NUCLEATED RED BLOOD CELL COUNT AS EARLY PROGNOSTIC MARKER FOR ADVERSE NEONATAL OUTCOME IN NEONATAL SEPSIS

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CERTIFICATE

This is to certify that the dissertation entitled **"NUCLEATED RED BLOOD CELL COUNT AS EARLY PROGNOSTIC MARKER FOR ADVERSE NEONATAL OUTCOME IN NEONATAL SEPSIS"** submitted by **Dr. AARUSHI MANU** to the Faculty of Paediatrics, The Tamil Nadu Dr. M.G.R. Medical University, Chennai in partial fulfillment of the requirement for the award of M.D. Degree Branch VII –PAEDIATRIC MEDICINE is a bonafide research work carried out by her under our direct supervision and guidance.

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I, **Dr AARUSHI MANU** solemnly declare that the dissertation titled **"NUCLEATED RED BLOOD CELL COUNT AS EARLY PROGNOSTIC MARKER FOR ADVERSE NEONATAL OUTCOME IN NEONATAL SEPSIS"** has been conducted by me at the Institute of Child Health and Research Centre, under the guidance and supervision of Director and my unit chief **PROF. DR. M. KULANDAIVEL. MD, DCH.** This is submitted in part of fulfillment of the award of the degree of M.D., (Paediatrics) for the April 2018 examination to be held under the Tamilnadu Dr. M. G. R. Medical University, Chennai. This has not been submitted previously by me for the award of any Degree or Diploma from any other university.

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ABSTRACT

Introduction: The most common cause of neonatal mortality in the developing countries is neonatal sepsis, the diagnosis of which depends on blood culture, which has low sensitivity and takes time. We hypothesize that demonstration of elevated NRBC levels in neonatal sepsis might help in predicting an adverse neonatal outcome and hence we can improve the care by prioritizing them.

Aim of the study : To measure the nucleated RBC levels in blood samples of neonates with sepsis and to analyse whether it can serve as a prognostic marker for neonatal sepsis and an increased risk for adverse neonatal outcome.

Materials and methods: This is a hospital based prospective study done in neonates who are admitted in NICU of Madurai Medical College with risk factors or clinical features of sepsis. After getting informed consent, the maternal details and examination findings were recorded and blood sample taken for sepsis screen, blood culture and peripheral smear for NRBC.

Results: The sensitivity of NRBC in identifying sepsis was 81.5%, its specificity was 61.76%, positive predictive value was 70.4% and negative predictive value was 75%. In the neonates who expired, serial NRBC counts (mean – 22.4) were significantly increased from baseline value (mean 17.3).

Conclusion: NRBC is significantly elevated in the neonatal sepsis and is a predictor of adverse neonatal outcome

KEYWORDS: neonatal sepsis, NRBC, outcome

INTRODUCTION

The most common cause of neonatal mortality in the developing countries is neonatal sepsis. [1] Most of these deaths occur at home without coming to medical attention. The Millennium Development Goal for child survival cannot be achieved without substantial reductions in mortality due to neonatal sepsis. Neonatal deaths continue to a global health concern and account for 40% of all death in children under 5years.[2] A lot of effort needs to be put to bring down the neonatal mortality from levels as high as 40 to 60 per 1000 live births and to achieve Millenium Development Goal for child survival (Goal 4- to reduce child mortality by two-thirds between 1990 and 2015).

Majority of the estimated 4 million neonatal deaths occur in low and middle income countries. Three conditions namely, infections, perinatal asphyxia and preterm delivery account for majority of neonatal deaths.[3] The estimates suggests that infections including sepsis, pneumonia, diarrhea, meningitis and tetanus are the most common causes of neonatal death in developing countries.[4,5] Numerous factors contribute to such high incidence like lack of antenatal care, lack of safe delivery practices and cord care, unsupervised or

poorly supervised home deliveries, low birth weight, prematurity, lack of exclusive breast-feeding, and delays in recognition of danger signs of neonatal sepsis by the mother or caretaker.

Even in developed countries, despite major advances in neonatal care and increasing research, four out of every ten infants with sepsis die or experience major disability including significant neurodevelopmental impairment.[6] Premature neonates experience the highest incidence and mortality of sepsis among all age groups. Compared to term neonates, sepsis in preterm neonates is upto 1000-fold more common and is associated with higher mortality rates and lifelong neurodevelopmental disability[7].

Factors that delay diagnosis and initiation of therapy include lack of specific clinical features, as the infant often presents with subtle and nonspecific clinical signs and symptoms. Typical diagnostic parameters depend on conventional laboratory tests that are routinely serum based, such as white blood count (WBC), absolute neutrophil count (ANC), immature/total neutrophil (I/T) ratio, and C-reactive protein (CRP).[9] However, these conventional sepsis evaluation

parameters have low sensitivity and are nonspecific, often demonstrating increased level response to various other neonatal conditions such as meconium aspiration, prolonged rupture of membranes, asphyxia, and the birth process.

The definitive diagnosis of sepsis rests upon isolation of pathogenic bacteria in blood cultures, which has low sensitivity and takes time to influence initiation of antibiotic therapy.[28] Further diagnostic limitations of the blood culture method include a higher incidence of false negative results, due to low blood volume drawn for culture and antenatal antibiotic use that may influence subsequent bacterial growth. As a result, early antibiotic therapy is frequently initiated for presumed infection or delayed due to uncertainty increasing disease risk.

Thus, early, accurate, and rapid diagnosis of neonatal sepsis remains a major diagnostic challenge in neonatology, revealing the need for reliable and timely diagnostic biomarkers to enable clinicians to efficiently diagnose sepsis risk during the early phases of sepsis, provide effective antibiotic management tailored to causative organisms, and provide a useful guide for therapy during recovery.

Neonates with sepsis are showing excess nucleated RBC in peripheral blood and is correlating with adverse out come. We hypothesize that demonstration of elevated NRBC levels in neonatal sepsis might help in predicting an adverse neonatal outcome and hence we can improve the care by prioritizing them. If we come to know that the nucleated RBCs are increased at an early stage, we can resort to higher antibiotics or other intensive management to prevent poor outcome. There are limited studies evaluating the role of nucleated RBCs in neonatal sepsis and hence this study has been undertaken.

REVIEW OF LITERATURE

Epidemiology: Indian data

According to the National Neonatal Perinatal Database (NNPD, 2002-03), the incidence of neonatal sepsis in India is 30 per 1000 live births. The NNPD network comprising of 18 tertiary care neonatal units across India found that sepsis is one of the commonest causes of neonatal mortality contributing to 19% of all neonatal deaths[8].

Definition of neonatal sepsis

Neonatal sepsis consists of infection with or without bacteremia occurring in the first month of life. It includes various systemic infections like septicemia,

pneumonia, arthritis, osteomyelitis, meningitis, and urinary tract infections. Superficial infections including conjunctivitis, oral thrush etc are not classified under neonatal sepsis[9].

Classification of neonatal sepsis

Depending on the day of onset of symptoms, neonatal sepsis can be classified into two broad categories [9,10].

1. Early onset sepsis (EOS)
2. Late onset sepsis (LOS)

Early onset sepsis: It usually presents within the first 72 hours of life. The presentation can be asymptomatic bacteremia, generalised sepsis, pneumonia or meningitis . The most common symptom will be respiratory distress which can range from mild tachypnea and grunting to respiratory failure. The source of infection is usually the maternal genital tract which the baby acquires during vaginal delivery.

The following risk factors are associated with early onset sepsis.

1. Low birth weight (<2500 grams) or prematurity (<37 wks)

2. Febrile illness in mother (>38^{o}C) with evidence of bacterial infection within 2 weeks of delivery or signs of chorioamnionitis

3. Foul smelling and/or meconium stained liquor

4. Rupture of membranes > 24 hours

5. Single unclean or > 3 sterile vaginal examination(s) during labour

6. Prolonged labour (sum of 1st and 2nd stage of labour > 24 hrs)

7. Perinatal asphyxia (Apgar score < 4 at 1 minute)

Presence of foul smelling liquor or any of the three above mentioned risk factors should be started on antibiotics. Infants with two risk factors without clinical evidence of sepsis should be investigated and then treated accordingly.

Late onset sepsis (LOS)*:* It presents after 72 hours of age. Almost half of cases of LOS is caused by CONS, followed by other gram positive organisms (S. aureus, enterococcus) (22%), gram negative organism (18%) and fungi (12%).The source of infection in LOS can be nosocomial (hospital-acquired) or community acquired. Factors that predispose to an increased risk of nosocomial sepsis include low birth weight, admission in intensive care unit, prematurity, invasive procedures, administration of parenteral fluids, mechanical ventilation

and use of stock solutions. A lumbar puncture should be done in the evaluation of LOS as meningitis is common in this age group.

The risk factors for community acquired LOS include poor hygiene, bottle-feeding, poor cord care and prelacteal feeds. Breast feeding is protective against late onset sepsis.

Clinical features

Non-specific features:

A high index of suspicion is needed for early diagnosis of neonatal sepsis. Neonates with sepsis may present with one or more of the following symptoms and signs

- Temperature instability (former is more common in preterm and low birth weight infants)
- Lethargy, poor cry, refusal to suck
- Poor perfusion, prolonged capillary refill time and septic shock
- Hypotonia, absent neonatal reflexes, flushed AF
- Brady/ tachycardia
- Respiratory distress, grunting and respiratory failure

- Hypo/ hyperglycemia
- Metabolic acidosis

Investigations

Since treatment should be initiated without any delay in a neonate suspected to have sepsis, only minimal and rapid investigations should be undertaken initially[11].

Blood culture: It is the gold standard investigation in the diagnosis of neonatal septicemia. A blood culture should be done in all cases of suspected sepsis before starting antibiotics. Blood should be collected for culture by following strict aseptic precautions and proper procedure.

The resident doctor/ staff must wear sterile gloves before the procedure. A patch of skin approximately 5 cm in diameter is prepared over the proposed veni- puncture site. This area must be cleansed thoroughly with 70% isopropyl alcohol, followed by povidone-iodine, and then again by isopropyl alcohol. Povidone-iodine must be applied in concentric circles moving inward out. The skin must be allowed to dry for at least one minute before the sample collection.

One-mL sample of blood will be adequate for a blood culture bottle containing 5-10 mL of culture media.

Cultures should be collected from a fresh veni-puncture site only to prevent contamination. All blood cultures should be observed for at least 72 hours before they are reported as sterile. Now bacterial growth can be detected within 12-24 hours by using improved bacteriological techniques such as BACTEC and BACT/ALERT blood culture systems. These techniques can detect bacteria at a concentration of 1-2 colony-forming unit (cfu) per mL.

Septic screen: A sepsis screen is done for all suspected sepsis cases while awaiting the culture reports. If there is a strong clinical suspicion of sepsis, antibiotics can be started irrespective of the sepsis screen reports and can be modified later. The various components of the septic screen are total leukocyte count (TLC), immature to total (IT) neutrophil ratio, absolute neutrophil count (ANC), micro-erythrocyte sedimentation rate (micro-ESR) and C reactive protein (CRP). The cut-off values are given in the below table.

Components of sepsis screen

Components	Abnormal value
Total leukocyte count	<5000/mm3
Absolute neutrophil count	Low counts as per Manroe chart[12] for term and Mouzinho's chart[13] for VLBW infants
Immature/total neutrophil	>0.2
Micro-ESR	>15 mm in 1st hour
C reactive protein (CRP)	>1 mg/dl

The age specific ANC values in the immediate neonatal period and the normal reference ranges for term babies are available from Manroe's charts.[12] Immediately after birth, the lower limit for normal ANC begins at 1800/mm^3, then increases to 7200/mm^3 at 12 hours of age and declines and persists at 1800/mm^3 after 72 hours of age. For very low birth weight infants, the normal reference ranges are available from Mouzinho's charts.[13]

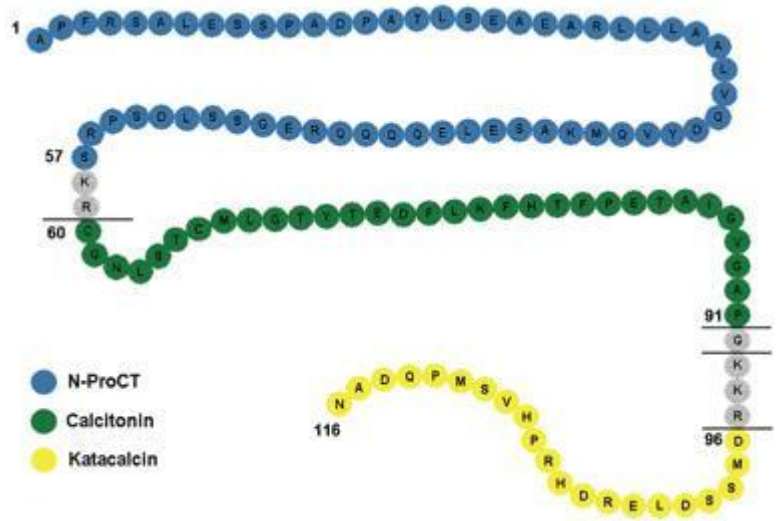
The normal I/T ratio at birth is 0.16 which then declines to a peak value of 0.12 after 72 hours of age. If two parameters are abnormal in a screen, then the sensitivity of the test in detecting sepsis is 93-100%, specificity of 83%, positive

and negative predictive values of 27% and 100% respectively. Hence, if two (or more) parameters are abnormal, then the sepsis screen is considered positive and the neonate is started on antibiotics. But if the sepsis screen is negative and clinical suspicion persists, it should be repeated within 12 hours. If the screen is still negative, sepsis can be excluded with reasonable certainty.

C-Reactive Protein : CRP is the most valuable and extensively used lab parameter in NICUs. It is an acute phase reactant produced in the liver in response to inflammation and infection. CRP production is stimulated by cytokines released during the inflammatory reaction such as IL-6, IL-1 & TNF-alpha. It is also produced in response to trauma, injury and surgery. CRP production changes with gestational age. Due to the immaturity of liver, the ability to produce CRP is decreased in preterm infants. Sensitivity and specificity of CRP in various studies are variable and in the ranges of 42-90% & 70-76% respectively. Serial CRP values are better in detecting severity of sepsis, to know the prognosis, and response to treatment compared to a single value. The main disadvantage of CRP is its lower sensitivity in early sepsis [15].

Procalcitonin: Procalcitonin is also a promising marker in diagnosing neonatal sepsis. Procalcitonin is the precursor of calcitonin and it contains 116 amino acids. It is produced by parafollicular C cells of thyroid gland[16]. Cytokines like IL-6 and tumour necrosis factor alpha induce production of procalcitonin from the monocytes and macrophages of various organs[17].

Bacterial lipopolysaccharide also induces the release of calcitonin into circulation. The optimum cut off value of procalcitonin is 1.1 ng/ml and it starts rising within 3-4 hour after onset of sepsis, attains peak value at 6 hours and remains elevated for 24 hours[19].



In a study done in Najafabad, during a period of May 2005-April 2006, it was shown that sensitivity of procalcitonin is 68-70% higher than CRP [45%] for diagnosis of neonatal sepsis[20].

PCT also correlates with the extent and severity of bacterial infections. It is considered to be more specific in diagnosing bacterial infections. PCT also correlates with the bacterial load. PCT also helps to differentiate bacterial from viral infections, because in viral infections, the increase in PCT is blocked by INF -Gamma released [18].

