PARAMETERS PREDICTING CLINICAL SEPSIS IN NEONATES

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PARAMETERS PREDICTING CLINICAL SEPSIS IN NEONATES

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LIST OF ABBRIVIATION

AB	Antibiotics
ABW	Appropriate birth weight
AGE	Acute gastroenteritis
ANC	Absolute neutrophil count
AUC	Area under curve
BC	Blood culture
+ve BC	Positive blood culture
-ve BC	Negative blood culture
BiPAP	Bi-level positive airway pressure
BP	Blood pressure
CBC	Complete blood count
cfu	Colony-forming unit
CNS	Central nervous system
CoNS	Coagulase negative staphylococci
CPAP	Positive airway pressure
CRP	C-reactive protein
CSF	Cerebrospinal fluid
CXR	Chest X-Ray
DIC	Disseminated intravascular coagulation
DNA	Deoxyribonucleic acid
EOS	Early onset sepsis
ELBW	Extreme low birth weight
ET	Exchange transfusion
ETT	Endotracheal intubation
FISH	Fluorescence in situ hybridization
GBS	Group B streptococci
GW	Gestational week
HIE	Hypoxic ischemic encephalopathy
HRPZ II	Hospital Raja Perempuan Zainab II
HUSM	Hospital Universiti Sains Malaysia
IT ratio	Immature to total neutrophil ratio
IL1	Interleukin 1

IL6	Interleukin 6
IPA	intra-partum antibiotic prophylaxis
INFRS	Increase need for respiratory support
LBW	Low birth weight
LOS	Late onset sepsis
LOGNS	Late onset gram negative sepsis
LVS	Lower vaginal swab
MAS	Meconium aspiration syndrome
NEC	Necrotising enterocolitis
NICU	Neonatal intensive care unit
NNJ	Neonatal jaundice
PCT	Procalcitonin
PCRT	Prolonged capillary refilling time
PCR	Polymerase chain reaction
PROM	Prolonged rupture of membrane
PREM	Prematurity
PPV	Positive predictive value
RD	Respiratory distress
RDS	Respiratory distress syndrome
ROC curve	Receiver operator characteristic
SGA	Small for gestational age
SEA URCHIN	South East Asia Using Research for Change in Hospital acquired Infection in Neonate
STD	Sexually transmitted diseases
TNF	Tumour necrosis factor
TPN	Total parenteral nutrition
URTI	Upper respiratory tract infection
UTI	Urinary tract infection
VLBW	Very low birth weight
WBC	White blood count
WHO	World health organization

PARAMETER MERAMALKAN SEPSIS KLINIKAL DALAM NEONAT

ABSTRAK

Pengenalan:

Sepsis neonatal merupakan masalah kesihatan utama di seluruh dunia. Pentakrifan tentang sepsis masih kekal sebagai satu isu yang amat sukar. Kajian ini bertujuan untuk menentukan parameter yang paling berguna untuk meramal sepsis yang disahkan atau sepsis klinikal.

Kaedah:

Ini adalah kajian keratan rentas yang dijalankan di wad rawatan tapi bayi(NICU) di Hospital USM dan Hospital Raja Perempuan Zainab II(HRPZ II) antara Julai 2012 dan Julai 2013 yang melibatkan semua bayi baru lahir yang diberi rawatan antibiotik. Satu pelekat khas dan folder bayi yang terlibat dengan kajian ini akan disemak semula bagi setiap episod jangkitan yang disyaki.

Berdasarkan keputusan kultur darah dan tempoh antibiotik bang di peskripsi bayi dibahagikan kepada: sepsis disahkan, sepsis klinikal dan bukan sepsis. Tiga puluh empat parameter berkaitan sepsis telah diperiksa untuk meneliti perkaitan mereka dengan kultur darah yang positif dalam setiap kumpulan menggunakan khi-kuasa dua dan ujian tepat Fisher. Kepekaan dan kekhususan parameter individu ditentukan menggunakan jadual 2x2 dan bagi model ramalan penentuaanya ditentukan dengan menggunakan perisian Stata.

Keputusan:

Sejumlah 1514 episod yang disyaki sepsis dari 1325 bayi telah dimasukkan kedalam kajian ini. Nisbah lelaki kepada perempuan adalah 1:1.3. Sebanyak enam puluh empat peratus bayi yang matang terlibat dalam kajian ini dan lebihnya 36% adalah bayi pramatang. Kebanyakan bayi (61%) mempunyai berat badan >2500g. LBW (1500-2500g), VLBW (<1500g) dan ELBW (<1000) dicatatkan sebanyak 31.4%,6.2% dan 1.1% secara berturut.

