respiratory rate  
- **Temp.:** Temperature  
- **SBP:** Systolic blood pressure  
- **HS:** Highly significant

This table shows highly significant difference between the three groups regarding HR at time of sepsis diagnosis where it was higher in cases of ET than cases of IVIG and antibiotic therapy.
Results

Table 13: Comparison between the study groups regarding investigations 24hrs. before treatment

<table>
<thead>
<tr>
<th>Investigations</th>
<th>IVIG (No=10)</th>
<th>ET (No=10)</th>
<th>Antibiotic (No=10)</th>
<th>p</th>
<th>significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSS</td>
<td>5.8±0.78</td>
<td>6.7±0.80</td>
<td>5.4±0.5</td>
<td>&lt;0.05</td>
<td>S</td>
</tr>
<tr>
<td>CRP</td>
<td>76 ± 3.4</td>
<td>116± 40</td>
<td>66.5 ± 5.2</td>
<td>&lt;0.001</td>
<td>HS</td>
</tr>
</tbody>
</table>

- HSS: Hematological scoring system
- CRP: C reactive protein

This table shows highly significant difference between the three groups regarding CRP 24hrs. before treatment where it was higher in cases of ET than cases of IVIG and antibiotic therapy. Also there is significant difference between the three groups regarding HSS 24hrs. before treatment where it was higher in cases of ET than cases of IVIG and antibiotic therapy.

Fig. 11: This bar chart shows comparison between the study groups regarding HSS 24hrs. before treatment

Fig. 12: This bar chart shows comparison between the study groups regarding CRP 24hrs. before treatment
**Results**

Table 14: Comparison between the study groups regarding blood culture results at time of sepsis diagnosis.

<table>
<thead>
<tr>
<th>Item</th>
<th>IVIG (No=10)</th>
<th>ET (No=10)</th>
<th>Antibiotic (No=10)</th>
<th>p</th>
<th>significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>%</td>
<td>No</td>
<td>%</td>
<td>No</td>
</tr>
<tr>
<td>Gram +ve cocci</td>
<td>3</td>
<td>30</td>
<td>3</td>
<td>30</td>
<td>3</td>
</tr>
<tr>
<td>Gram -ve bacilli</td>
<td>1</td>
<td>10</td>
<td>2</td>
<td>20</td>
<td>2</td>
</tr>
<tr>
<td>Candida</td>
<td>3</td>
<td>30</td>
<td>1</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>No growth</td>
<td>3</td>
<td>30</td>
<td>4</td>
<td>40</td>
<td>4</td>
</tr>
</tbody>
</table>

This table shows no significant difference between the three groups regarding blood culture results at time of sepsis diagnosis.

**Fig. 13:** This bar chart shows comparison between the study groups regarding blood culture results at time of sepsis diagnosis.
Results

Table 15: Comparison between the study groups regarding vital data 24hrs. after treatment of sepsis.

<table>
<thead>
<tr>
<th>Vital data</th>
<th>IVIG (No=10)</th>
<th>ET (No=10)</th>
<th>Antibiotic (No=10)</th>
<th>p</th>
<th>significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR</td>
<td>141±14.5</td>
<td>136.9±13.9</td>
<td>141.2±9.6</td>
<td>&gt;0.05</td>
<td>NS</td>
</tr>
<tr>
<td>RR</td>
<td>59.1±10.7</td>
<td>46.5±7.6</td>
<td>53.1±14.1</td>
<td>&gt;0.05</td>
<td>NS</td>
</tr>
<tr>
<td>Temp.</td>
<td>36.9±0.21</td>
<td>36.8±0.21</td>
<td>36.7±0.42</td>
<td>&gt;0.05</td>
<td>NS</td>
</tr>
<tr>
<td>SBP</td>
<td>87.9±10.2</td>
<td>84±18.11</td>
<td>77.5±11.2</td>
<td>&gt;0.05</td>
<td>NS</td>
</tr>
</tbody>
</table>

This table shows no significant difference between the three groups regarding vital data 24hrs. after treatment of sepsis.

Table 16: Comparison between the study groups regarding investigations 48hrs. after treatment of sepsis.

<table>
<thead>
<tr>
<th>Investigations</th>
<th>IVIG (No=10)</th>
<th>ET (No=10)</th>
<th>Antibiotic (No=10)</th>
<th>p</th>
<th>significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSS</td>
<td>4.8±0.91</td>
<td>4.1</td>
<td>5.5±0.97</td>
<td>&lt;0.05</td>
<td>S</td>
</tr>
<tr>
<td>CRP</td>
<td>51.4±14.1</td>
<td>34.5±3.2</td>
<td>75.2±13.4</td>
<td>&lt;0.05</td>
<td>S</td>
</tr>
</tbody>
</table>

This table shows significant difference between the three groups regarding HSS & CRP 48hrs. after treatment of sepsis where it was markedly decreased after ET then after IVIG compared to antibiotic therapy.