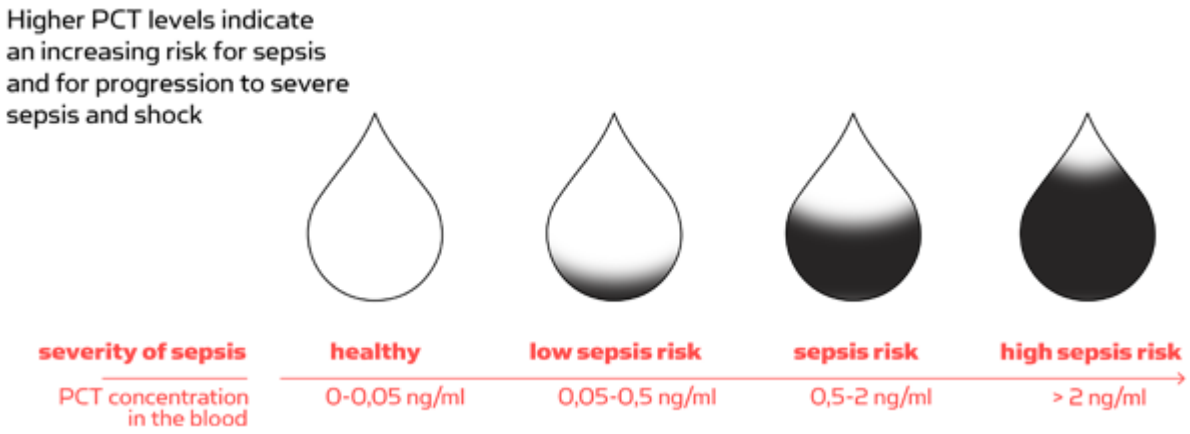
Stocker et al did a study in 2010, found that level of PCT concentrations in healthy newborn babies rise slowly after birth, reaching its peak levels at around 24 hours of life and then PCT levels decrease to less than 0.5ng/ml at around 48-72 hours of birth[21].

In another study, procalcitonin sensitivity in the early diagnosis of neonatal sepsis was found to be 82-100% & the specificity was shown to be 70-100%.

A study conducted by Chiesa et al found that after 1st 48 hours of life, serum PCT levels proved to be an ideal biomarker for neonatal sepsis. Similar study conducted by Altunhan et al in Turkey demonstrated that in Early onset sepsis, PCT levels were found to be normal initially at birth but then the serial

measurement taken at 24 hours of age was found to be more useful in diagnosing EOS[22].

So PCT is considered to be a more reliable and sensitive biomarker for infection than CRP during 1st 24 hours of birth.



Procalcitonin also rises in other non infective condition like

- Perinatal asphyxia
- Intracranial hemorrhage
- Pneumothorax
- After resuscitation[23]

THROMBOCYTOPENIA: Thrombocytopenia is one of the commonest laboratory findings seen in sick newborn admitted in NICUs. Thrombocytopenia is one of the earliest but non specific indicators of neonatal sepsis with or without DIC. Thrombocytopenia can be caused by bacterial, fungal & viral infections and also in other non-infectious conditions. Overall prevalence of thrombocytopenia in newborn varies from 2 - 5%, but the prevalence in neonates admitted to NICU care varied from 20%to 36%.

The major complication of thrombocytopenia is bleeding but is mostly seen in newborn with platelet count <30,000/mm^3. Studies also indicates that approximately 48-50% babies of Culture proven sepsis seem to get thrombocytopenia[24].

Mechanism of thrombocytopenia in neonatal sepsis:

Thrombocytopenia in sepsis with DIC is due to consumption of circulating platelets and coagulation factors. On the other hand, thrombocytopenia seen in

sepsis without DIC is debatable. The toll like receptors present over the platelets functions in recognising bacteria, viruses and fungi in sepsis. Bacterial products also produce endothelial injury which induces platelet adhesion & aggregation producing clearance of platelet from circulation. Immune complexes also play a pivotal role in producing thrombocytopenia in sepsis due to the presence of circulating immune complex[25].

Even though the onset of thrombocytopenia was found to be delayed in fungal sepsis, lower levels of platelet count and longer duration of thrombocytopenia are frequently observed in fungal sepsis[26]. Severe thrombocytopenia is seen more commonly in gram negative sepsis compared to gram positive sepsis.[27]

Lumbar puncture (LP): The incidence of meningitis in neonatal sepsis is from 0.3-3% in various studies .[8] In the setting of septicema, it is possible to have meningitis *without* any symptoms .so LP should be done in neonates with suspected sepsis. In EOS, lumbar puncture is done if culture is positive or if the clinical picture is consistent with septicemia . In LOS, LP should be done in all infants before starting antibiotics. In critically ill children LP can be done after stabilisation. The cerebrospinal fluid characteristics are different in the

newborn period compared to adults and normal values are given in the table below.

Normal cerebrospinal fluid examination in neonates[14]

CSF Components	**Normal range**
Cells/mm3	8 (0-30 cells)
PMN (%)	60%
CSF protein (mg/dL)	90 (20-170)
CSF glucose (mg/dL)	52 (34-119)
CSF/ blood glucose (%)	51 (44-248)

Radiology: Chest x-ray should be considered in the presence of respiratory distress or apnea. An abdominal x-ray is indicated in the presence of abdominal signs suggestive of necrotizing enterocolitis (NEC). Neurosonogram and computed tomography (CT scan) should be performed in neonates diagnosed to have meningitis.

Urine culture: Routine use of urine culture is not required. It is done in neonates with urogenital malformation or vesicoureteral reflex, at risk for fungal sepsis or suspected of UTI (crying during micturition). Urine cultures can be obtained by suprapubic puncture, bladder catheterization or clean catch sample from midstream of urine.

UTI is diagnosed by the following criteria:

(a) >10 WBC/mm^3 in a 10 mL of centrifuged sample

(b) $>10^4$ organisms/mL in urine obtained by catheterization or

(c) any organism in urine obtained by suprapubic aspiration.

Management

***Supportive*:** Adequate and proper supportive care is crucial in a sick neonate with sepsis. The baby should be nursed in a thermo-neutral environment thus avoiding hypo/hyperthermia. Oxygen saturation and blood glucose should be maintained in the normal range for the age group; other supportive measures include the use of mechanical ventilation, exogenous surfactant administration for pneumonia and respiratory distress syndrome, volume and pressor support

for shock, sodium bicarbonate for acidosis and anticonvulsants for seizures. Packed red cells and Fresh Frozen Plasma can be used in the event of anemia or bleeding diathesis.

Antimicrobial therapy: The choice of antibiotics depends on the prevailing flora in the given unit and their antimicrobial sensitivity in the unit. The decision to start antibiotics is based upon clinical features and/ or a positive septic screen. However, the duration of antibiotic therapy is dependent on the presence of a positive blood culture and meningitis.

Duration of antibiotic therapy in neonatal sepsis

Diagnosis	**Duration**
Meningitis	21 days
Culture positive sepsis without meningitis	14 days
Culture ***negative*** *sepsis* ***(sepsis screen positive*** *and clinical course* ***consistent with sepsis)***	5-7 days

Indications for starting antibiotics: The indications for starting antibiotics in neonates at risk of EOS include any one of the following:

(a) presence of >3 risk factors for sepsis

(b) presence of foul smelling liquor

(c) presence of > or = 2 risk factors *and* a positive septic screen or

(d) strong clinical suspicion of sepsis.

The indications for starting antibiotics in LOS include:

(a) positive septic screen and/or

(b) strong clinical suspicion of sepsis.

Choice of antibiotics: Empirical antibiotic therapy should be unit-specific and is determined by the prevalent spectrum of etiological agents and their antibiotic sensitivity pattern in the unit. Antibiotics once started need to be modified according to the sensitivity reports.

Empirical choice of antibiotics for treatment of neonatal sepsis

Clinical situation	**Septicemia & Pneumonia**	**Meningitis**
FIRST LINE Community-acquired (Resistant strains unlikely)	Penicillin or Ampicillin and Gentamicin	Add Cefotaxime
SECOND LINE Hospital-acquired Some strains are likely to be resistant	Ampicillin or Cloxacillin Gentamicin or Amikacin	Add Cefotaxime
THIRD LINE Hospital-acquired sepsis (Most strains are likely to be resistant)	Cefotaxime or Piperacillin-Tazobactam or Ciprofloxacin and Amikacin	Same (Avoid Cipro)

Add Vancomycin if MRSA is suspected.

The empirical choice of antibiotics is based on the probable source of infection. For infections that are community-acquired where resistant strains are not likely, a combination of ampicillin or penicillin with gentamicin may be a good choice as a first line therapy.

For hospital acquired infections, since resistant organisms are more likely, a combination of ampicillin or cloxacillin with gentamicin or amikacin may be started. If multiple resistant strains of klebsiella and other gram-negative bacilli are seen, then a combination of a third generation cephalosporin (cefotaxime or ceftazidime) with amikacin may be started. 3rd generation cephalosporins have very good CSF penetration and are thought to have excellent antimicrobial activity against gram negative organisms even though the recent studies show 60-70%of gram negative organisms are resistant to them[67-69]. Hence they were considered to be a good choice for the treatment of nosocomial infections and meningitis. The incidence of infections with ESBL (extended spectrum beta-lactamase) positive organisms are also increased due to routine use of these antibiotics. Therefore it is preferable to use antibiotics such as piperacillin-tazobactam or methicillin/ vancomycin in units with high incidence of resistant strains.

In case of pseudomonas sepsis, a combination of piperacillin- tazobactam with amikacin is a good choice. Penicillin resistant staphylococcus aureus should be treated with cloxacillin, nafcillin or methicillin. An aminoglycoside can be added in therapy against staphylococcus. Methicillin resistant staphylococcus aureus (MRSA) should be treated with a combination of ciprofloxacin or

vancomycin with amikacin. Ciprofloxacin is a good choice for gram negative sepsis but it doesn't cross blood-brain barrier. It may be used for the treatment of resistant gram-negative bacteremia after excluding meningitis.

For sepsis due to enterococcus, a combination of ampicillin and gentamicin is a good choice as initial therapy. Vancomycin should be used for treatment of enterococcus that are resistant to the first line of therapy.

Organism	**Antibiotic**
GBS	Ampicillin or Pencillin G
E-coli	Cefotaxime or ampicillin and gentamicin
CONS	Vancomycin
Klebsiella, Serratia	Cefotaxime or Meropenam and Gentamicin
Enterococcus	Ampicillin or Vancomycin or Gentamicin
Listeria	Ampicillin and gentamicin
Pseudomonas	Ceftazidime or Piptaz and Gentamicin or Tobramycin
Staphylococcus aureus	Nafcillin
MRSA	Vancomycin

Newer antibiotics like aztreonam, meropenem and imipenem should be kept as reserve antibiotics. Aztreonam has excellent activity against gram-negative organisms while meropenem is effective against most bacterial pathogens except methicillin resistant staphylococcus aureus (MRSA) and enterococcus. Imipenem is generally avoided in neonates because of increased incidence of seizures following its use. Empirical use of these antibiotics should be avoided.

Immunotherapies

1. Double volume exchange transfusion and granulocyte infusion- Currently it is not recommended for treatment of early or late onset sepsis because of the risk associated with transfusion. Only small trials are done in neutropenic septic neonates.
2. IVIG – in a definitive study including 3493 infants showed that there is no change in the primary outcome- death or major disability at 2yrs. It is not recommended in the treatment of neonatal sepsis.
3. Cytokines – Several studies suggest that G-CSF may result in lower mortality among neutropenic septic VLBW infants but currently there is insufficient data for its use.

4. Activated protein C and Pentoxifylline – they have been studied in in adults with severe sepsis. Neither of them is recommended in neonates without further study.

Newer Biomarkers of sepsis

Characteristics of an ideal biomarker are summarized in the below table.

Discriminate etiology of sepsis	Able to identify causes of sepsis such as viral, bacterial, or fungal
High sensitivity	The ability to detect sepsis in infants where sepsis is present (approaching 100%)
High specificity	The ability to rule out sepsis in infants where sepsis is absent (>85%)
High predictive value	Likelihood that the test accurately predicts presence or absence of sepsis (approaching 100%)
Rapid timely results	Necessary in early sepsis diagnosis generally in <60 minutes
Reliable and precise	Informs in early diagnosis, guides treatment decisions or prognosis
Readily available standardized method	Technology can be available from commonly obtainable small volume sample and expanded routinely among care institutions

The long-established and widely used diagnostic practice for neonatal sepsis evaluation is the white cell analysis, including the total WBC, ANC, and I/T ratio. The presence of thrombocytopenia can provide an alert for illness severity. The addition of biomarkers, like CRP, further enhance predictability of sepsis and assist in therapeutic management for the neonate.[26] But, these hematologic tests do not provide any absolute specific or sensitive diagnostic accuracy to assist in the decision to initiate treatment, particularly in the early phase of early-onset sepsis. As a result, newer biomarkers that target components of the complex early inflammatory response cascade help to target early immune host response in the early stages of sepsis.

Developing biomarkers

Acute phase proteins and other proteins

Serum amyloid A

Serum amyloid A (SAA) is an apo lipoprotein that is produced in the liver and an early acute phase reactant that is been studied in neonates. SAA is derived

from a variety of tissues such as endothelial cells, monocytes, and smooth muscle cells and is regulated by cytokines IL1 and IL6 as well as TNF-α.[29]

Serum levels of SAA have a wide range, which increase with age from birth to adolescence; SAA is released as a response to infection and injury.[30,31] SAA levels may be affected by hepatic function and host nutritional status, which may limit usefulness in late neonatal sepsis where hepatic dysfunction and nutritional status may be decreased.[32]

In a study of 104 full-term infants <72 hours of age, levels of SAA at 0, 24, and 48 hours rose earlier than CRP levels with better diagnostic accuracy in predicting early-onset sepsis, including a sensitivity of 96% versus 30%, similar specificity and greater positive predictive value (85% versus 78%), making SAA a better marker of infection.[33]

Lipopolysaccharide-binding protein

Lipopolysaccharide-binding protein (LPB) is mainly produced by liver but also by epithelial and muscle cells. It is a soluble pattern-recognition moleculethat is required for the interaction with endotoxin of gram-negative bacteria. Levels of LPB peak early, within 6 – 8 hours, after an acute infection. As a result, LPB

has higher sensitivity and negative predictive value compared to other reactants such as CRP and PCT and can be used as a diagnostic test in early-onset sepsis.

Cytokines and chemokines

Chemokines are cytokines that have the ability to direct WBC migration. Among the group of the pro-inflammatory cytokines, IL-6 had been widely investigated for its potential use as a biomarker of EOS.[34] During the acute phases of an infection, the B and T lymphocytes are stimulated to produce IL-6, which in turn induces the hepatic production of acute phase reactants like CRP.[35] As an early phase biomarker, IL-6 has good sensitivity (90%) compared to CRP, with a negative predictive value of 91%.[44] The main limitation of using IL-6 as an early biomarker of neonatal sepsis is its very short half-life.

IL-8 is also a frequently studied pro-inflammatory cytokine in neonatal sepsis, with good sensitivity of 90% and specificity in the range of 75%–100%. IL-8 regulates leukocyte migration and activation and is been extensively studied in neonatal infection.[36-39]

Anti-inflammatory cytokines

The inflammatory process is also regulated by anti-inflammatory mediators such as IL-10 and TGF-β. These cytokines prevent an exaggerated pro-inflammatory response in reaction to pathogen invasion.[40] But in the premature infant, the ability to mount an aggressive anti-inflammatory response is limited. Thus, in preterm neonates, serum values of anti-inflammatory cytokines have been studied to know their ability to prognosticate improvement and survival.[41,42] In infants, an elevated anti-inflammatory (IL-10) to pro-inflammatory (TNFα) cytokine ratio is associated with severe late onset sepsis and indicates poor prognosis.[41]

Other chemokines that are being studied as markers of early neonatal infection are IP-10, monokine induced by interferon-gamma, regulated on activation, normal T cell expressed and secreted (RANTES), and MCP-1.[43] Their use in combination with other inflammatory markers demonstrate better diagnostic utility in determining infection risk.

