A STUDY TO EVALUATE THE DIAGNOSTIC VALUE OF A PANEL OF INVESTIGATIONS IN EARLY DIAGNOSIS OF SEPTICEMIA IN NEW BORN

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CERTIFICATE

This is to certify that the dissertation entitled **"A Study to Evaluate the Diagnostic Value of a Panel of Investigations in Early Diagnosis of Septicemia in New Born"** is a bonafide work done by **Dr.VIJAY PRABHU.S** in **M.D BRANCH VII PAEDIATRIC MEDICINE** at Government Mohan Kumaramangalam Medical College, Salem, to be submitted to The Tamil Nadu Dr.M.G.R Medical University, in fulfilment of the University Rules and Regulation for the award of M.D. Degree Branch I Paediatric Medicine, under my supervision and guidance, during the academic period from May 2008 to April 2011.

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DECLARATION

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CONTENTS

ABBREVIATIONS

Hb%	-	HEMOGLOBIN
TC	-	TOTAL LEUCOCYTE COUNT
DC	-	DIFFERENTIAL COUNT
B/N	-	BANDFORM TO NEUTROPHIL RATIO
CRP	-	C-REACTIVE PROTEIN
ANC	-	ABSOLUTE NEUTROPHIL COUNT
m-ESR	-	MICRO ERYTHROCYTE SEDEMENTATION RATE
PPV	-	POSITIVE PREDICTIVE VALUE
NPV	-	NEGATIVE PREDICTIVE VALUE

INTRODUCTION

"Neonatal septicemia is defined as a bacterial infection documented by a positive blood culture in the first four weeks of life."

In developed countries, 0.2 to 11.2 per 1000 live births have evidence of sepsis with mortality of 9 to 15 %. In developing countries like India the values are around 10 to 30 per 1000 live births of which mortality is 30 to 40%. Neonatal sepsis is one of the leading causes of neonatal mortality and morbidity in newborns in our country.

Systemic bacterial infections during the first month of life have remained a major cause of infant morbidity and mortality despite the development of broad spectrum antimicrobial agents and technological advancements in life supportive therapy. In recent year's improvement in neonatal intensive care resulted in increased survival. However neonatal survivors of sepsis can have severe neurologic sequel due to CNS infection as well as from secondary hypoxemia resulting from septic shock, persistent pulmonary hypertension and severe parenchymal lung disease. Extremely low birth weight babies who had neonatal infection were more likely to have cerebral palsy and a range of adverse neurodevelopmental sequele[1].

Early recognition of neonatal sepsis is vital to the optimal outcome of this serious illness. The early diagnosis of neonatal septicemia still poses great difficulties. Early clinical symptomatology of neonatal septicemia is mimicked by lot of other disorders affecting the newborn. It is a major cause of morbidity and mortality and it accounts for half of all the neonatal deaths in this country.

Neonatal sepsis can be divided into two subtypes depending upon whether the onset of symptoms is during the first 72 hours of life or later. Although the term early onset sepsis had been used to refer to neonatal infections occurring as late as one week of age, it should be restricted to those infections with a perinatal pathogenesis, the usual onset of which occur within 72 hours.

Early - onset sepsis is caused by organisms prevalent in genital tract or in the labour room. Ascending infection, transplacental hematogenous spreads are important mechanisms of early onset sepsis. The organisms enter the body through the umbilicus, skin or mucosa. Due to poor immunological defense of the new born, even local infections tend to become generalised. Early onset sepsis can manifest as a fulminant disease with immediate onset of respiratory distress soon after delivery or on day one to three of postnatal life after an asymptomatic period. Infections are more commonly met with preterm and low birth weight babies.

To prevent serious morbidity and mortality caused by untreated or lately treated neonatal septicemia early diagnosis and treatment is vital. Even though the positive blood culture is diagnostic of neonatal septicemia, the technique of blood culture is time consuming that demands a well equipped laboratory and has a success rate of around 40%, culture reports are available only after 48 to 72 hours and therefore the blood culture has its own limitations.

Early treatment with rational antibiotic therapy is possible with the help of certain indirect markers such as Total leukocyte count, band form to neutrophil ratio, absolute neutrophil count, micro-ESR and C-reactive protein. These investigations are collectively known as **Sepsis Screen.**

The early diagnosis of neonatal sepsis by clinical examination is vital. In the presence of predisposing factors, early clinical suspicion coupled with sepsis screen will detect neonatal septicemia earlier, which will enable the clinician to treat the infection timely and adequately, which in turn will help to reduce the neonatal morbidity and mortality. The basic treatment of infant with sepsis has not changed substantially over the last 50 years (antibiotics with supportive care).So it is likely that further improvement in outcome will result from a greater understanding of perinatal factors responsible for sepsis, interventions that address these factors and better ways to identify the infected newborn.

So this study was done to evaluate the usefulness of absolute neutrophil count, band form to total neutrophil ratio and CRP as an early marker of neonatal septicemia because these are simple, readily available bed-side tests and can be done within a short time before starting the neonate on antibiotic therapy. Sepsis screen was cost-effective in decreasing antibiotic usage in the nurseries. (Philip)[2].

AIM OF THE STUDY

1. To study the diagnostic value of the combination of CRP, absolute neutrophil count, band form count to total neutrophil ratio in early diagnosis of neonatal septicemia.

2. To validate the combination of the above tests against the blood culture which is considered the gold standard in detecting sepsis in newborn.

HISTORICAL ASPECTS AND REVIEW OF LITERATURE

Septicemia as cause of neonatal mortality was first recognized by Yippo in 1919. He documented positive culture results from dying infants. Dunham described 33 cases of neonatal sepsis at new heaven hospital. In 60% of these cases onset of sepsis was in first week of life but only 10% were symptomatic at birth. By the end of the nineteenth century Budin and others had called attention to the four chief sources of infection; the skin, umbilicus, the gastrointestinal and respiratory tracts.

INCIDENCE :

Incidence of neonatal septicemia differs among hospitals, depending on the factors such as obstetric and nursery practices, prenatal care, the health and nutrition of the mother and the incidence of prematurity. But the important factors to be considered is that the overall incidence of sepsis neonatorum, while it may vary from year to year and from place to place, has fairly remained constant over the past 30-40 years (David Wilson) [3].