**Fig. 14:** This bar chart shows comparison between the study groups regarding HSS 48hrs. after treatment of sepsis

**Fig. 15:** This bar chart shows comparison between the study groups regarding CRP 48hrs. after treatment of sepsis.
Results

**Table 17:** Comparison between the study groups regarding investigations 1 week after treatment of sepsis.

<table>
<thead>
<tr>
<th>Investigations</th>
<th>IVIG (No=10)</th>
<th>ET (No=10)</th>
<th>Antibiotic (No=10)</th>
<th>p</th>
<th>significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSS</td>
<td>3.4±1.1</td>
<td>3±0.8</td>
<td>3.9±1.6</td>
<td>&gt;0.05</td>
<td>NS</td>
</tr>
<tr>
<td>CRP</td>
<td>38.4±3.5</td>
<td>18±8.4</td>
<td>39.3±3.6</td>
<td>&lt;0.05</td>
<td>S</td>
</tr>
</tbody>
</table>

This table shows significant difference between the three groups regarding CRP 1 week after treatment of sepsis where it was markedly decreased after ET compared to IVIG and antibiotic therapy, while there was no significant difference between the three groups regarding HSS 1 week after treatment of sepsis.

**Fig. 16:** This bar chart shows comparison between the study groups regarding HSS 1 week after treatment of sepsis.

**Fig. 17:** This bar chart shows comparison between the study groups regarding CRP 1 week after treatment of sepsis.
**Results**

*Table 18:* Comparison between antibiotic therapy group and IVIG group regarding treatment efficacy.

<table>
<thead>
<tr>
<th>Item</th>
<th>Antibiotic (No=10)</th>
<th>IVIG (No=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>%</td>
</tr>
<tr>
<td>Improved</td>
<td>5</td>
<td>50</td>
</tr>
<tr>
<td>Not improved</td>
<td>5</td>
<td>50</td>
</tr>
<tr>
<td>Relative risk reduction (RRR)</td>
<td>20%</td>
<td></td>
</tr>
<tr>
<td>Absolute risk reduction (ARR)</td>
<td>10%</td>
<td></td>
</tr>
<tr>
<td>Number need to treat (NNT)</td>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>

This table shows that IVIG treatment resulted in relative risk reduction of 20%, absolute risk reduction of 10% and 10 patients were needed to be treated for one to benefit compared with antibiotic therapy.

*Table 19:* Comparison between antibiotic therapy group and ET group regarding treatment efficacy.

<table>
<thead>
<tr>
<th>Item</th>
<th>Antibiotic (No=10)</th>
<th>ET (No=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>%</td>
</tr>
<tr>
<td>Improved</td>
<td>5</td>
<td>50</td>
</tr>
<tr>
<td>Not improved</td>
<td>5</td>
<td>50</td>
</tr>
<tr>
<td>Relative risk reduction (RRR)</td>
<td></td>
<td>30%</td>
</tr>
<tr>
<td>Absolute risk reduction (ARR)</td>
<td></td>
<td>20%</td>
</tr>
<tr>
<td>Number need to treat (NNT)</td>
<td></td>
<td>5</td>
</tr>
</tbody>
</table>

This table shows that ET treatment resulted in relative risk reduction of 30%, absolute risk reduction of 20% and 5 patients were needed to be treated for one to benefit compared with antibiotic therapy.
Results

Table 20: Comparison between IVIG group and ET group regarding treatment efficacy.

<table>
<thead>
<tr>
<th>Item</th>
<th>IVIG (No=10)</th>
<th>ET (No=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>%</td>
</tr>
<tr>
<td>Improved</td>
<td>6</td>
<td>60</td>
</tr>
<tr>
<td>Not improved</td>
<td>4</td>
<td>40</td>
</tr>
<tr>
<td>Relative risk reduction (RRR)</td>
<td></td>
<td>20%</td>
</tr>
<tr>
<td>Absolute risk reduction (ARR)</td>
<td></td>
<td>10%</td>
</tr>
<tr>
<td>Number need to treat (NNT)</td>
<td></td>
<td>10</td>
</tr>
</tbody>
</table>

This table shows that ET treatment resulted in relative risk reduction of 20%, absolute risk reduction of 10% and 10 patients were needed to be treated for one to benefit compared with IVIG.