Cell surface antigens

There are several cell surface antigens such as CD11b, CD 14, CD64, CD32, CD16, CD69, and sCD163 that have been identified as promising markers in the detection of congenital sepsis, as well as early and late onset neonatal sepsis.[43,44] Of this group, the most important is the neutrophil CD64.[43,45] CD64 is a high affinity Fc receptor for immunoglobulin G that increases its expression in response to infection.[46] CD64 along with IL-6 or CRP had demonstrated an excellent sensitivity and a negative predictive value close to 100% in diagnosing early-onset sepsis.[47]

Other recent biomarkers that are being explored include pentraxin 3 (PTX3), angiopoietins, suPAR, and soluble triggering receptor expressed on myeloid cells-1 (sTREM-1). Emerging molecular techniques using multiplex platforms that can measure multiple markers at a time such as protein, deoxyribonucleic acid (DNA), and ribonucleic acid (RNA) using various technologies such as fluorescence in situ hybridization (FISH), quantitative polymerase chain reaction (qPCR), 16 S rRNA, and miRNA detection can revolutionize the diagnosis of neonatal sepsis.[4]

NUCLEATED RBCS

Studies have shown that NRBC is an important marker of neonatal sepsis. Nucleated red blood cells are also called erythroblasts, normoblasts, or normocytes.

Units of reporting

Clinically it is best to express NRBCs as an absolute number of cells per unit volume, either "NRBCs/mm^3" or "NRBCs/l". However, Most laboratories report NRBCs relative to 100 white blood cells (WBCs).Because of the variability of leukocyte number after birth, there is a wide range of values for NRBCs when they are expressed relative to the WBC count. Processes that increase the leucocyte count will result in a low values of NRBCs when reported relative to WBCs, and processes that decrease the leucocyte count will produce high NRBC counts if reported relative to WBCs.

Normal newborn values

In 1924, Lippman[49] reported NRBCs in the blood of 41 of 42 newborns in the first day of life. These cells constituted about 500 NRBCs/mm^3 or 0.1% of the newborns' circulating red blood cells. Since then, many investigators have reported similar values at and shortly after birth. It is reasonable to conclude that the mean value of NRBCs in the first few hours of life in a healthy term neonate is about 500 NRBCs/mm^3, and that a value above 1000 NRBCs/mm^3 can be considered elevated. Or 0–10 NRBCs/100 WBCs are typical, and values above 10–20 NRBCs/WBC are considered elevated, although these values are highly dependent on the total leucocyte count.

The absolute NRBC count is calculated by using the following formulas.

$$\text{Corrected WBC count}(/mm^3) = \text{Total count} \times \left(\frac{100}{\text{NRBC} + 100}\right)$$

Absolute NRBC count= corrected WBC count x NRBC/100

Normal nucleated red blood cell (NRBC) count

Reference (first author)	*Sample size*	NRBCs	Age	*Gestation/birth weight*
Naeye[50]	84	919(1425) NRBCs/mm^3 560 (771) NRBCs/mm^3	1 hr 6 hr	Term/AGA
Green[51]	102	400(1300) NRBCs/mm^3	12-24 hrs	37-41 wks
Sinha[52]	84	2.3 NRBCs/100 WBCs	Birth (cord blood)	2501 and 3500 g
Shivhare[53]	33	4.1 NRBCs/100 WBCs	Birth (cord blood)	Term and near-term
Phelan[54]	83	3.4 NRBCs/100 WBCs	Birth (cord blood)	>37 weeks, >2700 g
Hanlon-Lundberg[55]	1112	8.5 (10.3) NRBCs/100 WBCs	Birth (cord blood)	37–41 weeks

Green[56]	26 37 86	2900 (3600) NRBCs/mm3 1200 (1800) NRBCs/mm3 1000 (900) NRBCs/mm3	Day 1 Day 1 Day 1	23–26 weeks 27–29 weeks 30-32 weeks
Buonocore[57]	47 185 105	8521 (1620) NRBCs/mm3 4548 (473) NRBCs/mm3 1689 (290) NRBCs/mm3	Birth (cord blood) Birth (cord blood) Birth (cord blood)	24–27 weeks 28–36 weeks 37–41 weeks
Axt[58]	304	3.7 (median) NRBCs/100 WBCs 6.5(median) NRBCs/100 WBCs	Birth (cord blood) Birth (cord blood)	261–289 days 290+ days

Results are mean (1 SD).

Differential diagnosis of increased nucleated red bloods cells in the fetus and newborn[59]

I. Physiological

- Labour and vaginal births
- Preterm newborns
- Post-term newborns

II. Increased erythropoiesis

- Chronic hypoxia
- Growth restriction
- Maternal pre-eclampsia
- Maternal smoking
- Anaemia
- Blood loss
- Haemolysis—ABO or Rh isoimmunization, other
- Maternal diabetes

Others

- Leukaemia

- Down’s syndrome
- TORCH infections

III. Acute stress release

- Acute hypoxia
- Subacute hypoxia
- **Chorioamnionitis and neonatal sepsis**

IV. Postnatal hypoxia

- Cyanotic heart disease
- Pulmonary failure

V. Idiopathic

Acute chorioamnionitis has been associated with increased levels of erythropoietin and increased NRBCs. Maier *et al*[60] found significantly elevated erythropoietin levels in neonates whose placentas showed signs of chorioamnionitis. Increased NRBCs have been reported in preterm infants born after pregnancies complicated by chorioamnionitis without cord acidosis or hypoxaemia.[61]

Leikin *et al*[62] found an increase in NRBCs when histological chorioamnionitis was present without signs of clinical chorioamnionitis. Salafia *et al*[61] postulated that the increase in NRBCs could be a fetal response to an inflamed environment and not due to fetal hypoxia.

In a study done by Antonette T. Dulay et al to determine if fetal inflammation is associated with an elevation of neonatal NRBC count in the setting of inflammation associated preterm birth, it has been found that neonates with early onset sepsis had higher absolute NRBC count($p=0.011$). NRBC counts were directly correlated with cord blood IL-6 levels ($p<0.001$) but not with erythropoietin, cortisol or parameters of acid-base status levels.[63]

In another article on the presence of nucleated red cells in the blood of critical care adult patients and its association with an increased mortality risk by Duțu Mădălina et al, it has been found that the daily screening of the presence of NRBCs seems to be a useful tool to estimate the mortality risk in adult population. Even though the incidence in critically ill patients was 20%, the mortality of NRBC-positive patients was 88.8% (16/18) and was significantly higher ($p <0.05$) than the mortality in NRBC-negative patients: 30.5% (22/72).[64] This study revealed that the daily screening for NRBCs can be used to estimate the patients' mortality risk.

In a study done by Tripati et al it was stated that activated macrophages releases cytokines that play an important role in stimulating NRBC in absence of hypoxia. She also revealed that NRBC were significantly high in early and late neonatal sepsis.[75]

In a review article on neonatal sepsis by Birju A Shah and James F Padbury, it has been stated that even though isolation of bacteria from blood is considered the gold standard for the diagnosis of sepsis, it takes 24–48 h for culture results. Inoculation of only 0.5–1.0 ml of blood decreased its sensitivity, as 60–70% of infants have a low level of bacteremia. Theoretically, for optimal results, 6 ml of blood would be required which is not feasible. Sepsis cannot always be excluded with certainty even when blood cultures are found to be negative. On the other hand, isolation of bacteria in a blood culture may be due to asymptomatic bacteremia or contamination.[66]

In another study done by Rathi R, Kapoor A et al on morphological changes in leukocytes in neonatal sepsis(2017), sensitivity of NRBCs was found to be 86.15%,specificity of 51.06%, positive predictive value 54.9% and negative predictive value of 84.21%.[73]

In a study done by Abhishek MG and Sanjay M on the diagnostic efficacy of NRBC in early diagnosis of neonatal sepsis, it was found that NRBC count was

higher in all sepsis cases. Sensitivity of the test in detecting proven sepsis was 35%, specificity 53.4%, positive predictive value 23.07% and negative predictive value 67.64%.[74]

Another study on NRBC as an auxiliary marker of intrauterine infection was done by Maria Szwajcowska et al to assess the usefulness of determining NRBC in fetal blood in the first 12 hrs of life as a marker of early onset sepsis. Those with generalised infection had higher NRBC levels than normal compared to controls (mean 15 per 100 WBC).

AIMS AND OBJECTIVES

SOURCE OF DATA:

The study was a prospective hospital based study conducted on neonates admitted in the NICU of Madurai Medical College for a period of 6 months from March 2017 to August 2017.

AIM OF THE STUDY

The aim of this study was to measure the nucleated RBC levels in blood samples of neonates with sepsis and to analyse whether it can serve as a prognostic marker for neonatal sepsis and an increased risk for adverse neonatal outcome.

INCLUSION CRITERIA

- All term live neonates admitted in Level II NICU with risk factors of sepsis or clinical features of sepsis will be included in the study

Risk factors of sepsis include the following:

1. Febrile illness in the mother with evidence of bacterial infection within 2 weeks prior to delivery
2. Foul smelling and/or meconium stained liquor
3. Rupture of membranes >24 hours
4. Single unclean or > 3 sterile vaginal examination(s) during labor
5. Prolonged labor (sum of 1st and 2nd stage of labor > 24 hrs)

Clinical features of sepsis include the following:

(a) Hypothermia or fever

(b) Lethargy, poor cry, refusal to suck

(c) Poor perfusion, prolonged capillary refill time

(d) absent neonatal reflexes, Hypotonia

(e) Brady/tachycardia

(f) Respiratory distress, apnea and gasping respiration

(g) Hypo/hyperglycemia

(h) Metabolic acidosis.

Specific features related to various systems:

- Central nervous system (CNS): Bulging anterior fontanelle, seizures, high-pitched cry, excess irritability, vacant stare, stupor/coma, neck retraction.
- Cardiac: Hypotension, shock ,poor perfusion
- Gastrointestinal: Feed intolerance, abdominal distension, paralytic ileus, vomiting, diarrhea, necrotizing enterocolitis (NEC)
- Hepatic: Hepatomegaly, direct hyperbilirubinemia (especially with UTI)
- Renal: Acute renal failure
- Hematological: Bleeding, purpura , petechiae
- Skin changes: Multiple pustules, mottling, sclerema, abscess, umbilical redness and discharge.

EXCLUSION CRITERIA

- Maternal pre eclampsia or eclampsia

- Gestational diabetes mellitus
- Intra-uterine growth retardation
- Birth asphyxia
- Pre-term and post-term babies
- Hemolytic anemia (ABO and Rh incompatibility)
- Maternal smoking

MATERIALS AND METHODS

METHODOLOGY

All babies who fulfilled the criteria and were admitted in NICU of Madurai Medical College were enrolled into the study. An informed consent was obtained from the mother or any other caretaker if mother was unable to give consent for any reason. Aim, objective and methodology are clearly explained in their own native language. Parents were also informed about only 3 ml of blood sample taken for complete blood count and culture. Parents were also assured that treatment will not be denied if they did not give consent.

Mothers detail regarding illness before and during pregnancy obtained from OG records. Risk factors for sepsis were also noted from mother case sheet.

Detailed clinical examination was done for all neonates enrolled in the study.

Blood sample was taken in all neonates for sepsis screen, blood culture and peripheral smear examination for NRBC. The blood sample was obtained while putting venflon or taking blood for other investigations.

CRP: CRP was taken only after 6-12 hrs in Early onset sepsis. CRP is estimated using qualitative and semiquantitative test by using latex agglutination test. When patient serum containing CRP combined with latex particle coated with anti CRP, agglutination reaction take place within two minutes.

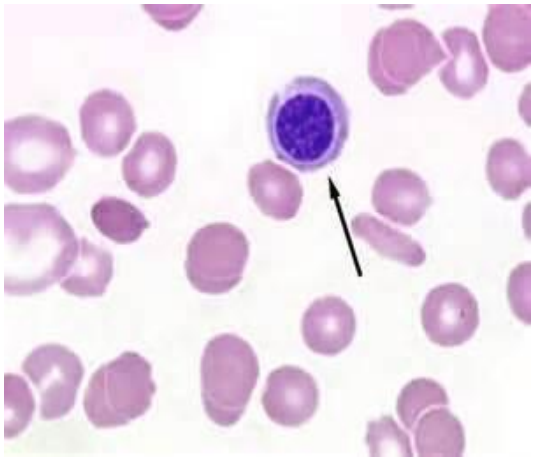
SEPSIS SCREEN:

The various components of the sepsis screen include Total leucocyte count, Absolute neutrophil count, I/T neutrophil ratio, micro-ESR and CRP. Due to non-availability of the test micro-ESR was not done in any patients. Presence of two or more abnormal parameter was considered as a positive screen and the neonate was started on antibiotics. If the screen was negative but clinical suspicion persisted, then it is repeated within 12hrs. If the screen was still negative, the diagnosis of clinical sepsis was excluded.

Preparation of peripheral blood film:

Peripheral blood film [PBF] visualises the morphology of different blood cell. Blood can be taken through venepuncture, sent to a laboratory in an EDTA tube, and processed within 2 hours. Or blood can be taken by finger prick also. Film is prepared by blood spreader or using cover slip and allowed for air drying for at least for 1 hour. Peripheral blood film is then stained with Leishmann stain for 10 minutes. Then the PBF is double rinsed with buffer solution and left for drying for 10 minutes. Then the PBF again left under running water for 5-10 min. PBF is then examined under microscope for morphology of cells and NRBC count per 100 WBCs.

A repeat peripheral smear was taken on day 3 of admission and the value was compared with the previous value. These neonates were followed up till discharge and repeat smear examination was done if the clinical condition of the neonate deteriorated.

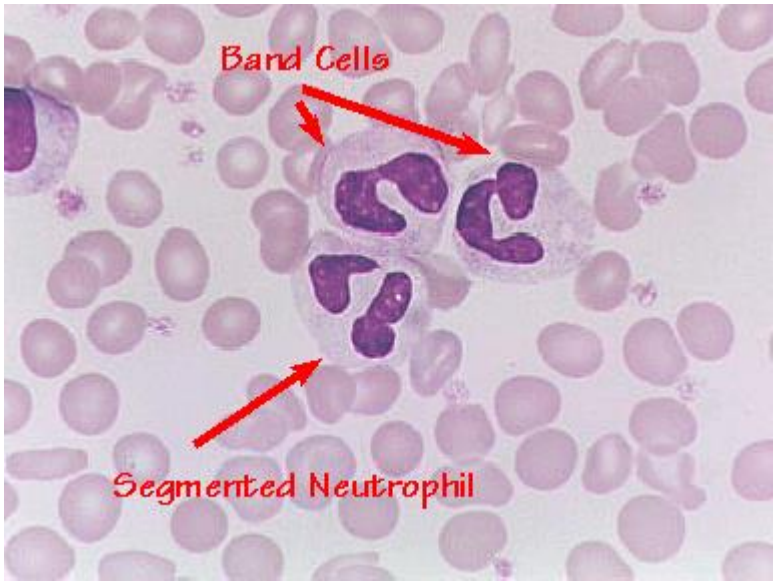


TOTAL COUNT:

Total count >5000 or 15000 calculated to be positive for sepsis.

I/T ratio:

High I/T ratio indicate the presence of sepsis. Neutrophil release more immature cells into circulation in response to sepsis to fight against infection.



Blood culture: The sample was collected by the resident doctor under strict aseptic precautions. A patch of skin approx. 5cm in diameter was prepared over the venepuncture site. This area was cleaned by 70% isopropyl alcohol, followed by povidone iodine and followed again by alcohol. The skin was allowed to dry for at least 1 min before the sample was collected. 1ml of blood sample was taken for a blood culture bottle containing 5-10 ml of culture media.

Other investigations like lumbar puncture,chest X ray, abdominal X ray and other radiological studies were done in indicated cases.