Diantara 1514 episod yang disyaki sepsis, sebanyak 51 (3.4%) disahkan sepsis, 612(4.04%) sebagai kinikal sepsis dan 851 (56.2%) bukan sepsis. Didapati 69% EOS dan LOS pula 31%. Bakteria gram negative mewakili majority patogen yang di isolat (60%).

Enambelas daripada 34 parameter mempunyai perkaitan statistik secara signifikan dengan yang disah sepsis dan 21 dengan disah sepsis dan sepsis klinikal, mereka ini diletakkan sebagai satu kumpulan. Apnoea, PCRT, kelesuan ,CRP meningkat dan thrombositopenia menunjukkan perkaitan positif yang paling kuat (0.001), diikuti dengan sawan, disyaki NEC dan leukopenia (p= 0.001). Hanya PROM > 12h menunjukkan perkaitan yang negatif.

Antara parameter individu yang cukup sensitif untuk mendiagnos dengan tepat permulaan disah sepsis yang lambat: sensitiviti yang paling tinggi dilihat berkait dengan pramatang adalah 64%. Sensitiviti model yang dibuat dengan 2 parameter signifikan

mempunyai ketepatan yang paling tinggi dengan kesensitifan sebanyak 61.3%, kekhususan 93.6% dan AUC 0.85%.

Kesimpulan:

Apnoe, PCRT, kelesuan , CRP meningkat dan trombositopenia merupakan parameter dalam kajian ini yang menunjukkan perkaitan positif yang paling kuat ($p<0.001$) dengan bayi disahkan sepsis. Namun begitu, ia jelas menunjukkan bahawa diagnosis sepsis adalah sesuatu yang kompleks, dan tiada satu parameter atau kombinasi parameter yang boleh meramal dengan tepat kepositifan kultur.

PARAMETERS PREDICTING CLINICAL SEPSIS IN NEONATES

ABSTRACT

Introduction:

Neonatal sepsis is a major health problem worldwide. The definition of sepsis remains an extremely difficult issue. This study aimed to determine the most useful parameters to predict confirmed sepsis or clinical sepsis.

Methods:

This was a cross sectional study conducted in NICUs at Hospital USM and HRPZ II between July 2012 and July 2013 involving all newborns who were started on antibiotics. A special sticker and the folders of enrolled neonates were reviewed for each episode of suspected infection.

Based on blood culture results and duration of antibiotics, babies were divided into: confirmed sepsis, clinical sepsis and no sepsis. Thirty four sepsis related parameters were checked for their association with positive blood cultures in each group using Chi-square and Fisher's exact tests. The sensitivity and specificity of individual parameters were determined using 2x2 tables and for the predictive model were determined using Stata software.

Results:

A total of 1514 episodes of suspected sepsis in 1325 neonates were included in the study. Male to female ratio was 1:1.3. Sixty four percent of involved babies were term,

while 36% were preterm. The majority (61%) had a birth weight of >2500g. LBW (1500-2500g), VLBW (<1500g) and ELBW (<1000) were recorded in 31.4%, 6.2% and 1.1% respectively.

Out of 1514 episodes of suspected sepsis, confirmed sepsis was found in 51(3.4%), clinical sepsis in 612 (40.4%) and no sepsis in 851(56.2%). EOS and LOS were found in 69% and 31% respectively. Gram negative bacteria represented the majority of isolated pathogens (60%).

Sixteen out of 34 parameters were statistically significantly associated with confirmed sepsis and 21 with confirmed and clinical sepsis, considered as one group. Apnoea, PCRT, lethargy, raised CRP and thrombocytopenia were showed the strongest positive association ($p<0.001$), followed by convulsions, suspected NEC and leukopenia ($p= 0.001$).Only PROM>12 h was showed a negative association.

None of individual parameters were sensitive enough to accurately diagnose the late onset confirmed sepsis: the highest sensitivity 64.0%, seen with prematurity. The sensitivity of the model made from at least 2 significant parameters had the highest accuracy with sensitivity 61.3%, specificity 93.6% and AUC of 0.85.

Conclusion:

Apnoea, PCRT, lethargy, raised CRP and thrombocytopenia were the parameters in this study that showed the strongest positive association ($p<0.001$) with confirmed neonatal sepsis. However it remains clear that the diagnosis of sepsis is a complex one, and no single parameter or combination of parameters can accurately predict culture positivity.

CHAPTER 1

INTRODUCTION

1.1 Background

Many terms, including “neonatal sepsis”, “neonatal septicaemia” and “sepsis neonatorum” are generally used to describe the infant’s systemic response to infection within the first 30 days of life, which requires the presence of both physical and supportive laboratory findings for its diagnosis.