Fig. 18: This bar chart shows comparison between the study groups regarding treatment efficacy.
Discussion
Discussion

Neonatal sepsis or septicemia is a clinical syndrome characterized by systemic signs of circulatory compromise (e.g., Poor peripheral perfusion, pallor, hypotonia and poor responsiveness) caused by invasion of the bloodstream by bacteria in the first month of life (Edmond and Zaidi, 2010).

Neonatal sepsis is a common disorder affecting 1.1 to 2.7% of all neonates. Thus; bacterial infections remain the most common cause for mortality and morbidity in early human life (Stoll et al., 2011).

Neonatal sepsis can broadly be classified into early onset sepsis (<72 hrs.) and late onset sepsis (>72 hrs.). Early onset sepsis (EOS) often presents as a fulminant, multi-system illness within 72 hrs. of delivery and is mainly due to bacteria acquired before and during delivery whereas late onset sepsis (LOS) is due to bacteria acquired after delivery (Nosocomial or community sources) and can present as either a fulminant or a smoldering infection (Sundaram et al., 2009).

Distal risk factors for neonatal sepsis include poverty and poor environmental conditions. Proximate factors include prolonged rupture of membranes, preterm labor, maternal pyrexia, unhygienic intrapartum and postnatal care, low birth weight and prelacteal feeding of contaminated foods and fluids (Bahl et al., 2009).

The most frequent microorganisms involved in EOS are *Streptococcus agalactiae* (GBS), *Escherichia coli* and *Hemophilus influenza*. Very recently a wide study reported on the possible role of *coagulase-negative staphylococci* (CoNS). The most frequent microorganisms involved in LOS are *Coagulase-negative staphylococci* (CoNS), *Enterobacteriaceae*, including *Escherichia coli*, *Klebsiella pneumoniae* and *Acinetobacter baumannii* (Paolucci et al., 2012).

Neonatal sepsis identification is difficult as the clinical signs of neonatal septicemia can be very similar to those of other life threatening diseases. However, recent studies in middle- and low-income countries
have provided seven danger signs which can be used to identify infants with very severe disease including neonatal sepsis (Difficulty feeding, convulsions, movement only when stimulated, respiratory rate >60 breaths per minute, severe chest indrawing and axillary temperature >37.5°C or <35°C) (Edmond and Zaidi, 2010).

The gold standard for diagnosis of neonatal sepsis remains blood culture. However, in many situations this test is fraught with practical problems, so hematological indices (e.g., Numbers of white blood cells, neutrophils and platelets) and biochemical markers of inflammation, such as C-reactive protein (CRP), and procalcitonin are now routinely used in clinical practice as they can aid in the diagnosis of neonatal sepsis. This is particularly useful in cases of persisting clinical symptoms and in the absence of a confirmatory positive blood culture, or in situations where localized sources of infection are being considered (Marchant et al., 2013).

The subtle, nonspecific nature of clinical signs and the rapid progression of neonatal sepsis make prompt diagnosis and antibiotic treatment crucial. Any delay in antimicrobial therapy places a neonate with sepsis at greater risk of mortality. Empirical antibiotic therapy should be based on knowledge of local epidemiology and antibiotic resistance patterns of neonatal sepsis, since geographic variation can be influential (Marchant et al., 2013).

Intravenous immunoglobulin (IVIG) has been considered as an adjuvant in the treatment of neonatal sepsis. Knowing the characteristics of the fetal immune system development and the defense mechanism failures to protect infants against neonatal pathogens has provided theoretical support for the use of IVIG. Although it has been demonstrated that the use of IVIG is safe, its effectiveness remains questionable (Franco et al., 2012).

Exchange transfusion may be beneficial as a last resort in babies with severe sepsis, particularly in babies who are failing appropriate antibiotic therapy and full supportive therapy, but the evidence is weak (Tarnow-Mordi et al., 2010).
Discussion

Multiple studies show that neonatal sepsis has major long term neurodevelopmental consequences in survivors, particularly in preterm neonates (Marchant et al., 2013).

This study was conducted to evaluate the role of intravenous immunoglobulin and exchange transfusion as an adjunctive therapy in treatment of neonatal sepsis compared to antibiotic therapy alone, aiming to set up protocol for management of neonatal sepsis in neonatal intensive care unit (NICU) of Benha University Hospital.