The study group were divided into the following 3 groups based on the clinical findings and investigations.

1. Proven sepsis (Group I) – Neonates with positive blood culture
2. Probable/ clinical sepsis (Group II) – Neonates with strong clinical features, a positive sepsis screen but a negative blood culture.
3. No sepsis (Group III) – Neonates with negative blood culture and a sepsis screen. They presented with features of suspected sepsis or with associated risk factors. On further evaluation they were found to be suffering from other disorders.

STATISTICAL ANALYSIS

Data was entered into MS excel and analysed using SPSS v20. Qualitative data were summarised as frequencies and percentages. Quantitative data were checked for normality. Normally distributed data were summarised using mean and standard deviation.

Pearson Chi-square/ Fisher Exact test has been used to find the significance of study parameters on categorical scale between two groups. Significance is assessed at 5 % level of significance. P value: < 0.05 is statistically significant.

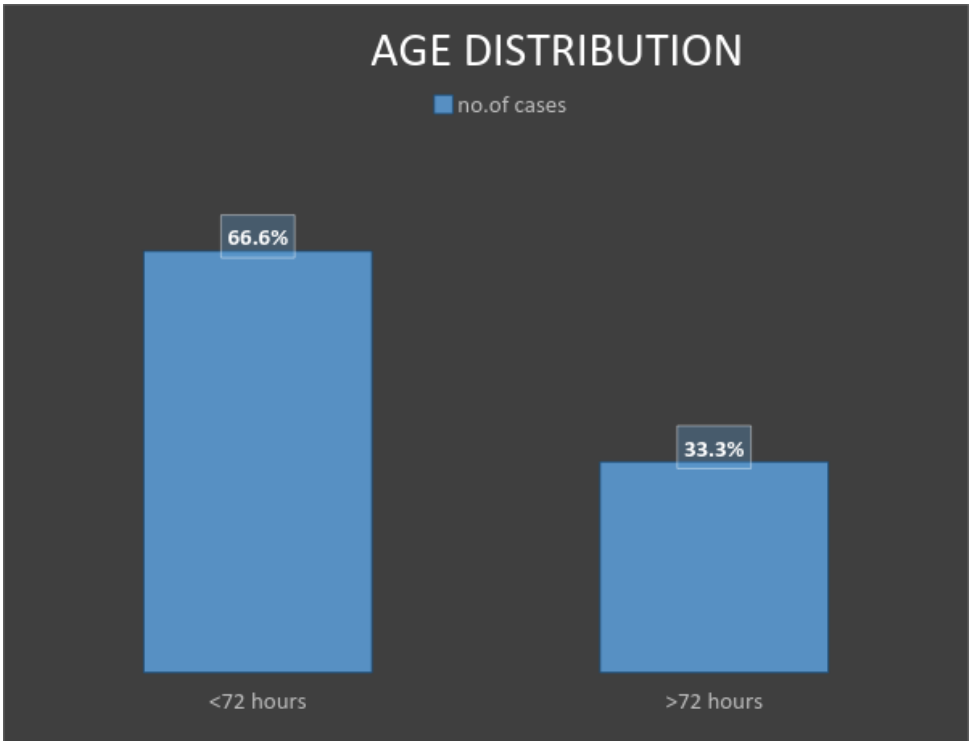
RESULTS

This study was a hospital based prospective study to evaluate whether a significant increase in NRBC is seen in neonatal sepsis.

A total of 72 neonates were included in the study of which, 48 neonates (66.6%) were less than 72 hrs old and were suspected to have early onset sepsis depending on the risk factors. The rest 24 neonates (33.34%) were more than 72 hrs old who presented with clinical features of sepsis or associated risk factors and suspected to have late onset sepsis.

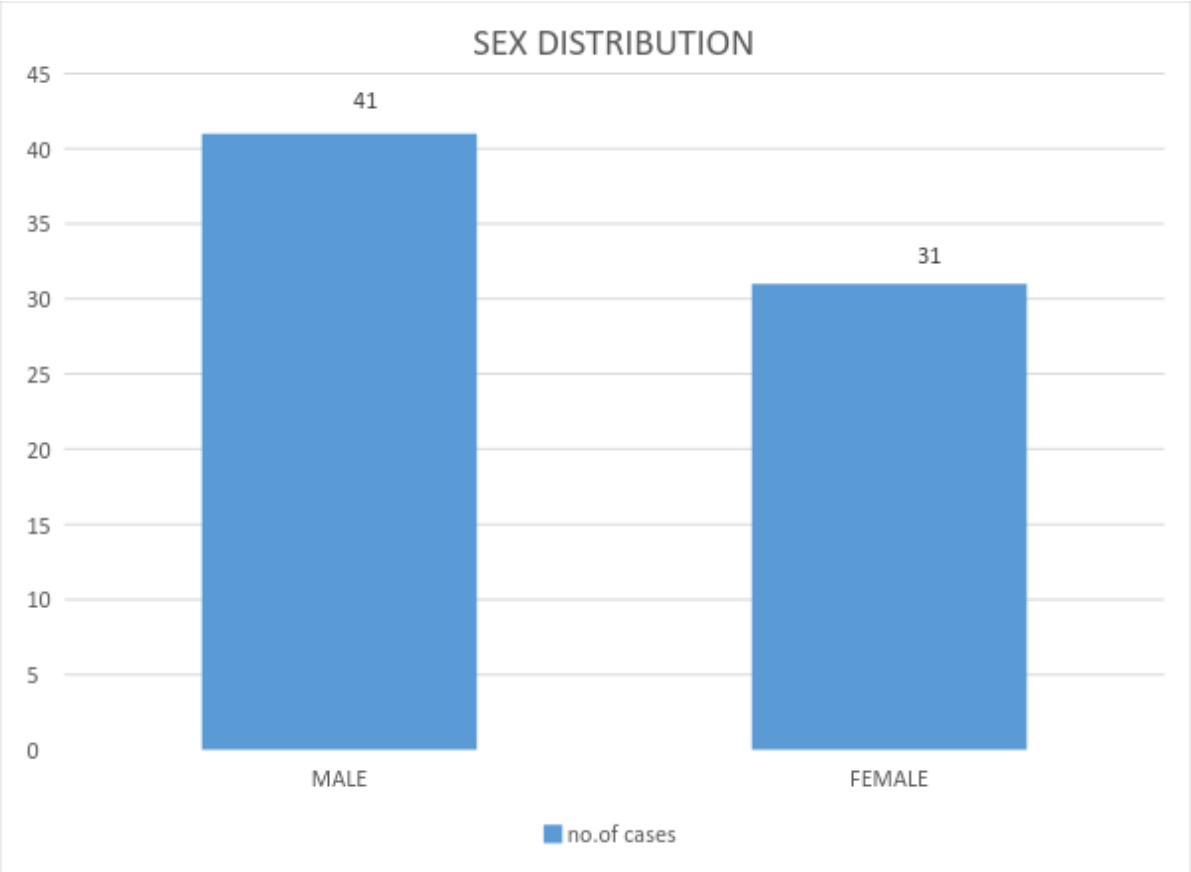
Age distribution among study population

AGE	final diagnosis	
	Sepsis	no sepsis
<72 hours	25	23
>72 hours	13	11
Total	38	34
p value	0.933 Not significant	



Of the 72 cases, 41 (56.9%) were male and 31 were female. The gender distribution among sepsis and no sepsis group was not statistically significant (p value -0.94)**.**

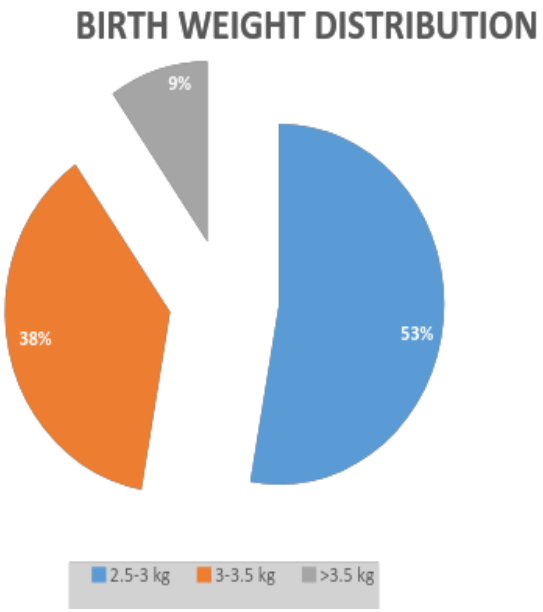
Sex distribution among the study population



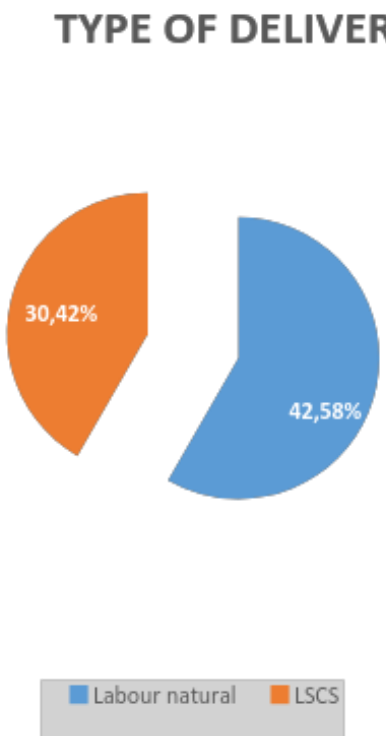
SEX	sepsis	no sepsis
MALE	21	20
FEMALE	17	14
Total	38	34
p value	0.947 Not significant	

All the cases included in the study were term babies who were appropriate for gestational age. Majority of the cases fit into birth weight of 2.5-3.5kg.

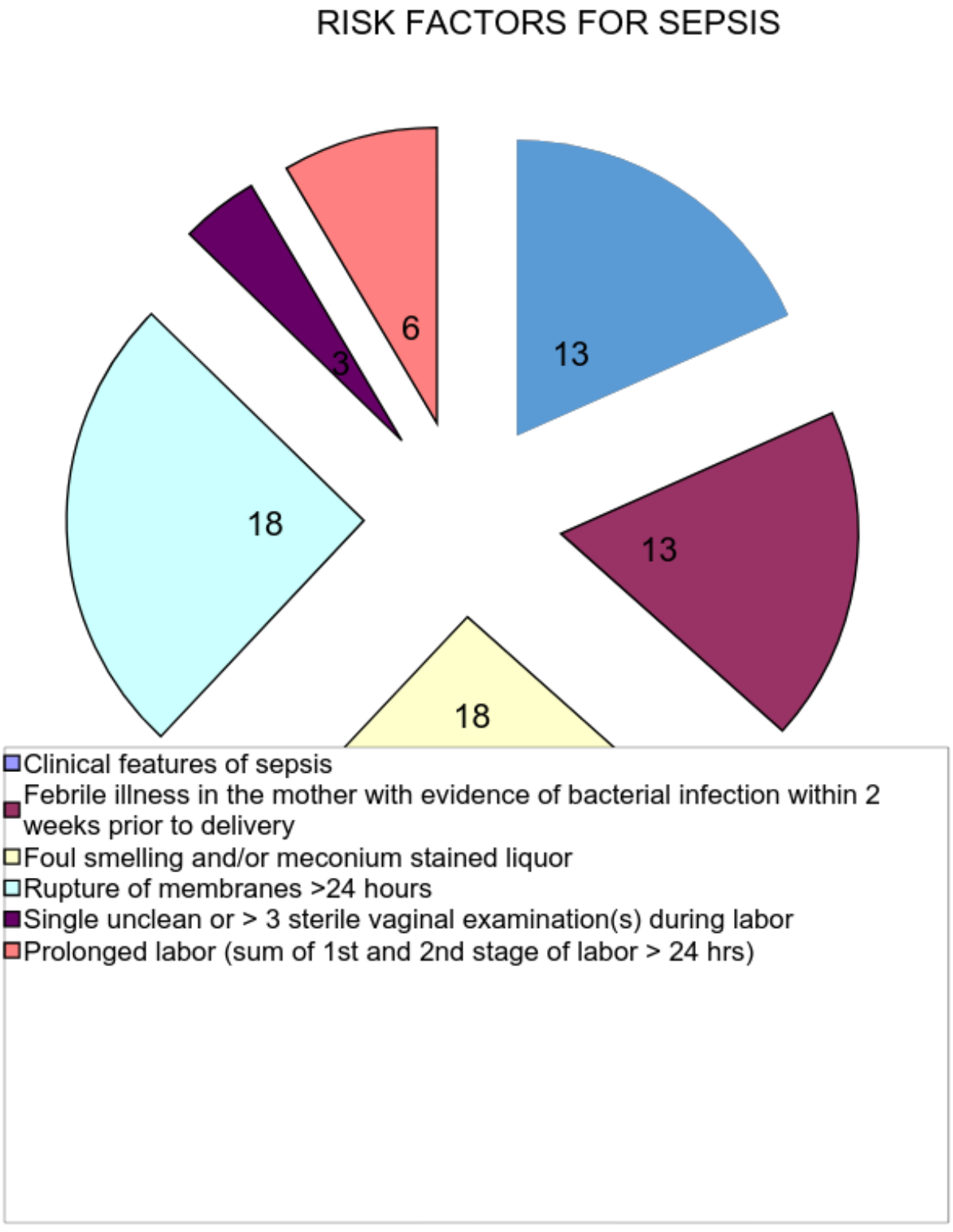
BIRTH WEIGHT	no.of cases
2.5-3 kg	38
3-3.5 kg	27
>3.5 kg	7
total	72



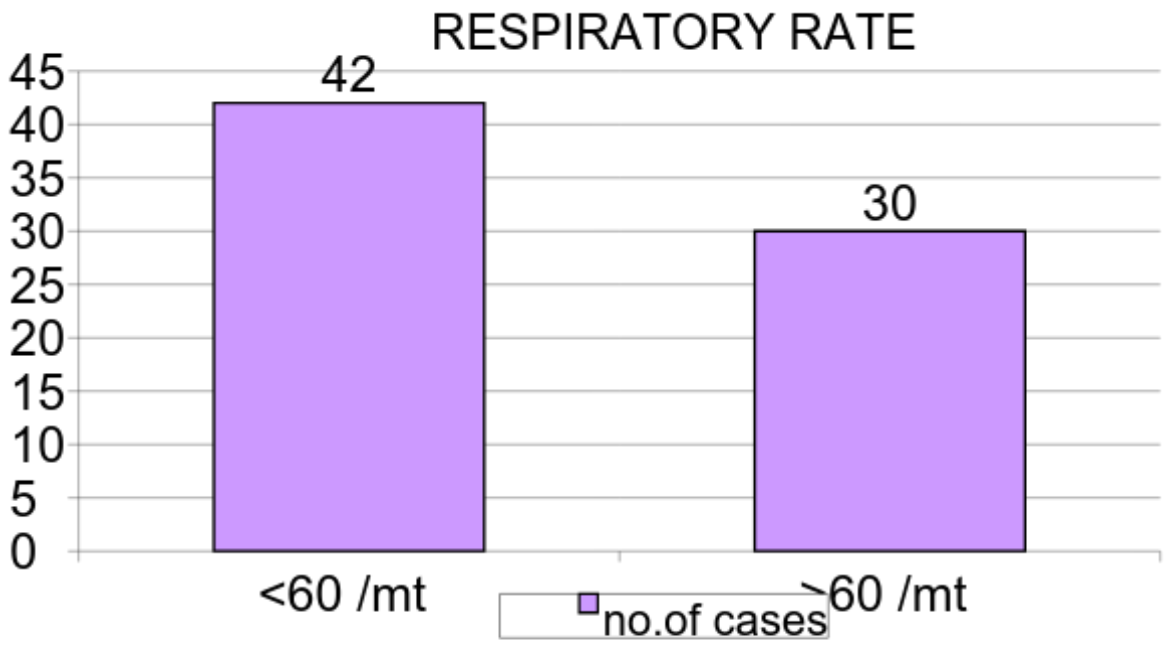
Of the 72 cases, 42 were born by labour natural of which 2 were assisted delivery (vacuum delivery).The rest 30 cases were LSCS. The difference in the two groups were not found to be statistically significant.(p value =0.57)