The role of a positive blood culture has been that of a golden standard for diagnosis with the exception of blood cultures positive with common skin flora or non-virulent organisms. The Centres for Disease Control and prevention and National Healthcare Safety Network CDC/NHSN have come out with two criteria to define the laboratory confirmed blood stream infection (LCBI) which are:

(1) isolation of a recognized organism from one or more blood cultures plus either the presence of at least one suggestive sign or symptom or positive laboratory result

(2) presence of suggestive signs and symptoms of sepsis and isolation of common skin contaminant cultured from 2 or more blood cultures drawn on separate occasions (Horan and MPH, 2008).

There may be a lot of reasons however for the blood culture to be negative even in infected babies. Among these are:

- The low predictability of blood culture if intra-partum antibiotics had been given to the mother
- Postnatal blood cultures might fail to detect bacteraemia in a significant number of cases when the bacteraemia is transient or intermittent
- Blood cultures may be falsely negative when an insufficient amount of blood has been obtained for culture, and /or processing of the sample and the culture process are sub-optimal.

Because of this and because babies with infection who are not or only partially treated can deteriorate very fast, several authors (Chiesa et al., 2004; Sankar et al., 2008) believed that the sepsis (clinical sepsis) should be considered if there is any systemic response to infection even in the absence of evidence of bacteraemia or clear focus of infection. Most clinicians tend to consider the infant is having sepsis based on risk factors, clinical signs and laboratory parameters (haematological and acute phase proteins) rather than on the blood culture result alone (Sivanandan et al., 2011)

Until now, neonatologists have not achieved any standardized definition for neonatal blood stream infection (BSI), which is perhaps mostly due to the insensitivity of blood culture tests in the neonates, the variable presentations of sepsis in neonates and the lack of sensitive and specific laboratory diagnostic tests as well as the discrepancies among the tests (Haque, 2005). Therefore, researchers in this field were pushed to create their own definitions to outfit the aim of their own particular studies. These definitions had to

be clear with regard to type of studied infant (defined by gestational age and birth weight), baby's age, culture results, clinical condition, and severity of the illness (Chiesa et al., 2004)

Fleming et al, affirmed that the inclusion of clinical criteria in the definition of bacteraemia helped to improve the evaluation of early onset neonatal sepsis (Fleming et al., 2012).For babies who are thought to have culture negative sepsis, the term clinical sepsis has been used widely and this will be the term used in the rest of this thesis as well. A large variety of clinical manifestations are deemed to be important to be part of the definitions of clinical sepsis. Haque (Haque, 2005) suggested the clinical features of respiratory distress, temperature instability, and prolonged capillary refill time in addition to the suggestive laboratory tests as abnormality of white blood cell (WBC) count and raised C-reactive protein (CRP).

CRP is however a late marker and the search for early and more sensitive and specific markers of infection has been quite extensive. Efforts have included proteins such as procalcitonin, several interleukins and cytokines and detection of bacterial antigens in the blood. Most of these newer diagnostic methods have been not become widely available for clinical use in middle and low income countries because they were either too expensive or still in an experimental stage.

This study was conducted to determine which of the easily available clinical and laboratory parameters that make the clinicians suspect infections in the neonate are most commonly associated with culture positive infection and with clinical sepsis as

determined by the neonatologist. It was hoped that this study could help in making the definition of clinical sepsis for the clinician working in the setting of middle or low income countries a bit easier.

1.2 LITERATURE REVIEW

1.2.1 Neonatal infection and mortality

According to a World Health Organization (WHO) Report, the global neonatal mortality rate is 28 per 1,000 live births with a great variation between regions, being highest in Africa with 40/1,000 and lowest in Europe 10/1,000. A wide variation is evident between different countries: - while it is only 1/1,000 in Iceland and Singapore, while, in Liberia it is up to 66/1,000 (Geneva, 2010).

The huge majority of all neonatal deaths occur in low- or middle-income countries with a neonatal mortality rate range from 41/1000 and 27/1000 -in low-income regions and lower-middle income, respectively, compared to 4/1000 in high-income regions (Lawn et al., 2005).

About 25 to 50% of neonatal deaths occur in the first 24 h while 75% within the first week. Sixty to eighty percent of neonatal deaths arise in low birth weight infants (< 2500 g). It has been found that, the neonatal mortality reduced by 30 – 50% with an increase in the mean birth weight by 100g (Bizzarro et al., 2005).

Globally, infections, prematurity and asphyxia are the major leading causes of neonatal death while congenital malformations account for of the remaining (Qazi and Stoll, 2009; Black et al., 2010; Liu et al., 2014). More than 1 million newborns die of infections each year. Most of these occur in the late neonatal period. However, in high-

resources countries, infections have a less important role. Instead, congenital malformations becoming the most significant cause of neonatal death (Saugstad, 2010).