According to National Neonatal-Perinatal Database (2002-03)[4], the incidence of neonatal sepsis in India was 30 per 100 live births. Klebsiella pneumoniae and staphylococcus aureus were the two most common organisms isolated.

The database comprising 18 tertiary care neonatal units across India found sepsis to be one of the commonest causes of neonatal mortality contributing to 19% of all neonatal deaths. Septicemia was the commonest clinical category with an incidence of 23 per 1000 live births while the incidence of meningitis was reported to be 3 per 1000 live births.

- Various authors have given different rate of incidence in their reviews. Freedman et.al. in 1981[5] reported that the incidence was 2-4 cases / 1000 live births , fairly constant over many years.
- T.Vesikari in 1985[6] reported the annual incidence of neonatal septicemia was 3/1000 live births and the overall mortality was 23%.
- Lokeshwar in 1988[7], reported that incidence in the developing countries like ours was much higher, 10-12/1000 live births compared to developed countries 1-10 cases /1000 live births.
- Moreno et.al. in 1994[8] observed overall incidence of 3.5 cases /1000 live births. Incidence was high in very low birth-weight and premature babies.
- Moro et.al. in 1996[9] found the incidence of nosocomial sepsis in neonatal intensive care units to be 2.9 cases / 100 newborns and 0.2cases / 100 days of stay. Sepsis represented 15.4% of all infections.
- Dawodu et.al. in 1997[10] found the incidence of neonatal septicemia 4.9 cases / 1000 live births.

- Berger et al. in 1998[11] found the incidence of neonatal bacterial sepsis 9.8 cases/1000 live births.
- Mc Cracken et al.[12] reported incidence of septicemia as 1 per 1000 live births and 1 per 230 premature births.

Risk factors of neonatal infections:

Maternal factors:

1. **Prolonged rupture of membrane**

- 49% of infection with GBS and 79% of other infants with sepsis had one more of three major maternal risk factors like signs and symptoms of chorioamnionitis PROM>18 hrs, colonization of GBS (Schachter et.al. 2000[13]) .
- Microbial pathogens can be recovered from amniotic cavity in 10-15% of case of spontaneous preterm labour and in 32-35% of women with PPROM and chances of neonatal infection increases by three fold in prolonged rupture of membrane. (N. Mehrotra[14)] .

2. **Prolonged labor**

- Prolonged labor found to be increasingly associated with neonatal sepsis.

3. **Infected Birth canal**

- Organisms that inhabit the cervix, vagina and rectum can spread upward in the amniotic cavity through intact or ruptured membranes and causes amnionitis. Intra amniotic infections are usually polymicrobial in etiology.
- Intra amniotic infection may be subclinical or clinical .But both types may result in serious neonatal morbidity. (Barton et. al.)[15]

4. **Unclean vaginal examinations.**

- Kuruvilla et.al. in 1998[16] found the maternal factors are significantly associated with early-onset sepsis. Those were meconium staining of liquor and multiple vaginal examinations.

Fetal factors:

1. **Prematurity**

Preterm babies have higher incidence of septicemia than term babies.

- Subclinical chorioamnionitis may be a common cause of preterm labour and PPROM (Hiller et al. 1988)[17].

2. **Low birth weight**

Low birth weight babies have higher incidence of septicemia.

- K.C. Buetow et al. (1965)[18] studied septicemia in preterm babies weighing 1000-2500 grams. They concluded that incidence of

septicemia was 54.3 per 1000 live preterm births. There was increasing mortality rate with decreasing birth weight.

3. Sex

Males have an approximately two fold higher incidence of sepsis than females. Females have greater genetic diversity resulting from random inactivation of one of the two X-chromosomes. Female resistance is due to her heterozygocity for genes of X-chromosomes.

- Neonatal sepsis is twice frequent in male infants as compared to females (Nelson) [19].
- Gluck (1966) [20] and N. Sinha et al. (1986) [21] observed that males were more prone to infection than females (ratio of 1.7%).
- Beutow and Gotoff[18] et al. agreed for male predominance.
- Khatua et al. [22] observed male predominance (70.7%) and concluded that presence of one X-chromosomes in the male confers less immunological protection compared to the female counterpart.
- Schaffer A.J. [23], Somu et al. [24] observed neonatal septicemia more common in males ranging from 59 to 82 percent. Female resistance is due to her heterozygocity for genes of X-chromosomes controlling immunoglobulin synthesis and thymic function which results in a greater heterozygocity of antibody response (Robert J. Schlepal et al. 1969) [25].

- Male infants have demonstrated a higher incidence of sepsis than female infants (wash burn et. al. 1965)[26].

4. Twin gestation

Twin gestations may be at increased risk for GBS sepsis even when corrected for prematurity[27].

5. Perinatal asphyxia

In a study by St. Geme et al[28] a 5-min Apgar score <6 in the presence of PROM was as strong a predictor of neonatal sepsis as chorioamnionitis. Similarly, a Danish study found that 27% of preterm infants with PROM and perinatal asphyxia have proven sepsis [29].

6. Mode of delivery

The incidence of positive blood culture was least in babies born by normal delivery and maximum in those delivered by caesarean section (Mehrotra)[14] . Observations of David[3] are same in this regard.

The incidence of positive superficial culture was higher in home delivery than hospital, while positive gastric aspirate culture was higher in the latter. Frequent superficial infections in the home delivered babies appear to result from many unhygienic surroundings of poor homes and also large number of visitors handling babies.

Higher rates of perinatal deaths due to infections, asphyxia and other causes are known with abnormal presentation of difficult labour. Higher incidence of infections in babies delivered by caesarian section appears to result from factors that necessitate it such as obstructed labour, prolonged second stage etc.

Classification of neonatal sepsis

Neonatal sepsis can be classified into two major categories depending up on the onset of symptoms[30].

Early onset sepsis (EOS):

It presents within the first 72 hours of life. In severe cases, the neonate may be symptomatic at birth. Infants with EOS usually present with respiratory distress and pneumonia. The source of infection is generally the maternal genital tract. Some maternal / perinatal conditions have been associated with an increased risk of EOS. Infants with two risk factors should be investigated and then treated accordingly.