Treatment was given according to the following protocol: All ill patients whatever the presentation and the postnatal age were admitted to NICU where blood samples were taken for blood culture, complete blood count (CBC) and CRP and empirical antibiotics were given. If hematological scoring system (HSS) and CRP were compatible with sepsis, the result of blood culture was awaited for three days and then antibiotics were changed according to the result of culture and sensitivity. If blood culture yielded no growth, clinical condition was assessed. If there is clinical improvement, same antibiotics were continued. If not, antibiotics were changed according to clinical suspicion, then after two days, the patient was reassessed. If there is clinical improvement, same antibiotics were continued. If not, IVIG was given, but if there was marked clinical deterioration, exchange transfusion (ET) was done.

In this study, 30 neonates with sepsis were investigated. All of them received antibiotic therapy and other supportive measures then were divided into three groups; group I (No=10) includes neonates who received also a single dose of 400 mg/kg of polyclonal intravenous immunoglobulin (IVIG) if they showed no clinical improvement in spite of five days of antibiotic therapy after admission, group II (No=10) includes neonates who received also exchange transfusion (ET) once with a double volume (170 ml/kg) of citrated, fresh (<72 hours old) cross matched adult whole blood if they showed marked clinical deterioration in spite of five days of antibiotic therapy after admission, and group III (No=10) includes neonates who received antibiotics and other supportive therapy alone without any adjunctive therapy.
Out of these thirty neonates, there were 14 males (46.66%) and 16 females (53.33%), there were no significant differences regarding sex between the three groups (P>0.05) (Table 8). This agreed with (Payashli et al., 2013) and (Labib et al., 2013), and disagreed with (Makkar et al., 2013) where there was significant difference regarding sex between the three groups (P 0.003) and this difference may be due to larger number of cases in their study (110 cases).

The mean gestational age was (36.1±2.9 weeks) of which 12 were preterm (40%) and 18 were term (60%), there were no significant differences regarding gestational age between the three groups (P>0.05) (Table 7). This agreed with (Payashli et al., 2013) and (Labib et al., 2013), and disagreed with (Makkar et al., 2013) where there was significant difference regarding gestational age between the three groups (P 0.0002) and this difference may be due to larger number of cases in their study (110 cases).

The mean birth weight was (2.5±0.61 kg.), there were no significant differences regarding birth weight between the three groups (P>0.05) (Table 7). This agreed with (Payashli et al., 2013) and (Labib et al., 2013).

The mode of delivery was normal vaginal delivery in 9 neonates (30%) and cesarean section in 21 neonates (70%), there were no significant differences regarding mode of delivery between the three groups (P>0.05) (Table 9). This agreed with (Labib et al., 2013).

The age of onset of sepsis in all cases ranged from day 1 to day 30. Only one case presented with EOS, while all others presented with LOS. Therefore, the distinction of early and late onset sepsis has not been made. This agreed with (Payashli et al., 2013).

All neonates enrolled in this study were admitted in NICU with different presentations then acquired sepsis through their stay. It was observed that respiratory distress syndrome (RDS) was the commonest presentation in 13 neonates (43.3%) followed by hypoxic ischemic encephalopathy (HIE) in 8 neonates (26.6%) (Table 6). This agreed with (Misra et al., 2013).
In this study, assisted ventilation and blood transfusion were required in all cases of ET (Table 1). This agreed with (Vain et al., 1980), where mechanical ventilator (MV) was required in 7/10 of cases of ET and blood transfusion was required in 6 cases.

In this study, there was a highly significant difference between the three groups regarding heart rate (HR) at time of sepsis diagnosis (P<0.01) where it was higher in cases of ET than cases of IVIG and antibiotic therapy (Table 12). This agreed with (Griffin et al., 2003) and (Moorman et al., 2011).

Blood culture of the study group showed that 9 cases were gram positive cocci (30%), 5 cases were gram negative bacilli (16.6%) and 5 cases were candida (16.6%), while the remaining 11 cases showed no growth (36.6%), there were no significant differences regarding blood culture results between the three groups (P>0.05) (Table 14). This agreed with (Stoll et al., 2011) and (Misra et al., 2013), and disagreed with (Labib et al., 2013) where out of 35 neonates included in their study, gram positive cocci represented (31.4%), gram negative bacilli represented (42.8%), while the remaining (25.7%) showed no growth, this disagreement may be due to younger age and birth weight of cases involved in their study where (68.8%) of cases were preterm and low birth weight.