The mortality rate, in a study from 8 Asian neonatal intensive care units (NICUs), was 13% for early onset sepsis and 8.9% for late onset. While, the overall mortality (as a direct outcome for sepsis) was stated as 0.69 deaths /1000 live births (Tiskumara et al., 2009).

1.2.2 Incidence of neonatal infection

Generally, the incidence of serious bacterial sepsis in developed countries is 1–4/1000 live births and 2–21/1000 live births in developing and low-income countries. The incidence of infection and sepsis in pre-term infants is 3–10 fold greater than that in full-term infants of normal birth weight (Yeung and Davies,2005).

A study by Tiskumara by analysing data from eight NICUs in seven countries in Asia (two from China and one each from Hong Kong, India, Kuwait, Iran, Malaysia and Thailand) stated that, the overall figure of sepsis was 11.6 per 1000 live births, with the rates varying between 2 per 1000 live births in Hong Kong up to 22 per 1000 live births in Thailand. The overall rate of a baby having either early or late sepsis in the same study varied from 3.0 per 1000 live births in Hong Kong to 15.0 per 1000 live births in Kuwait (Tiskumara et al., 2009).

A study done over 3 years in England found that the incidence of early onset sepsis (EOS) was similar over the whole period of the surveillance and it was 0.9 per 1000 live births and 9 per 1000 neonatal admissions .The overall incidence of late onset sepsis (LOS) was 3/1000 live births and 29/1000 neonatal admissions and it was similar in each of the years of surveillance (Vergnano et al., 2011). The overall incidence of nosocomial infection in a six-year surveillance study done in Spain was 25.6% per admission (Molina-Cabrillana et al., 2006).

A significant discrepancy was noted between the incidence of proven and clinical sepsis. Clinical sepsis found to be more common than culture confirmed sepsis in a study done in Korea. The estimated incidence rate of neonatal sepsis during the study period was 30.5 per 1000 live births for clinical sepsis whereas for sepsis with positive culture, it was only 6.1 per 1000 live births (Shin et al., 2009).

The rate of clinical sepsis in developing countries was reported to be ranging from 49 to 170 per 1000 live births in population based studies (Thaver and Zaidi, 2009). Other studies have found that the average incidence of culture confirmed sepsis in these countries was only 16 per 1000 live births. This was consistent with a result from Bangladesh in a recent population-based surveillance study: the incidence rate of clinically suspected neonatal sepsis was found to be 50 per 1000 live births, and the incidence of culture confirmed sepsis was 3 per 1000 live births (Ganatra and Zaidi, 2010)

A recent review of 5 studies reporting the incidence of early onset sepsis in developing countries found that, the incidence of culture confirmed EOS was ranging from 2.2 - 9.8 per 1000 live births, while the incidence of clinical sepsis was ranging from 20.7 to 50 per 1000 live births (Mehmet Satar, 2012).

1.2.3 Classification of neonatal sepsis

1.2.3.1 According to the onset of sepsis

Neonatal sepsis is usually categorized as early onset or late onset sepsis depending upon the onset of symptoms and signs of the sepsis. So far, the exact cut-off points in time used to define early and late have been the subject of controversy.

Classically, if symptoms appear before 48-72 hours after birth the sepsis is considered early onset sepsis (EOS); if later than that it is a late onset sepsis (LOS). However, some investigators as (Seale et al., 2009; Mukhopadhyay and Puopolo, 2012; Mayor-Lynn et al., 2005) considered infection occurring in the first 7 days of life as EOS.

For the purpose of this study the cut-off point between early and late onset sepsis was taken as 48 hours.

The classification between early and late onset sepsis has contributed greatly to a better diagnosis and treatment by identifying which microorganisms are likely to be responsible for sepsis during these periods.

1.2.3.1(A) Early Onset Sepsis (EOS)

Early onset sepsis is an infection that occurs within the first 48 to 72 hours of life and the microorganisms are generally acquired from the mother while passing through a colonized birth canal at delivery (Bhutta and Yusuf, 1997; Chacko and Sohi, 2005)

In a study that considered a cut-off point at 72 hours after birth, the majority of new-borns (85%) with EOS presented within the first 24 hours, 5% presented at 24-48 hours. while 10% presented between 48-72 hours. Onset was much more rapid in premature neonates (Anderson-Berry et al., 2006).

Infants with EOS present frequently with pneumonia or enterocolitis and less frequently with meningitis and septicaemia (Hasanov, 2010).

A study by Mukhopadhyay and Puopolo, found that the incidence of EOS among very low birth infants (VLBW) was 15-19 per 1000 live births, and had remained stable despite a change in the causative pathogens over the time. This was in contrast with the overall incidence which had dramatically declined with the advances in obstetrical and neonatal care.