Late onset sepsis (LOS):

It usually presents after 72 hours of age. The source of infection in LOS is either nosocomial (hospital-acquired) or community-acquired and neonates usually present with septicemia, pneumonia or meningitis[34, 35].

Various factors that predispose to an increased risk of nosocomial sepsis include low birth weight, prematurity, admission in intensive care unit, mechanical ventilation, invasive procedures and administration of parenteral fluids.

Clinical features

Non-specific features:

The earliest signs of sepsis are often subtle and nonspecific; indeed, a high index of suspicion is needed for early diagnosis. Neonates with sepsis may present with one or more of the following symptoms and signs

(a) Hypothermia or fever

(former is more common in preterm low birth weight infants)

(b) Lethargy, poor cry, refusal to suck

(c) Poor perfusion, prolonged capillary refill time

(d) Hypotonia, absent neonatal reflexes

(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, vacant stare, high-pitched cry, excess irritability, stupor/coma, seizures, neck retraction. Hypotonia, hyporeflexia, abnormal Moros & lethargy

Presence of these features should raise a clinical suspicion of meningitis.

Cardiac	:	Hypotension, poor perfusion, shock.
Respiratory system	:	Apnea, dyspnea, tachypnea, chest retractions, nasal flaring grunting & cyanosis.
Gastrointestinal	:	Feed intolerance, vomiting, diarrhea, abdominal distension, paralytic ileus, necrotizing enterocolitis (NEC).
Hepatic	:	Hepatomegaly, direct hyperbilirubinemia.
Renal	:	oliguria,Acute renal failure
Hematological	:	Bleeding, petechiae, purpura.
Skin changes	:	Multiple pustules, abscess, sclerema, mottling, umbilical redness and discharge.

Investigations

Selecting a screening test:

Four important characters of lab test when evaluating an infant for possible sepsis are

1. Sensitivity
2. Specificity
3. Positive predictive accuracy.
4. Negative predictive accuracy

- Sensitivity of a lab test is defined as the proportion of infant with proven sepsis in whom the result is abnormal. A sensitive test will rarely miss an infant with sepsis.
- Specificity is the proportion of healthy infants in whom the result is normal. A specific test will rarely miss diagnose an infant who is healthy.
- Positive predictive accuracy is the probability that an infant with abnormal screening test is infected.
- Negative predictive accuracy is the probability that an infant with normal screening result is healthy.

For screening of a common and severe condition like septicemia we need to select the tests with good sensitivity and with good negative predictive accuracy so that chances of missing the babies with infection will

be very low. There may be reasonable negligible compromise in specificity and in positive predictive accuracy as over diagnosing and treating with antibiotics do not cause much harm. On the other hand missing an infant with infection will be detrimental .Moreover first line drugs used to treat septicemia usually have good safety profile. At the same time indiscriminate use of antibiotics can lead to antibiotic resistance in the community.

Panel of screening tests and their usefulness.

Direct methods of screening include these following.

- Examination of gastric aspirates for polymorphonuclear cells had been used as a screening procedure for noenatal sepsis. But a careful analysis of origin of cells in gastric aspirate had indicated a maternal origin (Vasan et al, 1977[33]). Therefore presence of polymorphonuclear cells does not indicate fetal infection. Several other studies have also shown that gastric aspirate examination is not a useful indicator of neonatal infection[34].
- Avery's text book[35] of paediatrics states that given the low yield of urine culture it should not be the part of traditional sepsis screening in the first 72 hrs of life.
- Microscopy of buffy coat smear stained with methylene blue.

A number of indirect markers have been used to diagnosis sepsis. Among them commonly available and widely used indirect markers of infection are

1. Total leucocyte count
2. Absolute neutrophil count
3. (Band forms) Immature to total neutrophil ratio > 0.2
4. C-reactive protein

(Manual of neonatal Care by Cloherty 6th edition)[36] .

When these are studied collectively, called ***'Sepsis Screen '.***

Other newer investigations

Procalcitonin assay. It superior to CRP in the way it can distinguish infection and inflammation and differentiate between bacterial and viral infections with high specificity.

Fibronectin

Alpha 1 antitrypsin

Alpha 1 chymotrypsin

Haptoglobulin.

Fibrinogen

IL-1

IL-6

IL-8

TNF-α

G-CSF

Leucocyte α3 protienase inhibitor

CD116

CD64

PCR for genetic DNA of microbes

These markers have not yet made progress from laboratory to clinical application.[40]

Total leucocyte count:

Normal count ranges from 9000 to 30,000 cells/mm^2 at the time of birth and the differences in the site of sampling and activity of the baby can affect this value.

- Christensen and Rothstein[38] in 1979 found venous blood leucocyte count were 82% of simultaneously drawn capillary blood values. These investigators also reported that in samples collected after violent crying WBC count increased to 146% of baseline values.
- Total leucocyte count are not very helpful in diagnosis of septicemia in newborn.[39,40].

- Blood leukocytes count in the newborn babies have been considered to be so variable and unpredictable as to be of little value for clinical diagnosis[39,40].
- M.Xanthou in 1970[41] studied leucocyte blood picture in healthy full term and premature babies during neonatal period. Serial leukocyte counts were done on 15 full term during first 10 days of life and on 14 preterms during first 30 days of life. The main changes in the leucocyte count during the neonatal period were as follows – an increase in polymorphonuclear neutrophils after birth reaching a peak at 12 hours, thereafter dropping to a figure which remains fairly constant from 72 hours onwards.
- Leukocyte count varies from 8000 to 20000 per cu,mm during the 1st 28 days of life without demonstrable disease (steigbiggel R.T[42])

The micro-Erythrocyte sedimentation rate:

Micro ESR is performed by measuring in millimeters ,the settling of erythrocytes in a vertically placed capillary tube in 1 hr. Normal value increases with post natal age and are equal to the day of life plus 3mm/hr, upto a maximum of 15mm/hr. The micro-Erythrocyte sedimentation rate is a nonspecific indicator of tissue damage and is known to be elevated in infective states.

The rate of increase depends on the severity of the morbid process. False positive reactions can occur with hemolysis and even in physiological jaundice, whereas false negative results may be due to disseminated intravascular coagulation with consumption of fibrinogen which decreases roleaux formation[43].