In this study, ET was done in cases showing marked clinical deterioration in spite of five days of antibiotic therapy after admission. Also hematological scoring system (HSS) and C reactive protein (CRP) were significantly high in these cases (Table 13), indicating that these cases were critically ill. This agreed with (Vain et al., 1980), (Dalvi et al., 1991) and (Sadana et al., 1997) where ET was done only in septic neonates with sclerema or clinical deterioration despite antibiotic therapy, and disagreed with (Gunes et al., 2006) where they included non-critically ill patients in their study then divided them into three groups (IVIG, ET, antibiotic therapy groups) and this can be explained by the aim of their study that was to compare immunoglobulin level before and after treatment in these groups.
After ET, temporary clinical improvement and significant reduction of HSS and CRP were noticed compared to antibiotic group (Table 16, 17), but the overall mortality was (80%) indicating that ET had a short term effect but didn't significantly affect the final outcome. This agreed with (Tollner et al., 1977), and disagreed with (Vain et al., 1980) where overall mortality was (30%), this may be due to repetition of ET done with160 ml/kg of citrated fresh (<48 hrs. old) blood every eight to 12 hours up to four times or until complete clinical stabilization or death of the patient occurred. This also disagreed with (Dalvi et al., 1991) where overall mortality was (22.6%), this may be due to the use of double volume, citrated, fresh (<24 hrs. old) blood and repetition of ET every 12-24hrs. up to a maximum of four times or clinical improvement or death of patient occurred. This disagreed with (Gunes et al., 2006) where overall mortality was (21%), this can be explained by the enrollment of non-critically ill patients in their study.

This study also demonstrated that IVIG treatment resulted in clinical improvement and significant reduction of HSS and CRP compared to antibiotic group (Table 16, 17), with overall mortality (30%). This agreed with (Jenson and Pollock, 1997), (Haque, 2010) and (Ohlsson and Lacy, 2010). But disagreed with (Brocklehurst et al., 2011) where there was no significant reduction in mortality rate. This also disagreed with the review of seven articles done by (Franco et al., 2012), where mortality rate reduction showed no differences between the IVIG and antibiotic groups. The variation of the reported benefits of the effectiveness of IVIG in these studies may be due to inclusion of all instances of infection including localized soft-tissue infections, pneumonia, urinary tract infections, gastroenteritis, and necrotizing enterocolitis even when not associated with sepsis, and included outcomes such as suspected sepsis, presumed sepsis, or very probable sepsis in addition to proven sepsis, or due to the variations in composition of IVIG of different manufacturers in these studies.
Summary
Summary

Neonatal sepsis is a clinical syndrome of bacteremia characterized by systemic signs and symptoms in first month of life. It is a common disorder affecting 1.1 to 2.7% of all neonates.

Neonatal sepsis identification is difficult through clinical presentation alone, so hematological indices and biochemical markers of inflammation, such as C-reactive protein (CRP) are now routinely used in clinical practice as they can aid in the diagnosis of neonatal sepsis. However the gold standard for diagnosis of neonatal sepsis remains blood culture despite its problems.

As neonatal sepsis can be rapidly fatal if left untreated, so once it is suspected, highly effective antibiotic therapy must be used and delay in the provision of care must be minimized.

Polyclonal intravenous immunoglobulin (IVIG) significantly reduces mortality and is a promising adjuvant in the treatment of neonatal sepsis and septic shock. However, the totality of the evidence is insufficient to support its benefit. Adjunctive therapy with monoclonal IVIGs remains experimental.

Exchange transfusion (ET) may be beneficial as a last resort in neonates with severe sepsis, particularly in those who are failing appropriate antibiotics and full supportive therapy, but the evidence is weak.

Hence, the aim of this study is to evaluate the role of intravenous immunoglobulin and exchange transfusion as an adjunctive therapy in treatment of neonatal sepsis compared to antibiotic therapy alone, aiming to set up protocol for management of neonatal sepsis in neonatal intensive care unit (NICU) of Benha University Hospital.

To achieve this target, we prospectively studied a series of 30 septic neonates born at or referred to Benha University Hospital and admitted to NICU from November 2012 to May 2013.

Sepsis was diagnosed using the hematological scoring system (HSS) and CRP. All neonates enrolled in this study received antibiotic therapy and other supportive measures then were divided into three groups: Group I: (No=10) Includes neonates who received IVIG in addition, group II: (No=10) Includes neonates who received ET with fresh whole blood in addition and group III: (No=10) Includes neonates who received antibiotics and other supportive therapy alone.
All neonates were subjected to the following: Careful history taking, full clinical examination and the following investigations: Complete blood count (CBC), CRP and blood culture.

The three groups were compared regarding: Gestational age, birth weight, sex, mode of delivery, provisional diagnosis and complications.

- Results revealed the following:

Out of these thirty neonates, there were 14 males (46.66%) and 16 females (53.33%). The mean gestational age was (36.1±2.9 weeks) of which 12 were preterm (40%) and 18 were term (60%). The mean birth weight was (2.5±0.61 kg.). The mode of delivery was normal vaginal delivery in 9 neonates (30%) and cesarean section in 21 neonates (70%). There were no significant differences regarding sex, gestational age, birth weight and mode of delivery between the three groups (P>0.05).