This was more evident for GBS-specific EOS after using the guideline recommendations of intra-partum antibiotic prophylaxis (IAP) to prevent perinatal group B Streptococcus (GBS) disease. The incidence had decreased from 3-4 cases/ 1000 live births to 0.3-0.4 cases/ 1000 live births (Mukhopadhyay and Puopolo, 2012).

A surveillance done by Hyde (Hyde et al., 2002) involved 408 infants aged less than7 days who were identified as having early-onset sepsis during a 3- year study period (1998 to 2000). It was found that the rate of antibiotic-resistant E coli infections had increased among preterm infants during the study period. While other pathogens, including GBS were low and did not change significantly. However, an analysis of trends in GBS and E. coli related early onset sepsis from longitudinal prospective surveillance done in Australia and in New Zealand during a 10-year period from 1992

through 2001 by Daley and Isaacs showed that, the use of intra-partum antibiotics was associated with a steady decline in GBS early onset sepsis in Australasia, as well as E. coli early onset sepsis in all babies.(Daley et al., 2004)

In a study done in Asia, it was found that the rate of EOS from any pathogen was 0.72 infections/1000 live births and EOS constituted about 10.4% of all infections reported (Tiskumara et al., 2009) .

Fortunately, EOS is uncommon but it is an important cause of morbidity and mortality in infants, especially in those with VLBW (Tsai et al., 2012) where it was associated with a 3 fold increased risk of mortality (Mayor-Lynn et al., 2005). Nnenna (Nnenna, 2012) claimed that, despite diagnostic and therapeutic advances, EOS is associated with substantial morbidity and mortality rate of 15%-50%.

The risk factors for EOS in VLBW infants may differ from those found in term infants. It is well known that the risk of EOS in newborn is inversely related to gestational age and birth weight. In addition to prematurity and low birth weight, several maternal factors and intra-partum events, including intra-partum fever, prolonged rupture of membranes, chorioamnionitis, maternal GBS colonization, urinary tract infection were all identified as risk factors for neonatal sepsis (Chacko and Sohi, 2005; Tsai et al., 2012). Furthermore, multiparty, multiple courses of prenatal steroids and use of tocolytic agents have been found to be associated with EOS in a recent cross sectional study (Klinger et al., 2009).

The microorganisms most commonly associated with EOS include the following: *Group B Streptococcus* (*GBS*), *Escherichia coli* (*E.coli*), *Haemophilus influenza* and recently, a large study reported on the possible role of *Coagulase-negative Staphylococci* (*CoNS*) (Paolucci et al., 2012). *Group B streptococcus* (*GBS*) was the predominant in the past, but the incidence of EOS caused by *GBS* decreased after the development of guidelines for intra-partum antibiotic prophylaxis for *GBS* infection. This lead to an increased incidence of non-GBS pathogens particularly antibiotic resistant E.coli (Hyde et al., 2002; Kuhn et al., 2010)

However, in the United States (US) *GBS* remains an important infectious cause of morbidity and mortality among new-born infants, which results in about 1600 cases of neonatal sepsis and about 80 deaths annually (Schrag et al., 2002) The 2007 report updates on (*GBS*) disease in the USA by the Active Bacterial Core surveillance system revealed that, the overall rate of early-onset *GBS* infection was increased from 2003 to 2006, owing to an increasing incidence among black term infants (Apostol et al., 2009).

A study by Al-Taiar aimed to estimate the incidence of neonatal sepsis and determined the main causative organisms in few Asian neonatal units. It found that the profile of EOS in two (Malaysia and Macau) units seemed to be comparable to that reported recently from the USA. The incidence of *GBS* was low but remained the most frequent EOS pathogen. Though, the role of *E.coli* in these two units appears to be less evident (Al-Taiar et al., 2013).

1.2.3.1 (B) Late onset sepsis (LOS)

LOS is commonly defined as sepsis occurring after 48 to 72 hours of birth (Chacko and Sohi, 2005; Samanta et al., 2010), suggesting a horizontal transmission of the organism. LOS usually occurs as nosocomial (an infection that occurs more than 48 hours after admission of a baby who on admission did not have evidence of infection) or as community-acquired infections. Neonates with LOS mostly present with septicaemia, pneumonia or meningitis (Sankar et al., 2008).

Despite the recent advancement in the neonatal care with considerable improvement of survival of preterm infants in general, LOS remains an important cause of mortality and morbidity particularly in very low birth weight (VLBW) infants. The incidence of LOS and the number of sepsis episodes increases with decreasing birth weight and gestational age. Globally, LOS affects around 25% of VLBW infants. In one study among VLBW infants, 10.2% had one episode and 2.8% had two episodes while among those with birth weight> 2500g, 3.4% had one episode and only 0.7% had two episodes (Joseph, 2012).