- Anita Sharma et al[44] in study of 65 clinically suspected cases of neonatal septicemia reported the elevated C-reactive protein and elevated micro – ESR compared to controls at the time of diagnosis, but micro – ESR had no prognostic significance and C-reactive protein levels decreased with treatment.

Absolute neutrophil count and Immature to total neutrophil ratio:

Neutrophil indices like absolute neutrophil count and the ratio of band form to total neutrophil count(I/T ratio)has proven more useful than other indices.

The lower level of neutrophil count is 1700cells/mm2 at birth raises to 7200cells/mm2 by 12 hrs of life. It declines to 1720/mm2 by 72 hrs of life[45].

Immature neutrophil or bandform is a neutrophil in which the width of the narrowest segment of the nucleus is not less than one third of the broadest segment. Immature neutrophils (Band cells + myelocytes + metamyelocytes) to total neutrophils ratio (I/T) > 0.20 means that immature

neutrophils are over 20 percent of the total neutrophils because bone marrow pushes even the premature cells into circulation, to fight infection.

The absolute band form count also undergoes similar changes postnatally. It attains it's peak value of 1400cells/mm3 at 12 hr of life and then declines. I/T ratio is maximum at birth 0.16 and then decline to a value of.12 at 72 hrs of life.

- The reference ranges of each of these indices were established by Monroe et al[45] in 1979.
- Mouzinho A et al[46] observed very low birth weight babies i.e <1500gm (<30 week) often had neurtophil indices that did not fall within the range of Manroe's[48] .His reference range is given in table 1

TABLE I

Mouzinho A et al[46] Revised reference ranges for circulating neutrophils	**Absolute neutrophil count**	
	Minimum	**Maximum**
Birth	500	6000
18hrs	2200	14000
60hrs	1100	8800
120hrs	1100	5600

- Robert D. Christensen in 1981[38] found that the band form to neutrophil ratio was more frequently abnormal during neonatal sepsis than the absolute netrophil count.
- Robert Boyle et al.(1978)[47] also observed the usefulness of absolute neutropenia and band neutrophil ratio in identifying septic from non septic infants with respiratory distress syndrome.
- Monroe et al[45]. in 1979 observed that mild or early-onset of infection caused a significant increase in absolute value of neutrophils. The values were as, high as 17,500/cmm.
- Monroe et al.[45] also observed a 100% negative predictive value if the total neutrophil count, immature neutrophil count, and I/T were all normal.

C-reactive protein:

CRP was first detected by Tillett and Francis[48] in 1930. The most widely used acute phase reactant is C-reactive protein (CRP) which has a high degree of sensitivity for neonatal sepsis. The CRP gene is located on the first chromosome (1q21-q23). CRP is a 224-residue protein[5] with a monomer molar mass of 25106 Da.

CRP was originally discovered as a substance in the serum of patients with acute inflammation that reacted with the C polysaccharide of

pneumococcus[48]. The substance responsible for reactivity was termed C-precipitin and it was protein.

It consist of 5 non covalently bound identical sub units each containing 187 amino acids with one intra chain di sulphide bond and no carbohydrate modifications.. Later it was designated as C-reactive protein.

CRP is a rapidly responsive acute phase reactant synthesized in liver by the 6 to 8 hrs of an inflammatory stimulus. CRP may not be elevated early in course of infection.

CRP is one of the best available diagnostic tests for diagnosis of neonatal septicemia. Since the protein is produced by the fetus and neonate and it does not pass the placental barrier, it can be used for early detection of neonatal sepsis.[49]

Mathers and Polhandt[50] found that the sensitivity of CRP>1.0 was 16% on admission for sepsis evaluation, but 92% at 24 hrs of age. C-reactive protein is present in response to variety of inflammatory stimuli. It appears early in the acute phase and declines during convalescence.

Normal values in newborn < 0.3 mg/100 ml.

- CRP may also elevate in conditions such as meconium aspiration syndrome, prolonged rupture of membrane and shock.
- Felix et al.[51] and Hansen et al.[52] observed increased C-reactive protein more constantly in septicemia. Sabel et al.[53]. studied in cases of neonatal septicemia increased C-reactive protein in 85.7% cases with

positive blood culture. Thus the C-reactive protein test is a good diagnostic indicator of infection as blood culture in case of septicemia.

- As the biological half life of CRP is only 24 hours, CRP accurately parallels the activity of the inflammatory process and its concentration decreases much faster than ESR or any acute phase parameter which is useful in providing appropriate treatment.
- Sann et. al[54] in 1984 studied 36 newborn infants with septicemia and observed the inverse change in C-reactive protein with infected neonates. The immediate decrease in C-reactive protein reflects effect of treatment. And the later decrease parallels the clinical course of the infection. Thus determination of these tests can help to guide the treatment of infection in newborn.
- Alistair G. S. Philip[55] reported efficiency of C-reactive protein in diagnosing early septicemia. It becomes positive when concentration of C-reactive protein is approximately 0.8 mg % and more than this value is significant. This test had specificity of 86%.
- P.Hindocha[56] in 1984 observed 11 out of 12 cases with raised C-reactive protein. He also observed that the C-reactive protein level decreased in many cases after treatment.
- K. Kalra[57] in 1985 in his study of 76 cases of neonatal infection showed C-reactive protein was positive in 69.7% cases. A positive C-

reactive protein test in the presence of negative culture after antibiotic therapy seen in 9.2% cases indicated the presence of neonatal infection.