The age of onset of sepsis in all cases ranged from day 1 to day 30. Only one case presented with early onset sepsis (EOS), while all others presented with late onset sepsis (LOS).

All neonates were admitted in NICU with different presentations then acquired sepsis through their stay. It was observed that respiratory distress syndrome (RDS) was the commonest presentation in 13 neonates (43.3%) followed by hypoxic ischemic encephalopathy (HIE) in 8 neonates (26.6%), there were no significant differences between the three groups.

Blood culture showed that 9 cases were gram positive cocci (30%), 5 cases were gram negative bacilli (16.6%) and 5 cases were candida (16.6%), while the remaining 11 cases showed no growth (36.6%), there were no significant differences between the three groups.

Cases that were subjected to exchange transfusion were critically ill. This is proved by significant higher heart rate, their need to assisted ventilation and blood transfusion and significantly high HSS and CRP.

After ET, temporary clinical improvement and significant reduction of HSS and CRP were noticed compared to antibiotic group, but the overall mortality was (80%).

IVIG treatment resulted in early clinical improvement and significant reduction of HSS and CRP compared to antibiotic group, with overall mortality (30%).
Conclusions
Conclusions

- Most of cases of neonatal sepsis in our department were late onset sepsis (LOS).
- The most common presentations were respiratory distress syndrome (RDS) followed by hypoxic ischemic encephalopathy (HIE) in our department.
- Hematological scoring system (HSS) and C reactive protein (CRP) are available, cost effective and highly sensitive and specific, so they are good screening tools for diagnosis of neonatal sepsis. Also they are sensitive for assessment of efficacy of treatment of sepsis.
- Blood culture showed that the most common causative organisms of LOS were gram positive cocci, then gram negative bacilli, then candida. Also there were a high percentage of negative cultures.
- Cases that were subjected to exchange transfusion were critically ill. This is proved by significant higher heart rate, their need to assisted ventilation and blood transfusion and significantly high hematological scoring system (HSS) and C reactive protein (CRP) and this was the reason behind the high mortality rate despite the early improvement after exchange.
- Intravenous immunoglobulin treatment (IVIG) resulted in early clinical improvement and significant reduction of HSS and CRP, with little effect on mortality rate.
Recommendations
Recommendations

- It is recommended to apply more strict infection control measures to decrease the rate of late onset sepsis in our department.
- The application of hematological scoring system (HSS) and C reactive protein (CRP) for diagnosis of neonatal sepsis and assessment of treatment efficacy is recommended to be done routinely in all cases admitted to neonatal intensive care unit.
- It is recommended to take blood cultures before empirical antibiotics are given to increase its positivity.
- Repetition of exchange transfusion (ET) with double volume, fresh (< 24 hrs. old) blood every 12 hours up to four times or until significant clinical improvement occurs is recommended for further studies.
- ET should be done as early as possible once there is severe clinical deterioration in septic neonates without waiting for the results of antibiotic therapy.
- The use of intravenous immunoglobulin treatment in neonatal sepsis should be subjected to the physician's decision after weighting its potential benefit against its cost for every patient. Also further studies are recommended before its routine use in treatment of neonatal sepsis.
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مقارنة بين دور العلاج بالمضادات الحيوية ونقل الأجسام المناعية وتبديل الدم في تعفن الدم الوليدي

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بكالوريوس الطب والجراحة
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كلية الطب - جامعة بنها

كلية الطب
جامعة بنها
2013
الملخص العربي

مقدمة البحث:
التعفن الوليدي هو متلازمة سريرية تتميز بعلامات وأعراض جهازية بسبب التجرثم تحدث في الشهر الأول من العمر. وهو مرض شائع يؤثر على الأطفال حديثي الولادة بنسبة 1-7, 2٪. إنه لمن الصعب تشخيص التعفن الوليدي من خلال الصور السريرية وحدها، لذلك نستخدم مؤشرات الدم والعلامات البيوبيميكانيكية للالتهاب، مثل بروتين سي التفاعلي بشكل روتيني الآن في الممارسة السريرية لأنها يمكن أن تساع في تشخيص التعفن الوليدي. إلا أنه ما زالت مزرعة الدم تعد هي المعيار الذهبي لتشخيص التعفن الوليدي على الرغم من مشاكلها.