LOS is a serious disease and continues to be a significant cause of morbidity and mortality with a mortality rate ranging from 17% to 27% depending on the studied population (Lutsar et al., 2011). LOS in premature infants is often secondary to prolonged hospital stay and is associated with significant mortality, neurodevelopmental impairment among survivors and with increased health care costs (Downey et al., 2010).

Like in EOS, Low gestational age and birth weight as a risk factors were highly associated with a significant and marked increase in the rate of nosocomial LOS. Other risk factors included central line insertion, mechanical ventilation, use and duration of total parenteral nutrition (TPN) / lipids and prolonged use of antibiotic usage (Samanta et al., 2010).

According to de Brito, central venous catheters are the most important risk factors for LOS in NICU patients, particularly in VLBW infants. They found that, moving to a temporary NICU with higher admission activity and poor hand washing facilities resulted in higher rates of hospital acquired infection (de Brito et al., 2007).
Factors that may increase community acquired LOS include poor hygiene, poor cord care, bottle feeding and parenteral fluids (Sankar et al., 2008).

The range of organisms causing LOS included Gram-positive, Gram-negative organisms and fungal infection with a predominance of bacterial causes (Joseph et al., 2012).
The predominant organisms in LOS are *coagulase negative staphylococci* (CoNS), accounting for 36-66% of cases, whereas the Gram-negative rods like *Klebsiella pneumoniae*, *Escherichia coli*, *Serratia* species and *Enterobacter cloacae* were responsible for about 26-36% of cases. In recent years the incidence of Gram negative sepsis appeared to be increasing in many NICUs worldwide (Lutsar et al., 2011).

1.2.4 Diagnosis of neonatal sepsis

The diagnosis of sepsis neonatorum has generally two phases. The first phase is suspected sepsis. Whenever the suspicion of sepsis is strong enough, the start of antibiotics is warranted because if the diagnosis of sepsis is missed or delayed, the consequence for the baby could be detrimental (Edmond and Zaidi, 2010)

There are several parameters that will increase the suspicion of sepsis in neonates and they can be categorised as risk factors, clinical signs or symptoms and laboratory parameters. Each of these will be discussed in some detail below.

The second phase of the diagnosis of sepsis is when the results of essential investigations such as CBC, CPR, as well as the result of blood culture are known. In several studies (Fleming et al., 2012; Griffin et al., 2007), based on these results the baby is diagnosed as;

- Confirmed sepsis in cases where the blood culture is positive with a pathogen
- Clinical sepsis in cases where the blood culture is negative but other clinical and laboratory evidence points strongly to the diagnosis of sepsis
- No sepsis if the blood culture is negative and subsequent clinical and laboratory evidence does not support the diagnosis of sepsis.

1.2.4.1 Suspected Sepsis

1.2.4.1(A) Risk factors (from history)

Risk factors play an important role in the diagnosis of suspected infection. The more risk factors a certain baby has, the lower the threshold for the diagnosis for suspected infection tends to be. Generally for term babies, rarely antibiotics are recommended to start if there are absolutely no physical signs of infection. However in the presence of mild signs of infection, the presence or absence of risk factors may be the determining factor to start or not to start antibiotics. One particularly strong risk factor for infection is prematurity and for preterm babies with multiple risk factors it may, even in the absence of clinical signs be justified to start antibiotics while awaiting the results of investigations into the presence of septicaemia.

A variety of risk factors are commonly used in several guidelines that are available to the medical practitioners (Sarkar et al., 2006; Swarnkar, 2012). These risk factors include the gestational age, birth weight, maternal *Group B Streptococcal* status, presence or fever or other signs of infection in the mother, duration between rupture of membranes and actual delivery and administration or non-administration of antibiotics during the delivery. There is online even one calculator (2014) available that based on the presence of the above mentioned risk factors calculates the risk of EOS in babies born at or after 34 weeks of gestation. The formulas were derived based on published work by Puopolo and Escobar (Puopolo et al., 2011; Escobar et al., 2014)

1.2.4.1 (B) Clinical signs (from physical examination)

The clinical signs that make doctors suspect infection in neonates are often non-specific. Many of the signs of sepsis can be caused by other common problems in neonates such as anaemia, electrolyte disturbances, persistent ductus arteriosus, intracranial complications, worsening respiratory distress due to any cause and a variety of other problems. Besides a lack of specificity of individual signs and symptoms, there tend to be also a lack of sensitivity. Most signs are only present in a fraction of the patients with sepsis. The presentation and the early signs of sepsis are quite variable among individual babies.