- S. M. Ali et al[58]. in 1988 studied serial estimation of micro ESR and C-reactive protein in assessment of therapy in neonatal septicemia. All cases had significantly elevated micro ESR and C-reactive protein at the time of diagnosis. With treatment C-reactive protein level showed significant decrease as early as 3rd day.
- Gupta et al[59] in 1989 in a study of 150 newborns, 100 clinically septic, and 50 clinically aseptic cases of newborn infants. Out of 100 clinically septic newborns C-reactive protein was positive in 64% and C-reactive protein was found to be the most specific (96%) of neonatal septicemia.
- Anita Sharma in 1993[44]studied serial estimation of C-reactive protein in clinically suspected cases of septicemia. She found that significantly increased levels of C-reactive protein in suspected cases as compared to controls. A persistently positive C-reactive protein tests indicated bad prognosis. With treatment a declining trend of C-reactive protein was seen in survivors but in deteriorating babies the levels kept on increasing.
- Wagle et al[60]. in 1994 studied C-reactive protein as a part of sepsis screen. C-reactive protein levels of 10mg/L or above was considered

abnormal. He concluded that the C-reactive protein level when elevated on day one and / or day two the diagnosis of neonatal sepsis is extremely likely. And when the C-reactive protein level was within normal limit on day one and two of the septic episode, neonatal sepsis can be confidently excluded. C-reactive protein has proved to be most useful in suspected sepsis. It starts to rise within 12-24 hours of the onset of sepsis, earlier than the other acute phase reactants. Serum C-reactive protein was elevated in 15-90% of cases of sepsis and returned to normal within 2-7 days of successful treatment persistent elevation of the serum C-reactive protein may indicate persistent bacterial infection.

- Posen et. al in 1998[61] studied C-reactive protein as an indirect indicator of the presence and resolution of infection C-reactive protein has gained more recent wide spread use. C-reactive protein usually increases in a delayed manner with the onset of inflammation and infection and decreases as inflammation and infection resolves.
- Sabel et al. (1974)[62] studied 14 cases of neonatal septicemia and meningitis and compared blood culture with the results of CRP test in these cases they found raised CRP in 85.7% of cases with positive bacterial culture.

Blood culture in neonatal septicemia:

Blood culture for detection and typing of organisms remained the most specific investigation in neonatal septicemia since beginning. The technique of blood culture is time consuming that demands a well equipped laboratory and has a success rate of about 40%.

- O. N. Bhakoo et al. (1968)[63] in study of 70 neonates found that Staphylococcus pyogenes (32%) was the common organism followed by E-coli (16%) and Pseudomonas pyocyaneus (09%)..
- James C. Overall[64] in 1970 found that the Gram negative enteric bacteria were the most frequent etiologic agent in neonatal bacterial meningitis.
- N. Somu[22] in 1976 in the study of 725 cases of neonatal septicemia found that Coliform group of organisms were the commonest cause closely followed by Staphylococcus aureus. He also found the other bacteria like Pseudomonas pyocyaneus and Proteus responsible for neonatal septicemia.
- K. Chugh et al. (1987) [65] studied 250 sick neonates, 43 blood cultures were positive Klebsiella species (10%) was the commonest organism followed by Pseudomonas and Staphylococcus aureus and E.coli was the least only 7%.
- M.M.Placzek and A.Whitelaw[66] in 1983 reported that the Group-B Streptococcus and E-coli were the commonest causative organisms in

early onset septicemia and Staphylococcus epidermidis. Staphylococcus aureus and E-coli were the commonest organism responsible for late – onset septicemia.

- Mishra et al in 1985[67] studied 120 cases of neonatal septicemia and reported that the Gram-negative organisms were more common (71%) than Gram-positive organisms (49%) and also reported Gram-negative infection was higher in babies under 2000 gram birth-weight. Common bacteria isolated were E-coli, Pseudomonas, Staphylococci and mortality was high in Pseudomonas (76%) infection.
- Mehrotra et al.[11] in 1985 studied 75 symptomatic newborns with septicemia and E-Coli was the commonest organism on blood culture.
- Sinha et al[68]. in 198621 studied 82 cases of neonatal septicemia, of those Pseudomonas aeruginosa was most common followed by Klebsiella and E-Coli.
- Khatua et al[69]. 1986 in a study of 92 cases of neonatal septicemia showed blood culture was positive in 98% cases; of which 76.3% were the main Gram-negative organisms like Klebsiella, E Coli, Citrobacter, Pseudomonas and 23.7% were Gram-positive organisms predominantly Staphylococcus and Streptococcus.

- Sharma et al. 1987[70] found that Gram-negative septicemia was more common in newborn infant. He observed that E-Coli has replaced Klebsiella as the predominant pathogen of neonatal septicemia.
- Namdeo et al. in 1987[71] in the study of 50 neonates with septicemia reported that the Gram-negative organisms were found to be predominant in early onset septicemia and were often fatal whereas Gram-positive organisms were more frequent in late onset septicemia and were associated with favorable outcome. They found predominant organisms were E-Coli, Klebsiella, Proteus, Pseudomonas and Gram-positive cocci.
- P. Chaturvedi. M. et al. in 1989[72] found that Gram-negative organisms were more common than Gram-positive organisms. The culture positivity rate was 73% of those 24.9% cultures were polymicrobial. Among 1059 growths obtained 60.1% were Gram-negative organisms, mainly Klebsiella, E-coli, Pseudomonas and Gram-positive organisms mainly observed were Coagulase negative staphylococci (24%). An increasing incidence of Coagulase negative Staphylococci and Pseudomonas were observed.
- Mathur et al. in 1991[73] found that the Klebsiella (38.6%), Staphylococcus aureus (21.5%) were the most commonly isolated pathogens with higher mortality in infections with Gram-negative (63.5%) than with Gram-positive organisms (19.1%).

- Piyush Gupta et al. in 1993[74] in their study concluded that Klebsiella septicemia continues to be on priority list of nosocomial neonatal infections as evidenced by the rising incidence. Klebsiella septicemia affects the most vulnerable, has more incidences of complications and carries high mortality rate.
- Moreno et al. in 1994[8] in their study of 577 cases with culture proved sepsis found that Gram-negative bacilli particularly species of Klebsiella and E-coli were responsible for 61% of infections, whereas Gram-positive isolates especially Staphylococci and Candida were responsible for 37% and 2% respectively. Case fatality rate was 32%. Mortality was greater in infants with early-onset sepsis than in those with late infection.
- Koutouby et al.[75] in 1995 in study of 106 cases of neonatal septicemia found that most common organisms were Group B Streptococcus (23%), E-coli (17%), Staphylococcus epidermidis (18%), Klebsiella pneumoniae (16%). Group B Streptococcus was the most common cause for early-onset sepsis while the Staphylococcus epidermidis was the common cause for late-oneset sepsis. Psedomonas aeruginosa and Klebsiella pneumoniae had highest mortality.
- Endo et al. in 1996[76] found that Klebesiella pnuemonae and E-coli have been replaced by Staphylococcus aureus and Pseudomonas aeruginosa as the predominant isolate in newborn with sepsis.