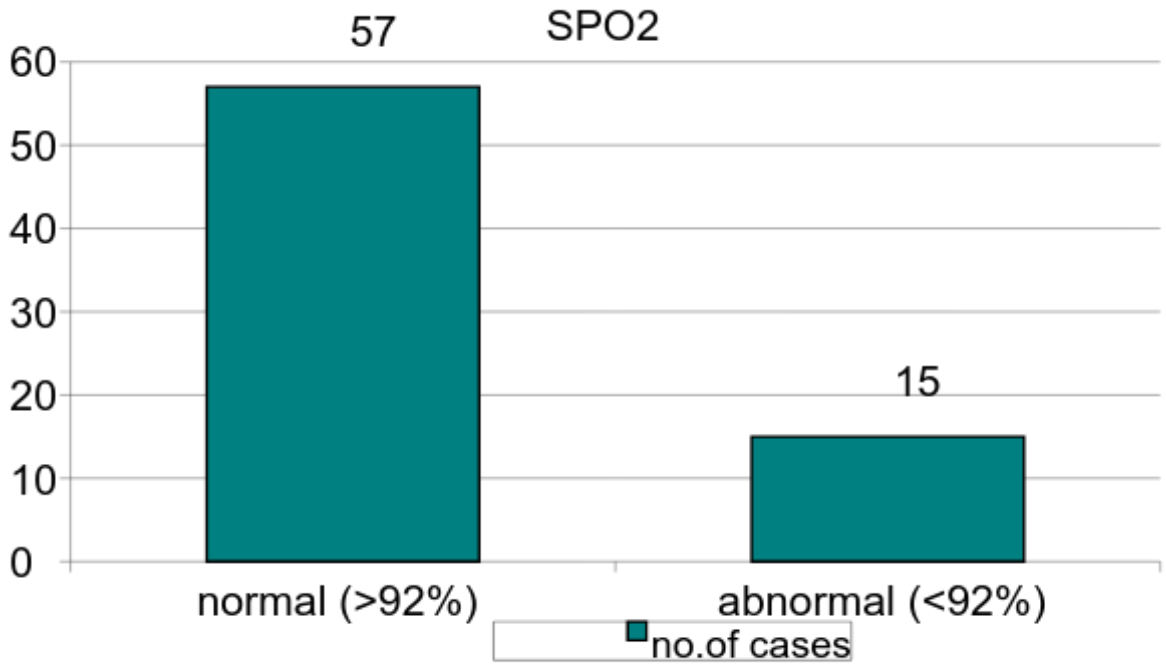


The most common risk factor for sepsis were foul smelling or meconium stained liquor(18 cases each) followed by maternal fever(13 cases).13 neonates presented with clinical features of sepsis. 3 cases had history of unclean vaginal examination and delivery. These were mostly home delivery or vehicle delivery.

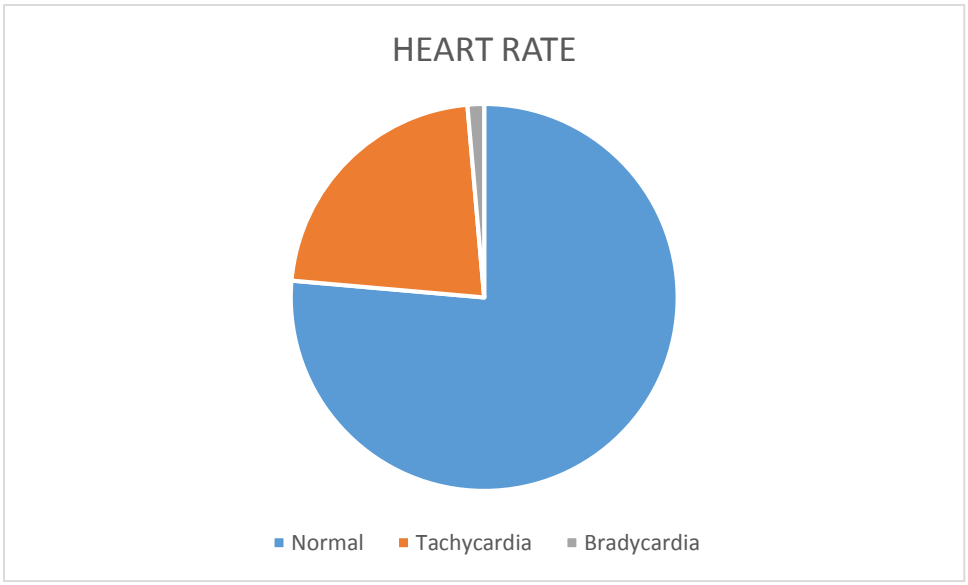

RISK FACTORS FOR SEPSIS
6
3
13
18
13
18
Clinical features of sepsis
Febrile illness in the mother with evidence of bacterial infection within 2 weeks prior to delivery
Foul smelling and/or meconium stained liquor
Rupture of membranes >24 hours
Single unclean or > 3 sterile vaginal examination(s) during labor
Prolonged labor (sum of 1st and 2nd stage of labor > 24 hrs)

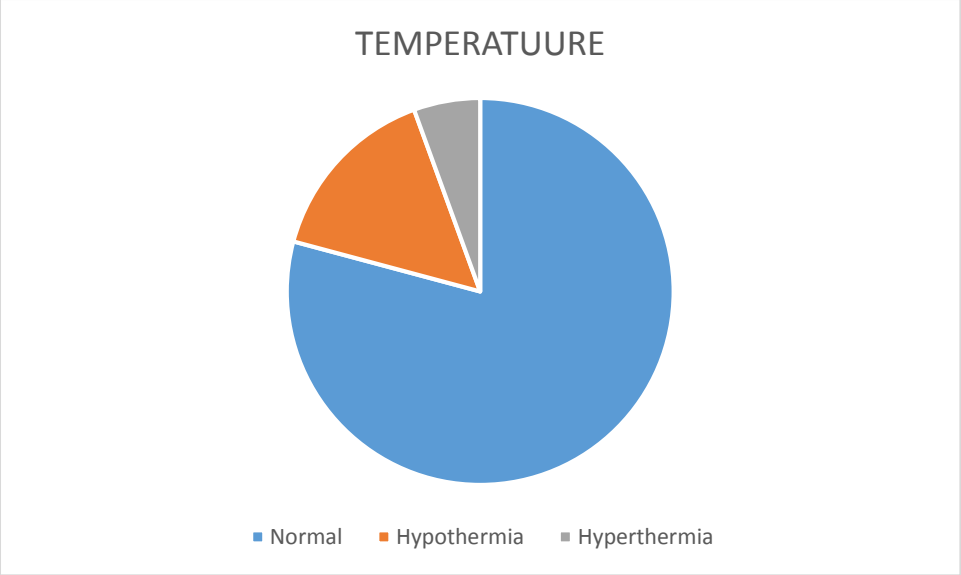
The most common symptomatology was respiratory distress seen in 30 cases of which 15 cases had desaturation. 13 cases presented with shock, 11 cases were hypothermic during admission and four cases had fever. Four cases presented with abdominal symptoms and two cases had convulsion.





Tachycardia was seen in 16 cases and 1 case had bradycardia.



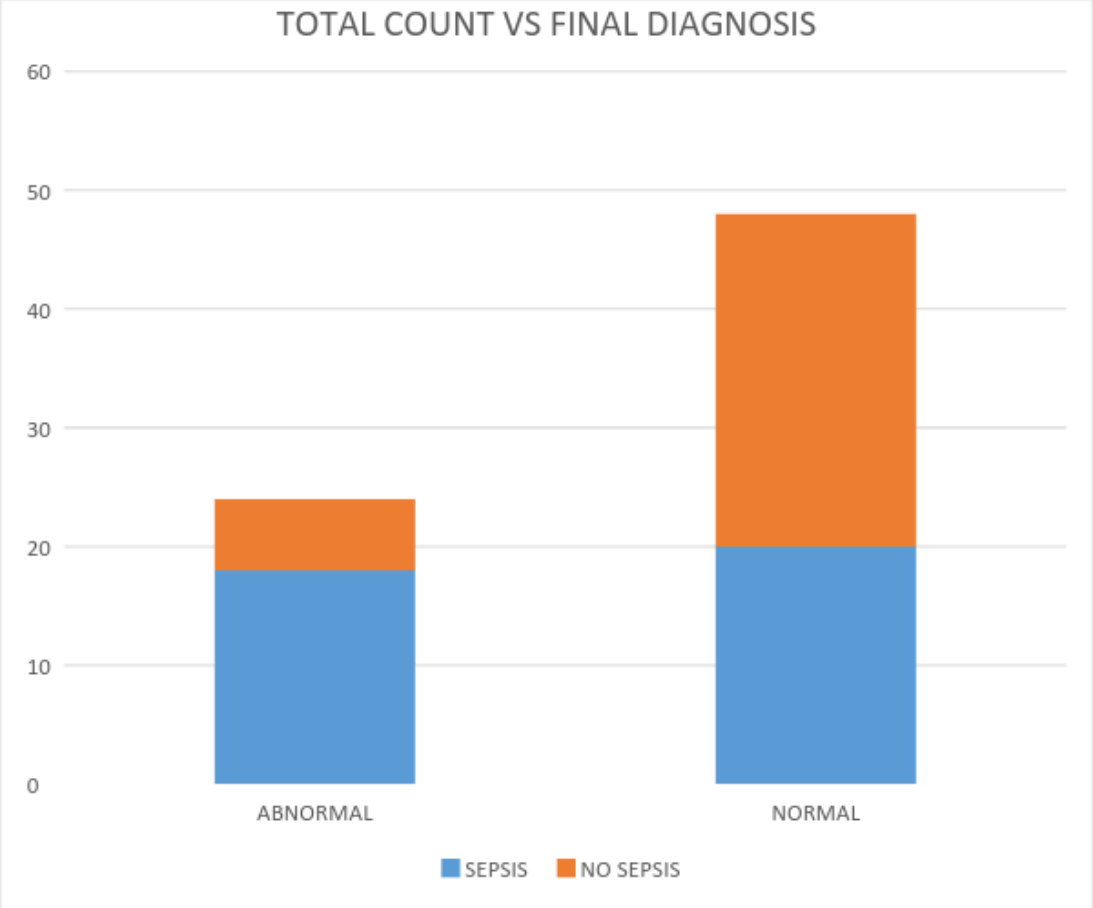


Comparison of baseline investigations in the sepsis and no sepsis group

Mean Hb in the sepsis group was 10.34 and in the no sepsis group the mean was 13.05 and the difference was statistically significant.

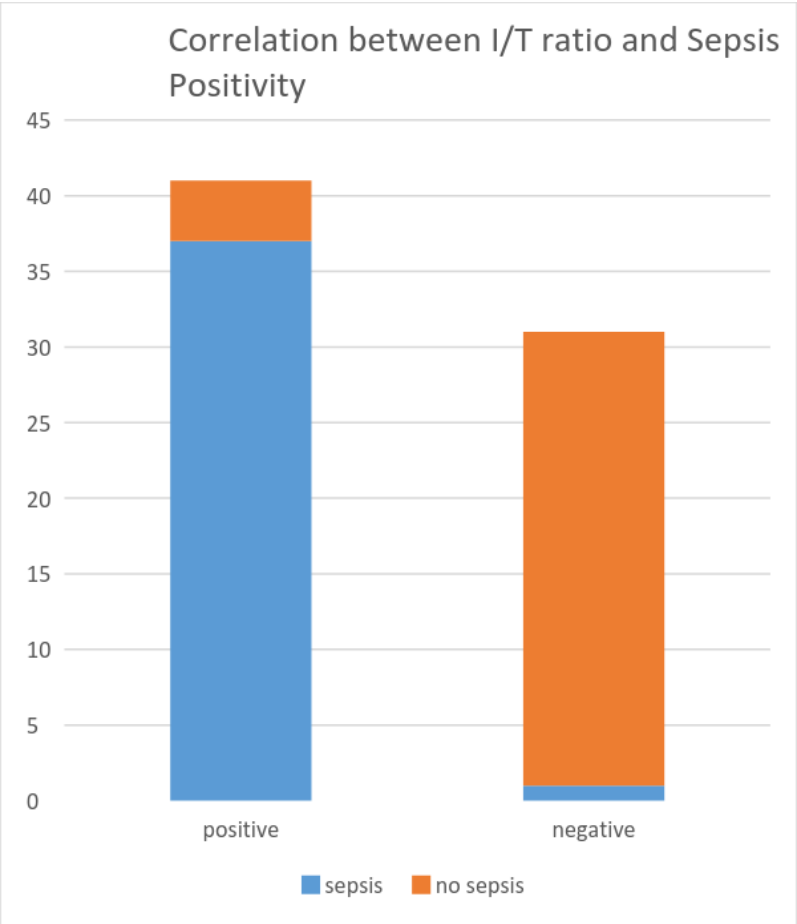
Total count was normal in 48 cases.19 cases had leucopenia of which 13 are in sepsis group and 6 in no sepsis group.5 cases had leucocytosis; all of them were in sepsis group. But the difference between the two groups were not statistically significant (p value=0.109).

Measure	Group	Mean	SD	P value
Hb	sepsis	10.34	2.41	0.002
	No sepsis	13.05	1.24	
Total count	Sepsis	8526	5778	0.1096
	No sepsis	6867	1593	
Platelet	Sepsis	1,83,000	87,872	0.0001
	No sepsis	3,34,000	93,111	
NRBC	Sepsis	13.13	5.48	0.0003
	No sepsis	4.97	4.6	



TOTAL COUNT	SEPSIS	NO SEPSS
ABNORMAL	18	6
NORMAL	20	28
P value=0.109		

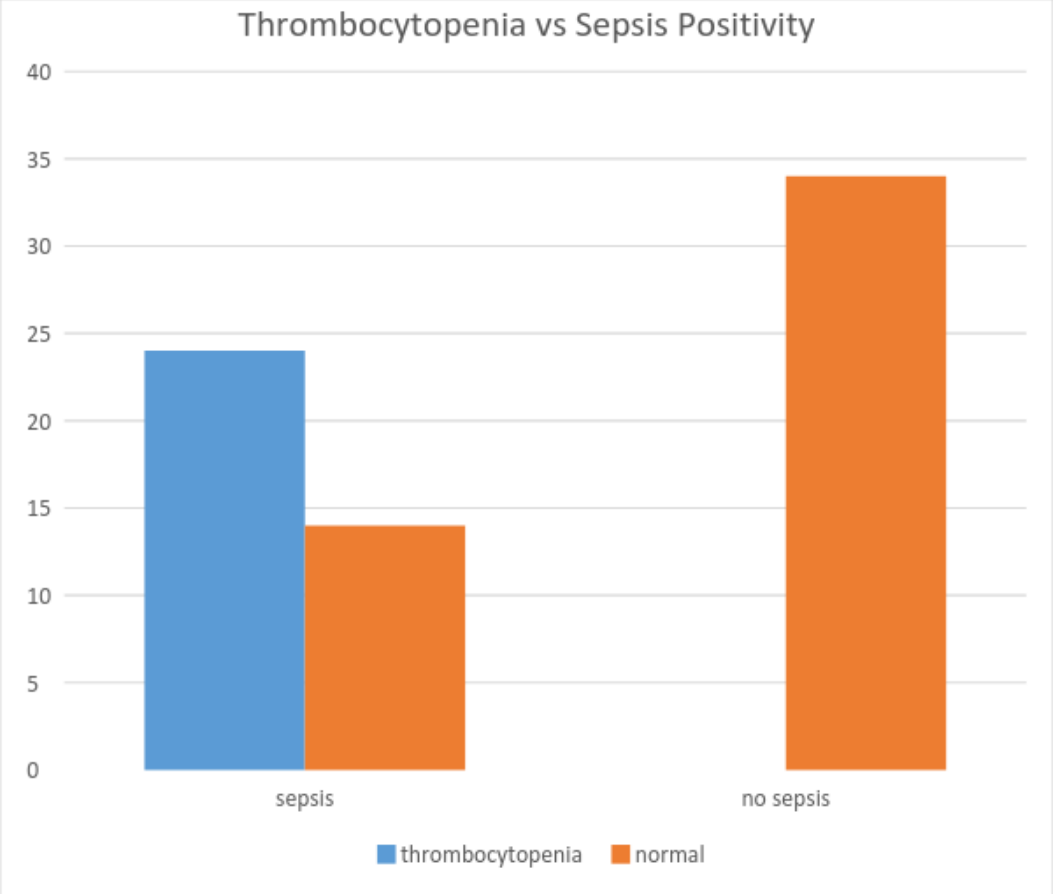
Immature to total cells (I/T) ratio was positive in 41 cases of which 37 turned out to be positive for sepsis and 4 were negative for sepsis and the difference is found to be stastically significant (p value<0.0001).