The above makes research into the diagnosis complex and more difficult. Since universal definitions of sepsis are generally lacking, many researchers in the past have used different definitions to define clinical sepsis. During clinical practice the decisions to continue antibiotics are more leaning towards experience based decisions than towards decisions made based on solid evidence. Most clinicians tend to look for combinations of risk factors, clinical signs and laboratory parameters to make their bed side decisions.

Clinical parameters that have been used for this purpose and which are incorporated in some guidelines on the use of antibiotics in the NICU are collected and listed in tables 2.1 and 2.2. Some of these parameters are general and do not point to any infection, table 2.1 while other parameters tend to point to certain localization which could be the port of entry or the major affected organ, table 2.2.

Table 2.1 Common non –specific clinical manifestations of neonatal sepsis

General manifestation ▪ Not looking well / Poor cry ▪ Lethargy / irritability ▪ Refusal to suck
Metabolic alteration ▪ Metabolic acidosis ▪ Hypoglycaemia ▪ Hyperglycaemia ▪ Neonatal jaundice
Respiratory problems ▪ Respiratory distress ▪ Apnoea
Temperature abnormality ▪ Hypothermia ▪ Hyperthermia
Heart rate abnormality ▪ Bradycardia ▪ Tachycardia
GIT manifestation ▪ Vomiting ▪ Increased per feed gastric aspirates ▪ Abdominal distension
Neurological manifestation ▪ Hypotonia ▪ Absent neonatal reflexes ▪ Convulsion
Skin manifestations ▪ Mottling ▪ Sclerema
Haematological &Coagulopathy ▪ Bleeding ▪ petechial, purpura ▪ Disseminated Intravascular Coagulation (DlC)
Cardiovascular: ▪ Hypotension, ▪ Poor perfusion ▪ Prolonged capillary refill time of >3 seconds ▪ Shock.

Table 2.2 Organ /system related clinical manifestations of neonatal sepsis

Central nervous system (CNS) ▪ Bulging anterior fontanilla ▪ Vacant stare ▪ High-pitched cry ▪ Excess irritability ▪ Stupor/ neck retraction
Pneumonia ▪ Chesty cough ▪ Respiratory distress ▪ Chest indrawing ▪ Grunting ▪ Pneumonic changes in CXR
Gastrointestinal (AGE / NEC) ▪ Vomiting/ diarrhoea/ dehydration ▪ Feed intolerance /Abdominal distension/Paralytic ileus
Skin infection ▪ Multiple pustules ▪ Abscess ▪ Umbilical stump swelling, redness, foul smelling and discharge.
Osteomyelitis ▪ Disability ▪ Local swelling or erythema ▪ Focal tenderness over a long bone

Several studies have been done worldwide aimed to determine the most common features associated with neonatal sepsis.

A study carried out in Bangladesh in 2006, to determine the clinical manifestation profile of neonatal sepsis, found that the most common clinical presentations included lethargy (73.4%), abdominal distension (70%), reluctance to feed (96.7%), jaundice (50%) and hypothermia (40%) (Waliullah et al., 2009).To some extent, this was in keeping with the results of a study by Kayange , where the inability to feed, lethargy, convulsion and cyanosis were found to be significantly associated with positive culture in both early and late onset sepsis. Tachypnea was found to be the only predictor of positive culture in early onset sepsis. Chest in-drawing, hypothermia, jaundice and umbilical redness were significant predictors in late onset sepsis (Kayange et al., 2010). Fleming has considered that respiratory distress is most common sign of neonatal infection (Fleming et al., 2012).

The results of a Longitudinal Analysis done in Korea by Shim et al indicate that, apnoea (19.7%) and fever (19.7%) were the most common symptoms of sepsis followed by bradycardia and hyperglycaemia. The study also found that very preterm infants born at less than 32 gestational weeks with sepsis tend to show non specific symptoms like apnoea, bradycardia and hyperglycaemia rather than typical signs such as fever which found to be the most common symptoms among those who were born beyond 32 gestational week (Shim et al., 2011).

In an effort to develop simple algorithms for early detection of serious neonatal illness including sepsis, by primary health care workers in developing countries, the Young Infants Clinical Signs Study Group recently conducted a large multi-country study to

identify clinical signs with high sensitivity and specificity for predicting the requirement for new-born referral using senior physician judgment as the gold standard. They declared that common clinical signs such as difficulties in feeding, convulsions, movement only when stimulated, tachypnoea, severe chest in-drawing, fever /hypothermia could be easily identified by health workers in primary care settings and indicated a need for referral (Ganatra and Zaidi, 2010).