- Samanci et al. in 1997[77] in their four year study of neonatal septicemia in neonatal intensive care unit concluded that the common causes of neonatal septicemia were Gram-negative bacilli and Staphylococci. Since the most common pathogen of hospital acquired sepsis was Gram- negative bacillus, higher mortality rates were observed in nosocomial sepsis. The overall mortality rate in neonatal sepsis was 44.2%. The mortality rate infants in whom nosocomial septicemia developed was significantly higher than in infants in whom early onset septicemia developed.
- Berger et.al. in 1998[78] found Coagulase negative Staphylococci, Gram-negative rods and Enterococcus faecalis as the major causative organisms for neonatal septicemia. Group B Streptococcus was the major pathogen of very early onset septicemia (within 24 hrs. of birth). Whereas late-onset infections were most commonly caused by Coagulase negative Staphylococci. Mortality rate was 14%.
- Kuruvilla et.al. in 1998[79] found that E-coli, E.faecalis were the predominant organisms causing early-onset sepsis, while Klebsiella and E. faecalis were the predominant organisms in late onset sepsis.

TABLE II

In Care of Newborn, 6th Ed. Meharban Singh observed the spectrum of bacterial pathogens analysed from hospital based data collected by National Neonatal Perinatal Database Network from different centers (1995)[80] in our country as follows.

Klebsiella pneumonae	29.7%
Staphylococcus aureus	14.7%
E.Coli	13.9%
Pseudomonas aeruginosa	09.2%
Enterobacter species	07.9%
Staphylococcus albus	07.2%
Candida species	04.8%
Acinetobactor	02.4%
Streptococcus viridans	01.4%
Others	08.7%

MATERIAL AND METHODS

Nature of the study: Prospective study

Period and place of study: This study was conducted in Department of Paediatrics, Neonatal unit, Government Mohan Kumaramangalam Medical College hospital over a period of 6 months between March to August 2010.

Study group: 75 babies who got admitted in Government Mohan Kumaramangalam Medical College hospital in newborn with age less than 3 days and with well defined maternal risk factors or clinical evidence of sepsis are included in the study.

Study method:

After admission detailed history was taken and thorough clinical examination was done. Then the babies satisfying the following inclusion and exclusion criteria were selected for the study.

Inclusion Criteria

Maternal risk factors

- PROM>18 hrs.
- Intrapartum temperature ≥ 100.4(38.0°C).

- Vaginal examination done > 3 times in labour.
- Foul smelling liquor.
- Untreated maternal UTI in last trimester.
- Babies with clinical evidence of sepsis.
    - Lethargy.
    - Refusal to feed
    - Abdominal distention
    - fever
    - Tachypnea.
    - apnea
    - Bradycardia.
    - Tachycardia.
    - Hypothermia
    - pallor
    - jaundice.

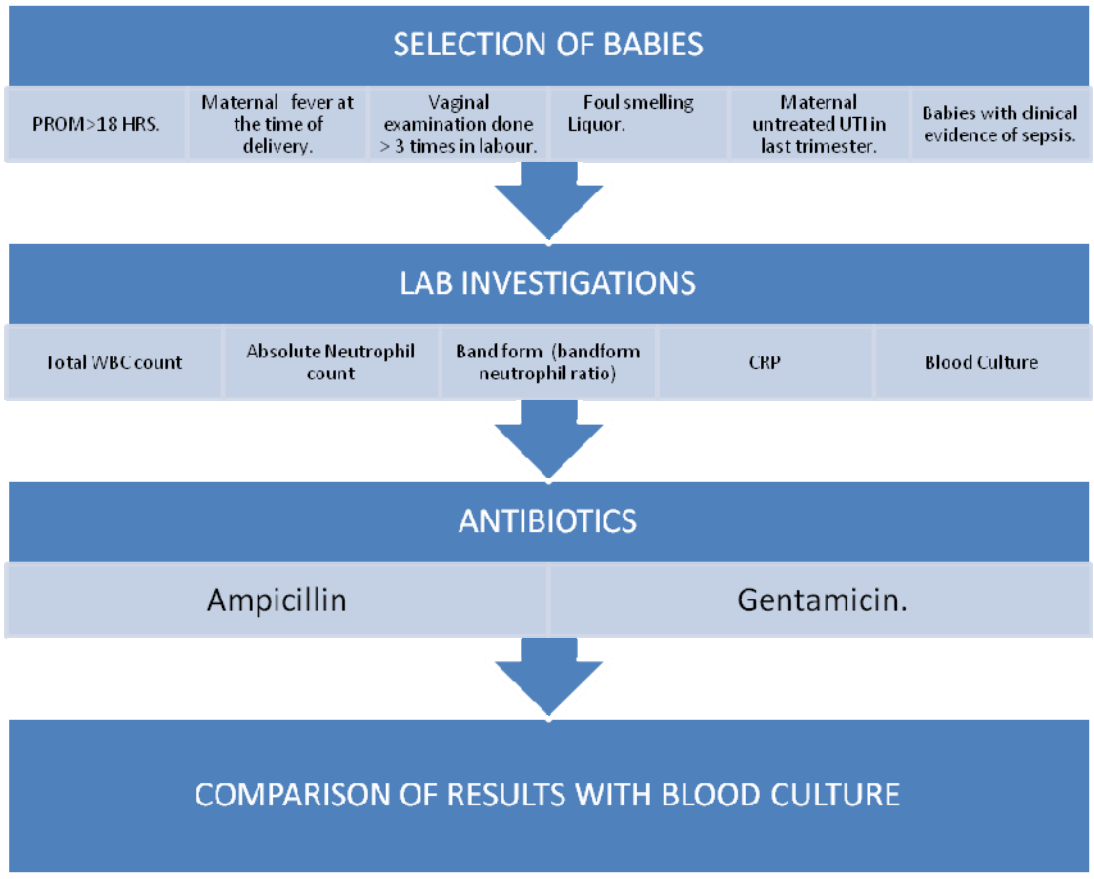
Exclusion Criteria:

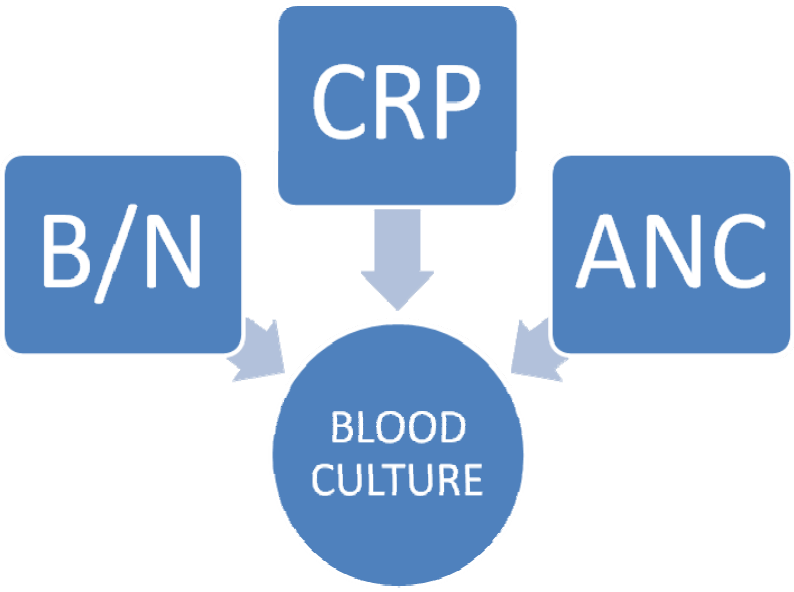
- Babies with RDS
- Perinatal asphyxia
- Meconium aspiration syndrome
- Babies weighing <1500gms
- Babies with congenital malformation.

- The following investigations were done in all the selected babies .
    - Total WBC count.
    - Absolute Neutrophil count.
    - Band form (bandform neutrophil ratio).
    - CRP.
    - Blood Culture.

All these details were recorded in a special proforma after obtaining informed consent from the parent/ guardian for registering the required data.

METHOD





Procedure:

In all neonates the blood sample was collected from peripheral vein with all aseptic precautions, prior to administration of any antibiotic therapy.

- Total count,
- Differential count(absolute neutrophil count),
- Band form(band form to neutrophil ratio)
- C-reactive protein and
- Blood culture

were all sent to respective labs.

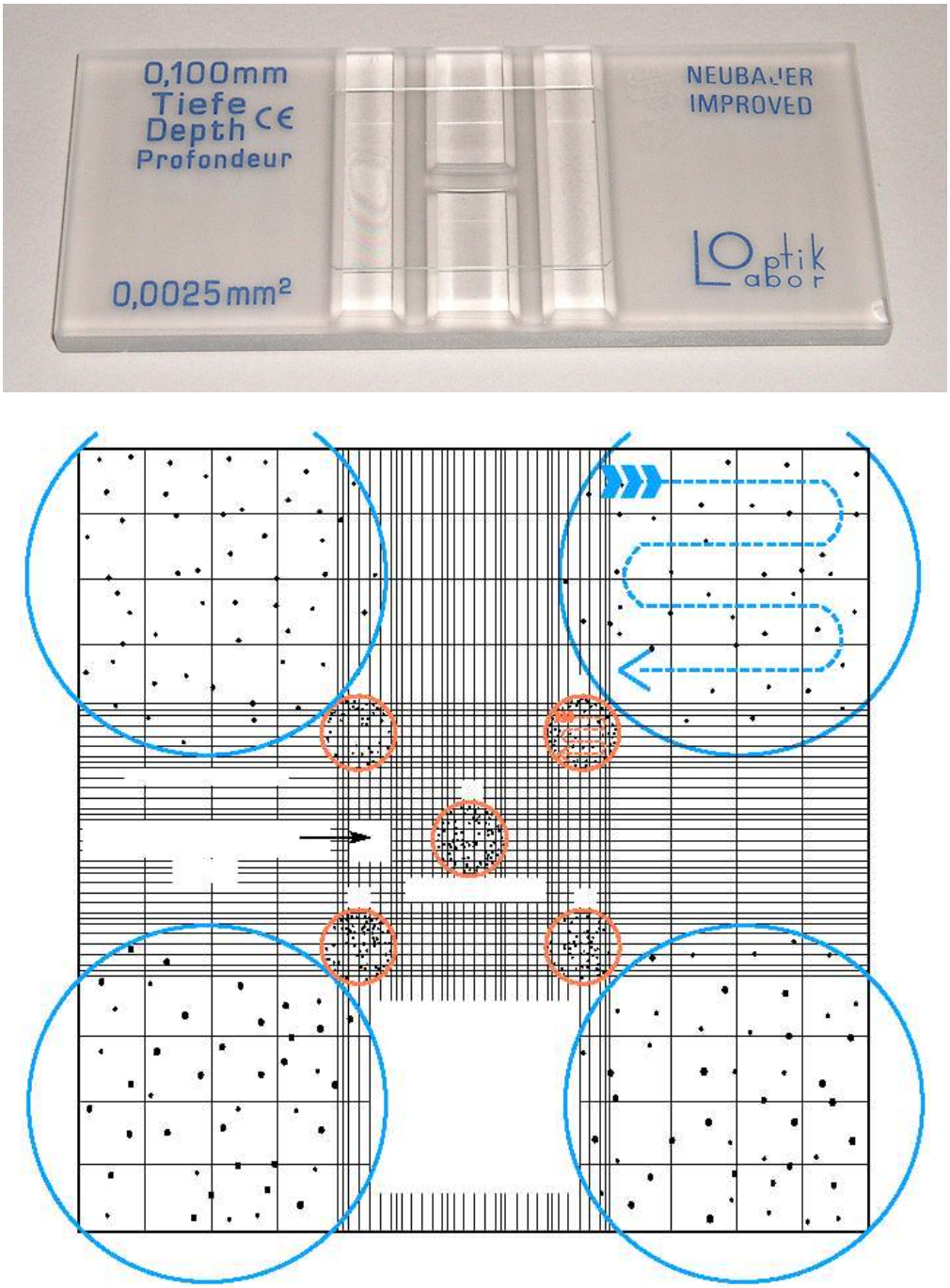


Fig. 1 : Neubauer's chamber

Total WBC Count:

Total WBC count was done using visual haemocytometer method. This was counting WBC in a calibrated chamber by diluting of blood to

1:20 dilution which causes lysis of RBCs and staining of WBCs. Diluting fluid used was Turk's fluid.