I/T RATIO	SEPSIS	NO SEPSIS
>0.2	37	4
<0.2	1	30

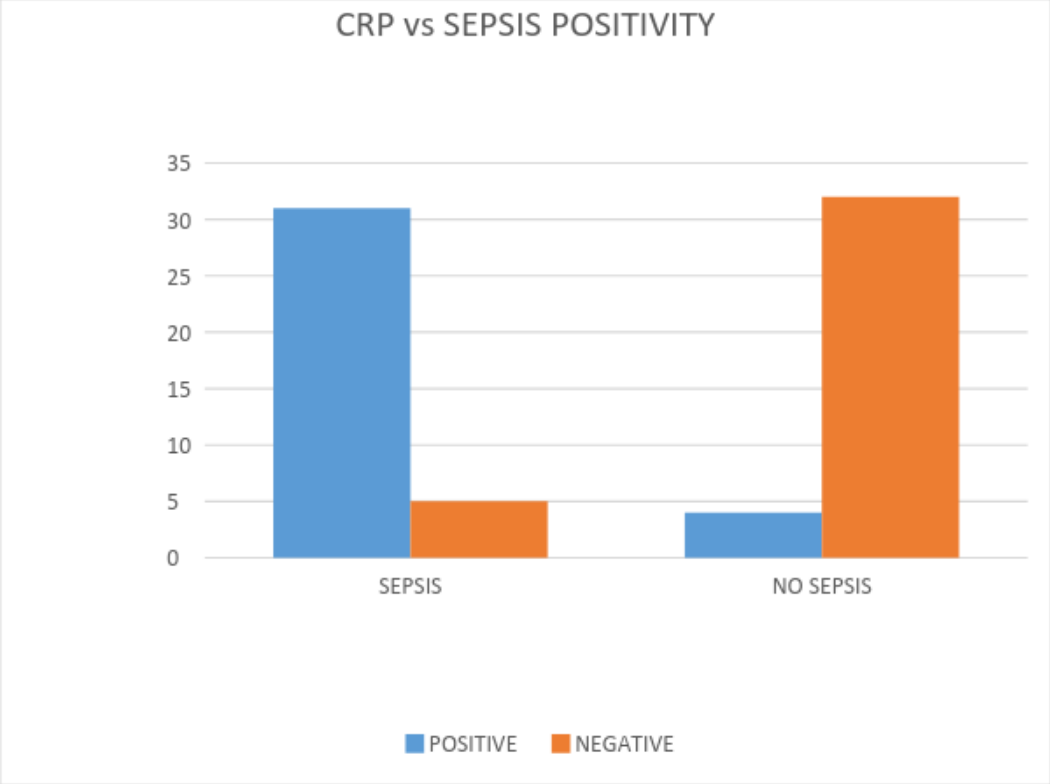
Mean platelet count was 1.16 lakh in the sepsis group and 3.34 lakh in the no sepsis group and the difference was statistically significant (p value=<.0001).24

cases had thrombocytopenia in the sepsis group but none of the cases in no sepsis group had thrombocytopenia.



	SEPSIS	NO SEPSIS
THROMBOCYTOPENIA	24	0
NORMAL	14	34
P VALUE = 0.0001		

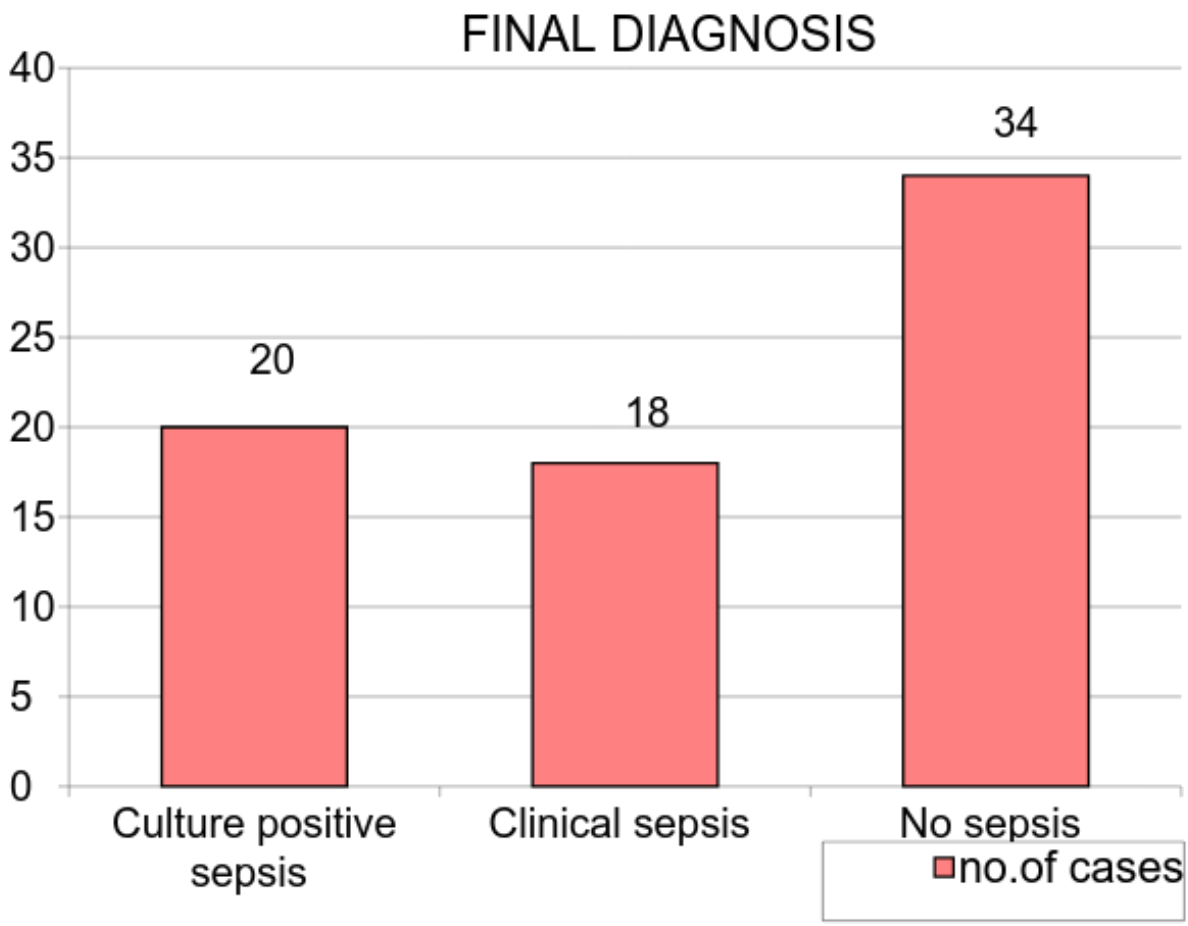
CRP was positive in 35 cases of which 31 cases were in sepsis group and 4 cases were in no sepsis group and the difference was found to be statistically significant.(p value <0.0001)



CRP	SEPSIS	NO SEPSIS
+VE	31	4
-VE	5	32
P VALUE = 0.0001		

The sensitivity of CRP in diagnosing sepsis in this study was 86% , specificity 88.8%, PPV 88.5% and NPV 86.4%.

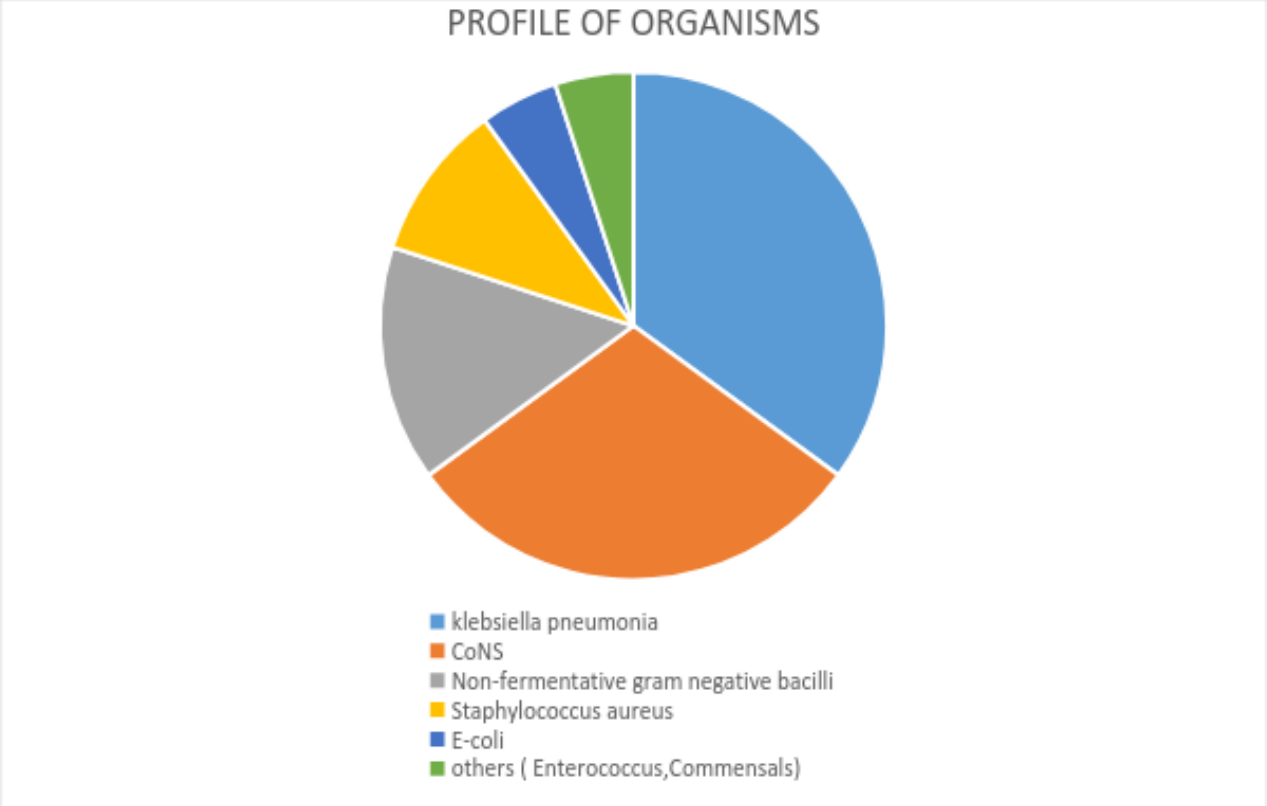
Of the 72 cases, 20 cases were culture positive sepsis, 18 cases were clinical sepsis or culture negative sepsis and the rest 34 cases were negative for sepsis (no sepsis).



PROFILE OF ORGANISMS ISOLATED FROM THE CULTURE POSITIVE GROUP

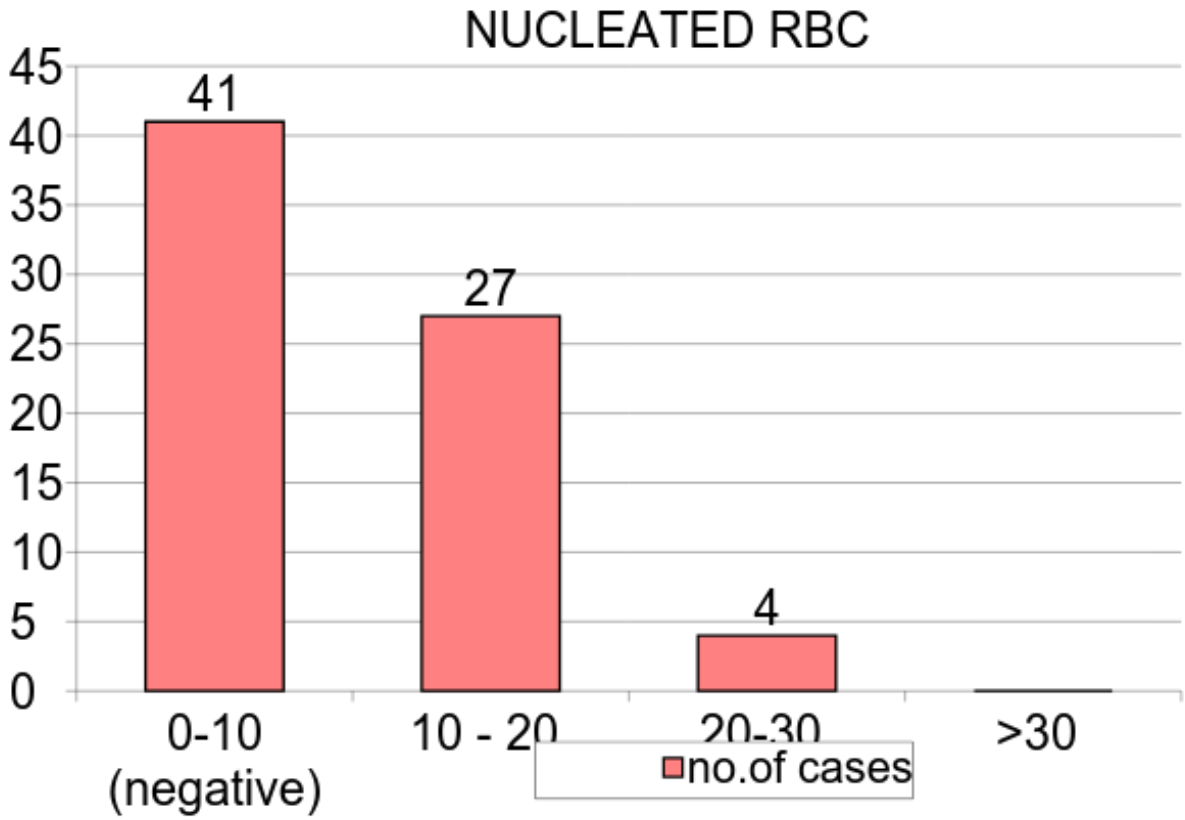
The most common organism isolated from culture is klebsiella pneumoniae (35%) followed by coagulase negative staphylococcus (30%).