1.2.4.1 (C) Laboratory parameters

Many laboratory parameters take some time to come back and will play a role only in the later decision to continue or discontinue antibiotics. There is however laboratory parameters that are readily available at the bedside or that can be obtained in general very fast from the laboratory. Such laboratory parameters may help in the decision on whether or not to start antibiotics when other factors that could be pointing to the presence of infection are present.

The blood sugar is commonly done as a bedside test and blood sugar abnormality (hypo/ hyperglycaemia) could be one of the factors playing a role in the decision to start antibiotics or not.

Hypoglycaemia is one of the metabolic signs that frequently accompany neonatal sepsis. The glucose requirement increased in neonates with septic state. Low level of cortisol secondary to inadequate response from the adrenal gland may be the cause of hypoglycaemia specially if associated with hypotension (Anderson-Berry et al., 2006).

Since long there are reported case series where hypoglycaemia was among the prominent presenting signs of neonatal sepsis (Yeung, 1970).

Hyperglycaemia has been reported as a presenting sign of bacterial or fungal sepsis, especially in extremely low birth weight and preterm babies (Shim et al., 2011; Fanaroff et al., 1998).

Another parameter that is readily available in the wards is the presence or absence of metabolic acidosis from the blood gas results (Cloherty et al., 2008). A report by Mathur showed that, metabolic acidosis was one of the significant independent predictors of mortality in neonatal sepsis (Mathur et al., 1996). Metabolic acidosis noted in many neonates with sepsis could explained by the poor tissue oxygenation which resulted in anaerobic metabolism with increased the production of lactic acid (Rubarth, 2008).

1.2.4.2 Definite diagnosis

When the results of the blood culture and other laboratory are available, usually within 48 hours, a more definite diagnosis is often possible. As stated above, the diagnosis of either culture confirmed sepsis, clinical sepsis is made. Below the value of individual laboratory parameters is discussed.

In view of the mortality and serious complications associated with neonatal sepsis, the most desirable characteristics for an ideal diagnostic test for neonatal sepsis, including early onset neonatal sepsis, is a very high sensitivity, rather than high specificity and a negative predictive value approaching 100% is wanted (Mishra et al., 2006; Chirico and

Loda,2011; Radulova, 2009). Such test is essential to differentiate infected and non infected infants and would allow safe withholding of antibiotics in babies at risk of or with a specific signs of sepsis (Zuppa et al., 2007).

Numerous tests have been evaluated looking for one that could be helpful in the diagnosis, one that rapidly confirms the diagnosis, and another which could rules it out. (Tappero and Johnson, 2010). Unfortunately there is no laboratory marker that has all of the characteristics of an ideal infection marker (Radulova, 2009).

Blood cultures remain the gold standard for diagnosis of neonatal sepsis. However, most district and community hospitals in developing countries do not possess facilities to perform blood cultures, and their reliability for the diagnosis of sepsis in neonatal period is still considered a hot topic for discussion.

It may be useful to use serial measurements of different markers of infection to screen for sepsis in the symptomatic and asymptomatic infants (Madan et al., 2003).

Likewise, the use of multiple markers, in particular, combining an early sensitive marker with a late specific test will promote the enhancement of the diagnostic accuracy of these mediators to identify infected babies. Serial measurement of such markers will certainly improve the diagnostic sensitivity of these tests (Radulova, 2009).

1.2.4.2.1 Blood culture

It is the golden standard for diagnosis of sepsis and should be taken in all cases of suspected sepsis prior to starting antibiotics. A positive blood culture with determination of antibiotic sensitivity of the isolated organism is the best guide to antimicrobial therapy (Sankar et al., 2008)

However, neonatal blood cultures present exceptional problems with regards to reliability (Døllner et al., 2001).Its sensitivity has long been considered controversial with early reports of sensitivity for identifying sepsis being only 50% to 80% (Tappero and Johnson, 2010).

Fastidious organisms, maternal intra-partum antibiotic treatment and limited specimen volumes were all blamed to decrease the sensitivity of blood cultures. Moreover, contamination by skin flora such as *CoNS* may be another problem (Venkatesh et al., 2010). Postnatal blood cultures may be sterile (false negative) owing to maternal administration of antimicrobial agents during labour as prophylaxis to GBS infections or to treat PROM or suspected intra-amniotic infection (Polin et al., 2012). Therefore, a negative culture could not exclude sepsis in the new-born (Tappero and Johnson, 2010).

Few and simple but important steps were recommended to optimize the reliability of blood culture in the new-born including: culturing early in the septic episode, applying of strict aseptic technique when collecting the specimen in order to minimize the possibility of contamination , collection of adequate volume of blood sample (0.5 - 1.0