وحيث أنه من الممكن أن يكون التعفن الوليدي قاتل بسرعة إذا ترك بدون علاج، لذلك بمجرد اشتباه حدوثه، يجب استخدام المضادات الحيوية شديدة الفعالية، ويجرب تجنب أي تأخر في تقديم الرعاية.

وقد أدى استخدام الأجسام المناعية الوريدية متعددة النسج إلى انخفاض كبير بالوفيات وهي تعد علاج مساعد واعد في علاج التعفن والصدمة السريرية. ومع ذلك، فإن مجال الأدوية غير كاف لتأكيد فائدتها. أما عن استخدام الأجسام المناعية الوريدية وحيدة النسيلة كعلاج مساعد فإنه ما يزال تحت التجربة.

ومن الممكن أن يكون تبديل الدم مفيدا كملاحظ آخر في الأطفال الذين يعانون من التسمم الحاد، وخاصة في الأطفال الذين خلقت المضادات الحيوية المناسبة والعلاج الداعم الكامل في علاجهم، ولكن الأدوية متاحة ضعيفة.

الهدف من البحث:
إن الهدف من هذه الدراسة هو تقييم دور الأجسام المناعية الوريدية وتبديل الدم كعلاج مساعد في علاج التعفن الوليدي مقارنة مع العلاج بالمضادات الحيوية وحدها. بهدف وضع بروتوكل لعلاج التعفن الوليدي بوحدة العناية المركزة بالأطفال حديثي الولادة (الحفلان) بمستشفى بنها الجامع.

المرضى و طرق البحث:
لتحقق هذا الهدف، قمنا بدراسة مستقلة على 30 وليدا يعانون من التعفن الوليدي ولدوا في المستشفى أو حولوا إليها وتم حجزهم بالحفلان خلال الفترة من نوفمبر 2012 إلى مايو 2013.

تم تشخيص التعفن الوليدي باستخدام نظام التقييم الدموي وبروتين سي التفاعلي. وتم تقسيم الأطفال حديثي الولادة المدرجين بالبحث إلى ثلاثة مجموعات: المجموعة الأولى: (عدد = 10) وتتمثل الأطفال الذين تلقوا الأجسام المناعية الوريدية بالإضافة إلى المضادات الحيوية، المجموعة الثانية: (العدد = 10) وتتمثل الأطفال الذين خضعوا لتبديل الدم بدم كامل طازج بالإضافة إلى المضادات الحيوية والمجموعة الثالثة: (العدد = 10) وتتمثل الأطفال الذين تلقوا المضادات الحيوية وحدها.

وتعرض جميع الأطفال حديثي الولادة إلى ما يلي: بعد تاريخ مرضي دقيق والفحص السريري الكامل والتحاليل التالية: صورة دم كاملة وبروتين سي التفاعلي ومزرعة الدم. قورنت المجموعات الثلاث فيما يتعلق: عمر الحمل والوزن عند الولادة والجنس وطريقة الولادة والتشخيص المبكر والمضاعفات.
نتائج البحث:
من الثلاثين طفلًا المدرجين بالبحث، كان عدد الذكور 14 (46.66%) وعدد الإناث 16 (53.33%). وكان متوسط العمر الحملي (36.1 ± 2.9 أسابيع) منهم 12 طفلًا متصرًا (40%) و18 طفلًا كاملاً النمو (60%). وكان متوسط الوزن عند الولادة (2.5 ± 0.61 كجم). وكانت طريقة الولادة في 9 حالات ولادة مهبالية طبيعية (30%) وفي 21 حالة ولادة قيصرية (70%). ولم تكن هناك فروق ذات دلالة إحصائية فيما يتعلق بالجنس والعمر الحملي وزن الولادة وطريقة الولادة بين المجموعات الثلاثة.

يتراوح عمر بداية التعرف الوليدي في جميع الحالات من يوم إلى 30 يوم. و كانت هناك حالة واحدة فقط بداية مبكرة، في حين كانت كل الحالات الأخرى ذات بداية متأخرة.

وقد تم حجز جميع الأطفال حديثي الولادة في الحضانة بأعراض مختلفة ثم اكتسبوا تعفن وليدي خلال إقامتهم. ولوجز أن متلازمة الضائقة التنفسية كانت أكثر الأعراض شيوعا حيث حدثت في 13 طفل (43.3%)، بلغها نقص الأوكسيجين الدماغي الإفقاري حيث حدث في 8 أطفال (26.6%) ولم تكن هناك فروق ذات دلالة إحصائية بين المجموعات الثلاث.