Blood culture	no.of cases
No growth	52
klebsiella pneumonia	7
CoNS	6
Non-fermentative gram negative bacilli	3
Staphylococcus aureus	2
E-coli	1
others (Enterococcus,Commensals)	1
total	72



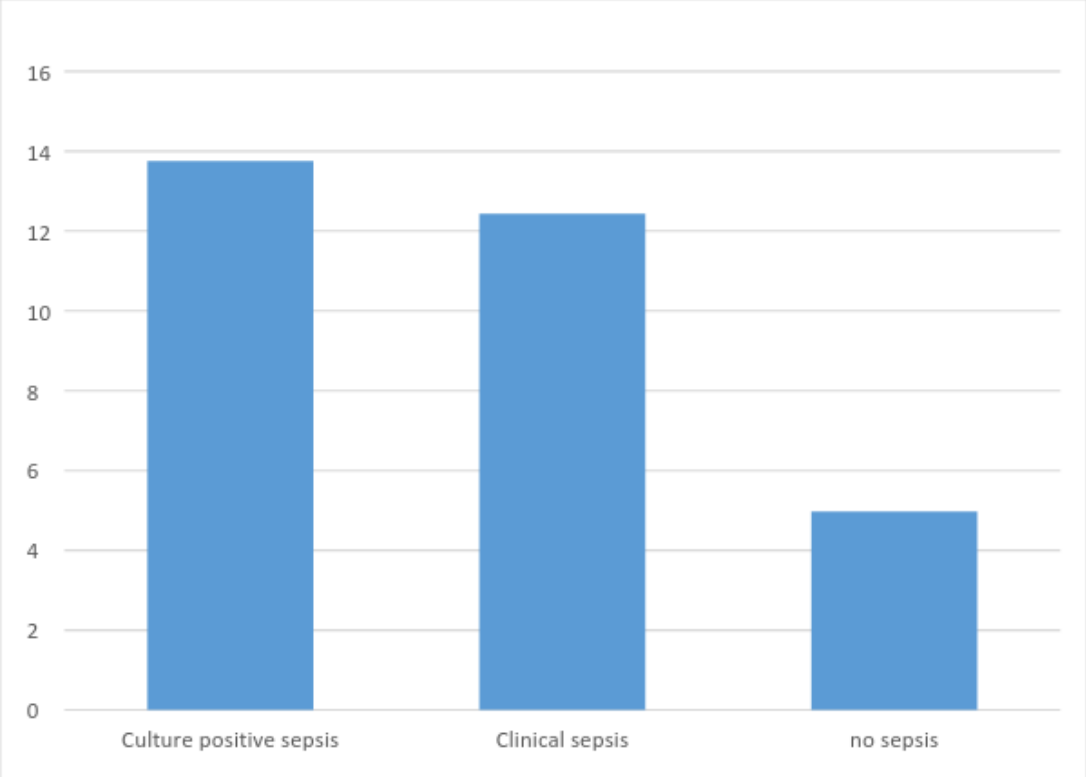
Klebsiella pneumonia is more common organism in EOS while CONS is seen commonly in LOS.

Of the total cases, nucleated RBC were negative in 41 cases and positive in 31 cases.



The mean NRBC value in each group is given in the table

Final diagnosis	mean
Culture positive sepsis	13.75
Clinical sepsis	12.44
no sepsis	4.97

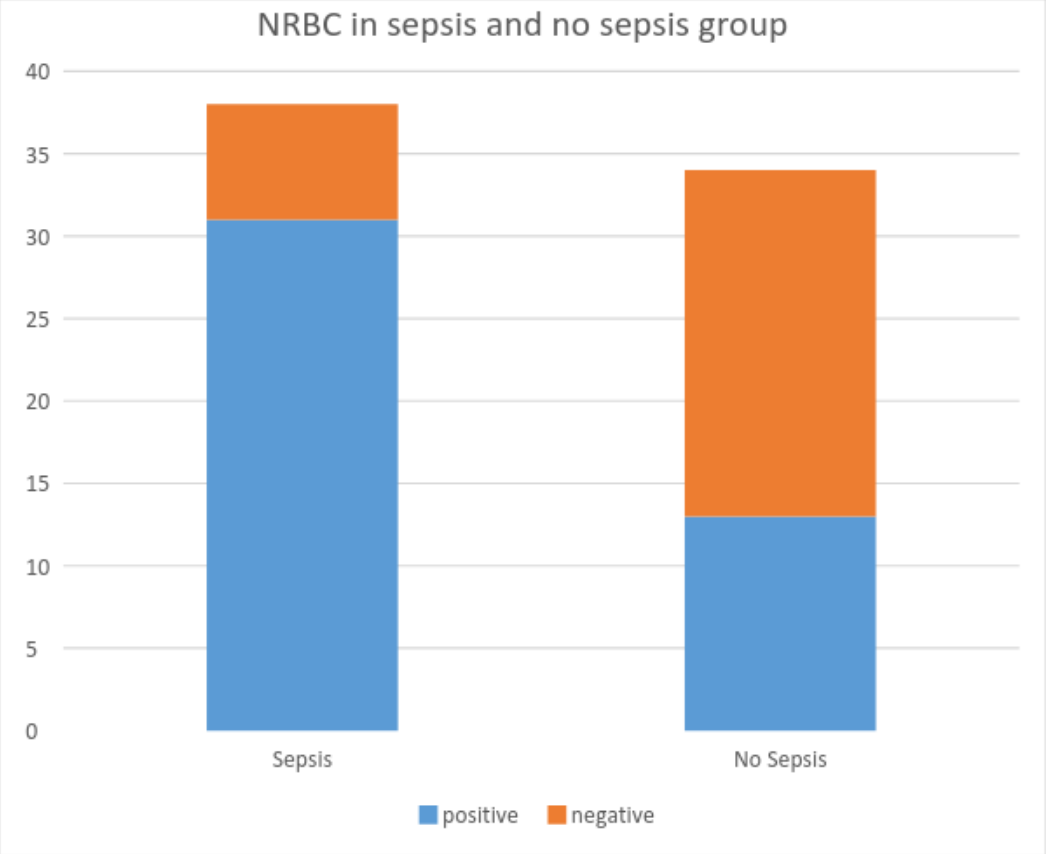


MEAN VALUES OF NRBC

NRBCs were positive in 16 culture positive cases, 15 culture negative cases and 13 cases with no sepsis.

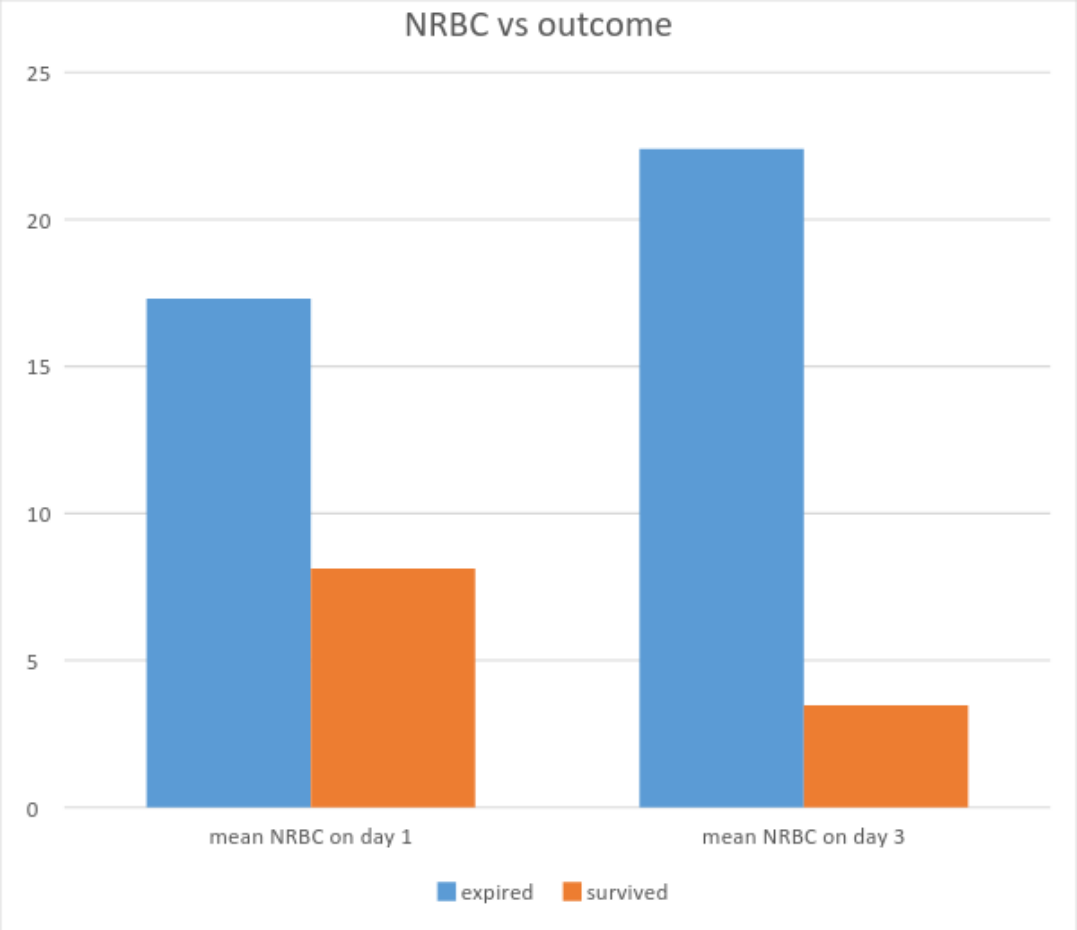
NRBC	Sepsis	No Sepsis
positive	31	13
negative	7	21
P value- 0.0001		

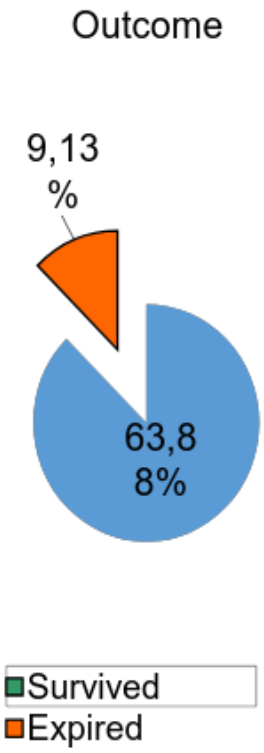
The sensitivity of NRBC in identifying sepsis was 81.5%, its specificity was 61.76%, positive predictive value was 70.4% and negative predictive value was 75%.



Of the 72 cases, 63 survived and 9 expired with a mortality of 13%. Of the 9 expired cases, 6 were culture positive. Mean NRBC in the mortality group was

17.3 on day1 while a repeat count on day 3 showed an increase in the number of circulating NRBC in the mortality group and the mean value was 22.4. In the group that survived, NRBCs decreased on day 3 and were undetectable in most of the cases with a mean value of 3.47. NRBC is a better predictor of mortality and adverse neonatal outcome.




Outcome
9,13
%
63,8
8%
Survived
Expired

DISCUSSION

This study was conducted in NICU, Government Rajaji Hospital, Madurai Medical College, Madurai. A total of 72 neonates were enrolled into the study of which 20 were culture positive, 18 were clinical sepsis or culture negative sepsis while the rest 34 did not had sepsis.

AGE OF BABIES ENROLLED IN OUR STUDY

Of the 72 cases, 48 cases were less than 3 days old of which 25 cases (65.7%) were classified into early onset sepsis (13 cases were culture positive sepsis). Of the 24 cases who were more than 3 days old, 13 cases (34.2%) were positive for sepsis and were classified as late onset sepsis, of which 7 were culture positive.

The difference in age was not found to be statistically significant.

COMPARISION OF MODE OF DELIVERY IN NEONATES ENROLLED IN OUR STUDY:

Mode of delivery is compared with the final diagnosis and difference was not found to be statistically significant. (P value = 0.57)

CLINICAL FEATURES :

Out of the 72 cases, 30 cases presented with respiratory distress of which 15 cases had desaturation. 13 cases presented with shock, 11 cases were hypothermic during admission and four cases had fever. Four cases presented with abdominal symptoms and two cases had convulsion.

DIAGNOSIS OF SEPSIS:

In our study, out of the 38 sepsis cases, 25 cases (65.7%) were early onset sepsis, of which 13 were culture positive and 13 cases (34.2%) were late onset sepsis of which 7 were culture positive. This is comparable to a study by Pramila et al shows 55.1% of EOS & 44.8% LOS. In another study from Egypt, 44.2% were classified as early onset sepsis EOS (≤72 hr) and 55.8% as late onset sepsis LOS (>72 hr). The association between the culture positivity and the onset of sepsis was not found to be statistically significant. (p value=0.924)

COMPARISON OF BASELINE INVESTIGATION IN NEONATES ENROLLED IN OUR STUDY

The mean Hb in the sepsis group was 10.3 and in the no sepsis group it was 13.05. The difference in the total count between the 2 groups was not found to be statistically significant (p value 0.109) while thrombocytopenia between the groups was statistically significant (p value 0.0001).

COMPARISION OF CRP IN NEONATES ENROLLED IN OUR STUDY:

The comparison of CRP positivity with sepsis positivity was found to be statistically significant.(p value <0.0001) The sensitivity of CRP in diagnosing sepsis in this study was 86% , specificity 88.8%, PPV 88.5% and NPV 86.4%.

In diagnosis of early-onset sepsis, previous studies reported widely differing sensitivities and specificities of CRP ranging from 29 to 100% and from 6 to 100%, respectively[71,72]. These extreme variations are a result of different reference values, test methodologies, patient characteristics and inclusion criteria, as well as different definitions of sepsis, numbers of samples taken, and sampling times.

Study	Our study	Naher et al	Koksal et al
Sensitivity	86%	55%	48%
Specificity	88.8%	100%	87%

COMPARISION OF I/T RATIO AND SEPSIS IN NEONATES ENROLLED IN OUR STUDY

The difference in I/T ratio between the sepsis positive and negative group was statistically significant (p value 0.0001).

PROFILE OF ORGANISM ISOLATED IN BLOOD CULTURE

The commonest organism isolated from culture is Klebsiella pneumonia (35%) followed by CoNS (30%).

COMPARISON OF BASELINE NRBC BETWEEN NEONATES ENROLLED IN OUR STUDY

The baseline NRBC in culture positive cases was 13.75, 12.44 in clinical sepsis and 4.97 in no sepsis group and the difference was found to be statistically significant. In a study done by Rathi R et al, 47 cases out of 56 neonates with proven sepsis had a NRBC score of >10/100 WBCs accounting to 83.9%.

The sensitivity of NRBC in identifying sepsis was 81.5%, its specificity was 61.76%, positive predictive value was 70.4% and negative predictive value was 75%. In a study done by Abhishek M G et al, the sensitivity of the test in detecting proven sepsis was 35%, specificity 53.4%, positive predictive value 23.07% and negative predictive value 67.64%.[74] In another study done by Rathi R,Kapoor A et al, sensitivity of nRBCs was found to be 86.15%,specificity of 51.06%, positive predictive value 54.9% and negative predictive value of 84.21%.[73]

	Present study	Abhishek M G et al	Rathi R et al
Sensitivity	81.5%	35%	86.15%
specificity	61.76%	53.4%	51.06%
PPV	70.4%	23.07	54.9%
NPV	75%	67.64%	84.21%

SERIAL NRBC VALUES IN NEONATES WITH SEPSIS WHO EXPIRED

Of the total 72 cases, 9 cases expired with a mortality of 13%. Mean NRBC in the mortality group was 17.3 on day 1 and on day 3, the mean value was 22.4. In our study, the mortality was high in cases with increased NRBC counts. NRBC is a better predictor of mortality and adverse neonatal outcome. In a study by Duțu Mădălina et al, it has been found that the daily screening for the presence of NRBCs seems to be a useful tool to estimate the mortality risk.

COMPARISON OF SERIAL MPV BETWEEN SURVIVORS AND NON SURVIVORS

Mean NRBC in the mortality group was 17.3 on day 1 while a repeat count on day 3 showed an increase in the number of circulating NRBC and the mean value was 22.4. In the group that survived, NRBCs decreased on day 3 and were undetectable in most of the cases with a mean value of 3.47. The difference was found to be statistically significant.

LIMITATIONS OF THE STUDY

The sample size was small

NRBC count was done by peripheral smear examination rather than by automated analyser, which can lead to inter-observer variation.

Micro-ESR was not done as a part of sepsis screening due to non-availability of the test.

CONCLUSION

Estimation of NRBC in suspected neonatal sepsis can predict sepsis earlier with day 1 NRBC count. The difference between controls and sepsis group was found to be significant .

Serial NRBC measurement between survivors and non survivors was found to be significant with p value <0.05.

NRBC count is helpful in assessing prognosis of sepsis in response to therapy.

It is a better predictor of mortality in neonatal sepsis.

The sensitivity of NRBC was 81.5%, its specificity was 61.76%, positive predictive value was 70.4% and negative predictive value was 75%. These results were comparable to a study done by Rathi R et al.

RECOMMENDATIONS

In future, further studies are needed to evaluate the usefulness of nucleated RBC as a good predictor of Neonatal sepsis using large sample sizes.

Nucleated RBC can be used as a prognostic marker for adverse neonatal outcome.

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Epidemiology of Neonatal Sepsis and Implicated Pathogens: A Study from Egypt

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ABBREVIATIONS

NRBC - nucleated red blood cells

CRP - C-reactive protein

PCT - Procalcitonin

NNPD - National Neonatal Perinatal Database

EOS - Early onset sepsis

LOS - Late Onset Sepsis

UTI - Urinary Tract Infection

NEC - Necrotizing Entero Colitis

WBC - White Blood cells

IL-1 - Interleukin 1

TNF-α - Tumor Necrosis Factor Alpha

PPV - Positive Predictive Value

NPV - Negative Predictive Value

TLC - Total Leukocyte Count

ANC - Absolute Neutrophil Count

I/T ratio - Immature to Total Neutrophil Ratio

Micro ESR - Micro Erythrocyte Sedimentation Rate

NICU -Neonatal Intensive Care Unit

MRSA- methicillin resistant staphylococcus aureus

SAA- Serum Amyloid A

LSCS - Lower Segment Caesarean Section

LBP- Lipopolysaccharide Binding Protein

ABBREVIATIONS TO MASTERCHART

Sl.no - Serial number

B.wt - Birth weight

GA - Gestational Age

HR - Heart rate

RR - Respiratory rate

SpO_2 - Oxygen saturation

CRT -Capillary refill time

Hb - Hemoglobin

TC - Total count

I/T ratio - Immature to total neutrophil ratio

CRP - C reactive protein

NRBC - Nucleated red blood cells

PROFORMA

NAME: **AGE/SEX:** **ADDRESS:**

DATE OF BIRTH :

DATE OF ADMISSION :

DATE OF DISCHARGE OR EXPIRY :

BIRTH WEIGHT :

GESTATIONAL AGE :

TYPE OF DELIVERY :

RISK FACTORS FOR SEPSIS :

EXAMINATION FINDINGS :

HR	RR	SpO2	CRT	TEMPERATURE

SYSTEMIC EXAMINATION:

INVESTIGATIONS:

Date	Hb	TC	IMMATURE/TOTAL CELLS	ANC	PLATELET	CRP	nRBC

BLOOD CULTURE :

PROVISIONAL DIAGNOSIS :

FINAL DIAGNOSIS :

OUTCOME :

Sl.NO	AGE	SEX	B. WT	GA	TYPE OF DELIVERY	RISK FACTORS	EXAMINATION FINDINGS					SYSTEMIC EXAMINATION	INVESTIGATIONS							BLOOD CULTURE	FINAL DIAGNOSIS	OUTCOME
							HR	RR	SPO2	CRT	TEMPERATURE		HB	TC	I/T ratio	PLATELET	CRP	NRBC/ 100 WBC on day 1	NRBC on day 3			
1	1	1	2	38	1	3	1	2	1	1	1	respiratory distress	10.4	5600	0.6	60,000	2	13	16	2	1	1
2	1	1	1	37	1	3	1	1	1	1	1		11.2	9600	nil	190,000	1	3	0	1	3	1
3	1	2	1	39	2	6	1	1	1	1	1		13.6	7900	nil	306,000	1	11	0	1	3	1
4	1	2	2	40	2	2	2	2	2	2	2	shock	8.2	1200		45000	2	24	30	4	1	2
5	1	1	1	39	1	4	1	2	1	1	1		10	4600	0.4	190,000	2	11	5	1	2	1
6	1	1	1	38	1	4	1	1	1	1	1		14	6600	nil	220,000	1	0	0	1	3	1
7	1	1	2	38	2	3	1	1	1	1	1		13.6	7200	nil	300,000	1	10	3	1	3	1
8	1	2	1	37	2	2	1	1	1	1	1		14.1	5200	nil	250,000	1	2	0	1	3	1
9	1	1	1	38	1	2	1	2	1	1	1	poor suck	11.2	11500	0.3	36,000	2	4	0	2	1	1
10	1	2	2	38	1	4	2	2	2	1	1	respiratory distress	9.6	10600	0.5	90,000	2	18	16	1	2	1
11	1	2	2	37	1	3	1	2	1	1	1		10.4	3200	0.4	260,000	2	13	0	1	2	1
12	1	1	1	38	2	3	2	1	1	1	1		11	7500	nil	320,000	1	10	0	1	3	1
13	1	1	3	40	2	4	1	1	1	1	1		12	8600	nil	520,000	1	11	0	1	3	1
14	1	2	2	38	1	3	1	1	1	1	1		14.6	5400	nil	480,000	1	0	0	1	3	1
15	1	1	1	39	1	2	1	1	1	1	1		13	6600	nil	395,000	1	1	0	1	3	1
16	1	1	1	38	2	4	2	2	1	2	3	shock	9	2400	0.8	45,000	1	12	7	3	1	1
17	1	2	1	37	1	4	1	1	1	1	1		12.6	4500	nil	270,000	1	0	0	1	3	1
18	1	2	1	38	1	5	1	1	1	1	1		14.3	5200	nil	400,000	1	0	0	1	3	1
19	1	1	2	37	2	4	1	1	1	1	1		12.6	6800	nil	460,000	1	4	0	1	3	1
20	1	1	1	39	1	6	2	2	1	1	1			7100	0.4	230,000	2	14	7	1	2	1
21	1	1	1	40	1	3	2	2	1	1	3	respiratory distress	10.5	4000	0.6	20,000	2	18	5	3	1	1
22	1	2	2	37	2	4,6	2	2	2	2	2	sclerema,shock	7.2	21000	0.8	55,000	2	22	28	5	1	2
23	1	2	2	39	2	4	1	1	1	2	2	shock	9.6	5300	0.2	190,000	2	12	5	1	2	1
24	1	1	1	37	1	2	1	2	2	1	1	respiratory distress	11.2	10600	0.4	98,000	1	15	0	1	2	1
25	1	1	1	38	1	3	1	1	1	1	1		12.5	8200	nil	536,000	2	3	0	1	3	1
26	1	1	1	39	1	4	1	1	1	1	1		14	7500	nil	270,000	1	11	0	1	3	1
27	1	2	2	38	2	4	1	1	1	1	1		15	6900	nil	250,000	1	2	0	1	3	1
28	1	1	1	37	1	5	1	1	1	1	1		13.6	8600	0.3	290,000	1	10	5	1	3	1
29	1	1	1	39	1	4	1	1	1	1	3	flushed AF	12.9	23000	0.7	40,000	2	15	6	3	1	1
30	1	2	2	40	2	2	1	2	2	1	2		11	17500	0.4	110,000	2	12	7	1	2	1
31	1	1	3	40	2	5	1	1	1	1	1		12	9400	nil	420,000	1	10	0	1	3	1
32	1	1	1	37	1	4	1	1	1	1	1		13	7600	nil	320,000	1	10	0	1	3	1

33	1	2	2	37	1	3	1	1	1	1	1		12.6	8600	nil	280,000	2	11	0	1	3	1	
34	1	2	1	38	2	3	1	1	1	1	1		14.1	3000	0.8	30,000	2	21	17	2	1	1	
35	1	2	1	37	1	6	2	2	2	1	1		7.5	15600	0.5	40,000	2	11	3	2	1	1	
36	1	1	1	38	1	2	1	2	1	1	1		11.5	10500	0.4	56,000	1	17	7	1	2	1	
37	1	1	3	40	2	4	3	2	2	2	2		6	1000	nil	78,000	2	21	30	1	2	2	
38	1	1	1	39	1	4	1	1	1	1	1		14	7200	nil	476,000	1	2	0	1	3	1	
39	1	2	2	38	2	3	1	1	1	1	1		12.2	4800	nil	420,000	1	11	0	1	3	1	
40	1	1	2	38	2	3	1	1	1	1	1		13.8	5900	0.2	425,000	1	10	4	1	3	1	
41	1	1	2	38	1	4	1	1	1	1	1		11.3	6800	0.35	250,000	2	11	4	1	2	1	
42	1	2	1	37	2	4	1	1	1	1	1		10.6	14400	0.5	72,000	2	12	6	1	2	1	
43	1	2	2	39	1	6	2	2	2	2	2	sclerema	9.8	9000	0.9	25,000	2	12	22	2	1	2	
44	1	1	2	38	2	2	1	2	1	1	1		12.6	5200	0.3	68,000	2	4	0	3	1	1	
45	1	1	1	38	1	2	1	1	1	1	1		11.6	5600	nil	270,000	1	10	4	1	3	1	
46	1	1	1	39	1	3	1	1	1	1	1		10.2	12600	0.6	160,000	2	22	20	4	1	1	
47	1	2	2	40	1	4	1	1	1	1	2		9	3200	0.4	230,000	2	11	6	2	1	1	
48	1	2	3	38	2	6	1	2	1	1	1		12	6900	0.3	225,000	2	3	0	1	2	1	
49	2	1	2	39	2	1	2	2	2	2	2	shock	6.2	2100	0.9	20,000	2	17	23	3	1	2	
50	2	1	1	38	2	1	2	2	2	2	1	respiratory distress	10.2	14600	0.42	50,000	2	10	12	5	1	1	
51	2	1	1	37	1	2	1	1	1	1	1		14	7600	nil	386,000	1	0	0	1	3	1	
52	2	2	2	37	1	1	1	2	1	1	1		11	9200	nil	325,000	1	0	0	1	3	1	
53	2	2	1	37	1	1	2	2	1	2	1	shock	12.3	4600	nil	298,000	1	1	0	1	3	1	
54	2	1	3	40	2	3	1	1	1	1	1		10.6	18000	0.5	278,000	2	12	8	2	2	1	
55	2	2	2	38	1	3	2	2	2	2	2	respiratory distress,shock	9.2	2500	0.7	35,000	2	14	18	1	2	2	
56	2	1	1	38	1	1	1	1	1	1	1		14.5	4800	nil	290,000	1	3	0	1	3	1	
57	2	1	1	37	1	1	1	1	1	1	1		12.9	6500	nil	254,000	1	4	0	1	3	1	
58	2	2	2	39	1	4	2	1	1	1	1		11.6	3500	0.3	297,000	1	17	15	5	1	1	
59	2	1	1	38	2	1	2	2	2	2	2	shock	9.6	1600	0.6	30,000	2	18	20	6	1	2	
60	2	1	1	37	1	3	1	1	1	1	1		11.2	10700	0.2	180,000	2	3	0	1	2	1	
61	2	2	3	39	2	1	1	2	2	1	1	respiratory distress	10.6	3200	0.8	22,000	2	13	19	1	2	2	
62	2	2	1	37	2	2	1	1	1	1	1		15.4	4900	nil	179,000	2	1	0	1	3	1	
63	2	2	2	38	1	6	1	1	1	1	1		14.2	5600	nil	410,000	1	3	0	1	3	1	
64	2	1	1	37	1	1	1	2	2	1	2	respiratory distress	10.9	9600	0.2	110,000	1	4	0	4	1	1	
65	2	2	1	39	2	3	2	1	1	2	1		12.6	11500	0.35	225,000	2	15	12	7	1	2	
66	2	1	2	39	2	1	1	2	1	1	1		11.2	8300	nil	300,000	2	12	7	1	3	1	
67	2	1	1	37	1	1	1	2	1	1	1		12	9600	nil	316,000	1	0	0	1	3	1	
68	2	2	1	38	1	1	1	2	2	1	1	respiratory distress	11.5	6500	0.2	229,000	1	3	0	1	3	1	
69	2	2	2	39	2	2	1	1	1	1	1		14.1	4500	0.25	321,000	1	0	0	1	3	1	
70	2	1	2	38	1	2	1	1	1	1	3	fever	12.3	9800	0.3	90,000	1	5	8	3	1	1	
71	2	2	2	37	1	3	1	1	1	1	1		12.5	6800	0.3	163,000	1	8	3	1	2	1	
72	2	1	3	40	2	1	1	2	1	2	1	shock	9.5	14300	0.4	225,000	2	15	13	1	2	1	

Key to masterchart	
Age	<72 hours-1
	>72 hours-2
sex	Male-1
	Female-2
Birth weigh	2.5-3 kg -1
	3-3.5 kg -2
	>3.5 kg -3
Type of deli	labour natural -1
	LSCS -2
Risk factors	Clinical features of sepsis - 1
	Febrile illness in the mother with evidence of bacterial infection within 2 weeks prior to delivery - 2
	Foul smelling and/or meconium stained liquor -3
	Rupture of membranes >24 hours - 4
	Single unclean or > 3 sterile vaginal examination(s) during labor - 5
	Prolonged labor (sum of 1st and 2nd stage of labor > 24 hrs) -6
Heart rate	Normal - 1
	Tachycardia - 2
	Bradycardia - 3
Respiratory	<60 /mt -1
	>60 /mt -2
SpO2	normal (>92%) - 1
	abnormal (<92%) -2
CRT	< 3 sec -1
	> 3 sec - 2
Temperatu	Normal -1
	Hypothermia -2
	Hyperthermia -3
CRP	negative -1
	positive - 2
Blood cultu	No growth - 1
	klebsiella pneumonia - 2
	CoNS - 3
	Non-fermentative gram negative bacilli - 4
	Staphylococcus aureus - 5
	E-coli -6
	others (Enterococcus,Commensals)- 7
Final diagnc	Culture positive sepsis -1
	Clinical sepsis - 2

		No sepsis - 3							
Outcome		Survived -1							
		Expired - 2							

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ETHICS COMMITTEE CERTIFICATE

Name of the Candidate	:	Dr.Aarushi Manu
Course	:	PG in MD., Paediatrics
Period of Study	:	**2015-2018**
College	:	MADURAI MEDICAL COLLEGE
Research Topic	:	Nucleated red blood cell count as an early prognostic marker for adverse neonatal outcome in neonatal sepsis
Ethical Committee as on	:	21.04.2017

The Ethics Committee, Madurai Medical College has decided to inform that your Research proposal is accepted.

Member Secretary

Chairman

Prof Dr V Nagaraajan
M.D., MNAMS, D.M., Dsc.,(Neuro), Dsc (Hon)
CHAIRMAN
IEC - Madurai Medical College
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However, these conventional sepsis evaluation

3 parameters have low sensitivity and are nonspecific, often demonstrating increased level response to various other neonatal conditions such as meconium aspiration, prolonged rupture of membranes, asphyxia, and the birth process. The definitive diagnosis of sepsis rests upon isolation of pathogenic bacteria in blood cultures, which has low sensitivity and takes time to influence initiation of antibiotic therapy. 28 Further diagnostic limitations of the blood culture method include a higher incidence of false negative results, due to low blood volume drawn for culture and antenatal antibiotic use that may influence subsequent bacterial growth. As a result, early antibiotic therapy is frequently initiated for presumed infection or delayed due to uncertainty increasing disease risk. Thus, early, accurate,