وكانت نتيجة مزرعة الدم كالآتي: 9 حالات بها ميكونوبات ميورس إيجابية (30%) و5 حالات بها عصيات سلبية (16.6%) و5 حالات بها فطريات (16.6%)، في حين لم تظهر الحالات 11 المتبقيّة أي نمو (36.6%)، ولم تكن هناك فروق ذات دلالة إحصائية بين المجموعات الثلاث.

وكانت الحالات التي تعرضت لتبديل الدم في حالة صحية حرجة. وقد ثبت ذلك من خلال معدل ضربات القلب العالي واحتاجتها إلى التنفس الصناعي ونقل الدم وارتفاع ملحوظ في نظام التقييم الدموي وبروتين سي التفاعلي لهذه الحالات.

لوحظ بعد تبديل الدم وجود تحسن سريري ملحوظ ونقص ملحوظ بنظام التقييم الدموي وبروتين سي التفاعلي مقارنة مع مجموعة المضادات الحيوية، ولكن كان العدد الإجمالي للوفيات 13 (9.7%).

كما أدى العلاج باستخدام الأجسام المناعية الوريدية إلى تحسن سريري مبدئي ونقص ملحوظ بنظام التقييم الدموي وبروتين سي التفاعلي مقارنة مع مجموعة المضادات الحيوية، وكان العدد الإجمالي للوفيات 3 (30%).

وقد استخلصت الدراسة:
- معظم حالات التعرف الوليدي في قسم وحدة العناية المركزية بالأطفال حديثي الولادة بمستشفى
- بنها الجامعي ذات بداية متأخرة.
- أكثر الأعراض شيوعاً في قسمنا: متلازمة الضائقة التنفسية. بلغها نقص الأوكسيجين الدماغي الإفقاري.
- إن نظام التقييم الدموي وبروتين سي التفاعلي متاحين وذوا فعالية وحساسية عالية وتكلفة محدودة، لذلك فيما أداة فحص جديدة لتشخيص التعرف الوليدي كما أنهما يتمتعان بحساسية عالية لتقديم فعالية العلاج من التسمم.
أظهرت مزرعة الدم أن الميكروبات الأكثر شيوعاً المسببة للتوفان الوليد ذات البداية المتاخرة كانت الميكروبات المكوره الإيجابية، ثم العصيات السلبية، ثم الفطرية. أيضاً كانت هناك نسبة عالية من المزارع التي لم تظهر أي نمو.

كانت الحالات التي تعرضت لتبديل الدم في حالة صحية حرجة. وقد ثبت ذلك من خلال معدل ضربات القلب العالي وحاجتها إلى التنفس الصناعي ونقل الدم وارتفاع ملحوظ في نظام التقييم الدموي وبروتينات التفاعلي لهذه الحالات وكان هذا هو السبب وراء ارتفاع معدل الوفيات على الرغم من التحسن المبدئي بعد تبديل الدم.

أدى العلاج باستخدام الأجسام المناعية الوريدية إلى تحسن سريري مبدئي ونقص ملحوظ بنظام التقييم الدموي وبروتينات التفاعلي مع تأثير ضئيل على معدل الوفيات.

وقد أوصت الدراسة:

- من المستحسن تطبيق تدابير أكثر صرامة لمكافحة العدوى لخفض نسبة التوفان الوليد ذات البداية المتاخرة في قسماً من المستحسن تطبيق تدابير أكثر صرامة لمكافحة العدوى لخفض نسبة التوفان الوليد ذات البداية المتاخرة في قسماً.

- ينصح بتطبيق نظام التقييم الدموي وبروتينات التفاعلي لتشخيص التوفان الوليد وتقديم فعالية العلاج الذي ينبغي القيام به بشكل روتيني في جميع الحالات المحجوزة بالعظام. من المستحسن أن تأخذ مزارع الدم قبل إعطاء المضادات الحيوية المبدئية لزيادة نسبة الإيجابية بها.

- ينصح تكرار تبدل الدم فورًا بممية مضاعف، بدء طارج (عمره > 24 ساعة) كل 12 ساعة بعد أقصى يصل إلى أربعة أضعاف أو حتى يحدث تحسن سريري ملحوظ وترك ذلك لمزيد من الدراسات.

- ينبغي أن يتم تبديل الدم في أقرب وقت ممكن عندما يكون هناك تدهور سريري شديد في الحاله دون انتظار نتائج العلاج بالمضادات الحيوية.

- يجب أن يخضع العلاج باستخدام الأجسام المناعية الوريدية في التوفان الوليد إلى قرار الطبيب بعد مقارنة الفوائد المحتملة للعلاج مع تكلفته لكل مريض. كما ينصح بعمل مزيد من الدراسات قبل الاستخدام الروتيني لهذا العلاج في علاج التوفان الوليد.