Anticoagulated blood was drawn up to mark 0.5 in WBC pipette. Tip and outside of pipette was wiped. It was mixed well by rotating the pipette for 2-3 minutes. Neubauer's chamber was charged with the mixture after discarding 1-2 drops from WBC pipette. Then the cells were allowed to settle down for 2-3 minutes. Then the WBCs were counted under low power (10X) in 64 small squares which are grouped into 4 large squares. Total WBC count was calculated from the formula.

- **Total WBC = X * 10 /4 *20** WBCs (*Where X is no. of WBCs counted in 4 corner groups of 16 squares and 20 is dilution factor*.)

Analysis of Differential leucocyte count

- The blood films were prepared and stained with Leishmans stain. Leishman stain is a compound dye –eosinate of methylene blue dissolved in acetone free methyl alcohol. Eosin is an acidic dye stains cytoplasm pinkish while methylene blue, a basic dye stains nuclear chromatin blue –violet.
- The stained blood film is examined under oil immersion and different types of neutrophils were identified one by one until 200 cells have been examined. The percentage distribution of each type of leucocyte is then determined.

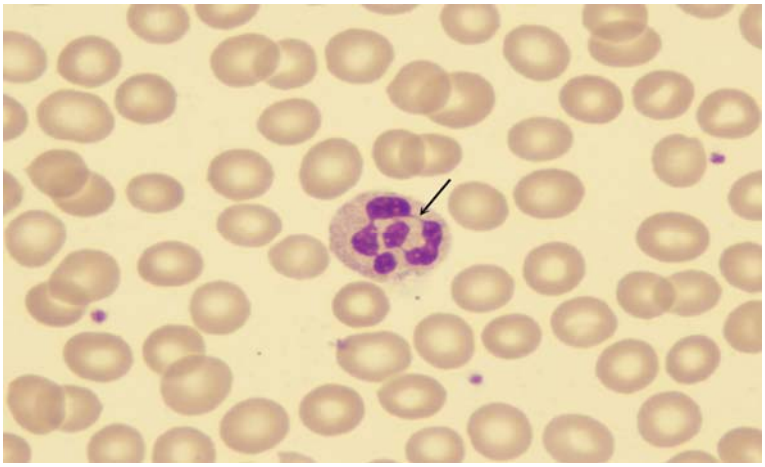


Fig. 2 : Neutrophil

Absolute neutrophil count

- Knowing the total leukocyte count and differential count, absolute neutrophil count can be calculated.
- Then the absolute neutrophil count and was noted in monroe's chart. Absolute neutrophil count outside the normal as seen in munroe's chart was taken as positive. Monroe's chart is the graph showing the normal range of absolute neutrophil according to age in hours. Shown in annexure no. 1.
- Band form neutrophil ratio was computed from differential count. Band form: total neutrophill ratio > 0.2 were considered as positive.

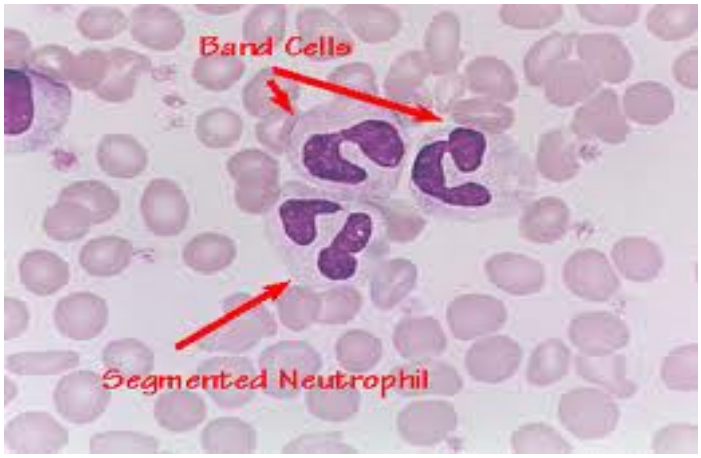


Fig. 3. : Band form (Segmented Neutrophil)

Measurement of CRP-

- C-reative protein was measured by latex agglutination test. The latex reagent, controls and serum specimens were are brought to the room temperature. The antigen suspension was mixed thoroughly prior to use.
- The semi quantitative latex agglutination assay involves the serial dilution of serum and saline. Each is mixed with latex reagent and observed for the presence of agglutination. The highest dilution in which agglutination is visualized corresponds to the concentration of CRP ligand complexes. Results are reported on mg/l concentration of CRP.
- CRP >8 mg/L were considered as positive.

Fig. 4 : C-Reactive Protein Kit

In all neonates the blood sample was collected from peripheral vein with all aseptic precautions, prior to administration of any antibiotic therapy. The area was cleaned thoroughly with alcohol, followed by povidone-iodine, and followed again by alcohol. Povidone-iodine was applied in concentric circles moving outward from the centre. The skin was allowed to dry for at least 1 minute before the sample was collected. One-mL sample of blood collected in a blood culture bottle containing 10 mL of culture media. This sample was immediately sent to Microbiology Department. Three subcultures were observed after 24, 48 and 120 hrs. If no growth was observed after five days culture was reported as negative. If growth was observed material was further analysed for specific organisms.

Positive blood culture was considered as gold standard.

Antibiotics were started immediately after blood culture was taken. The usual antibiotics were ampicillin and gentamicin.

TABLE III

SCREENING TEST RESULTS	Neonatal sepsis (Blood Culture Proven)		TOTAL
	PRESENT	ABSENT	
POSITIVE	a	b	a + b
NEGATIVE	c	d	c + d

a -- Individuals found positive on the test who have the disease. (i.e.true positive)

b -- Individuals found positive on the test who do not have the disease (i.e.false positive)

c -- Those with negative results but have the disease.

d -- Those with negative results who do not have the disease.

The specificity, sensitivity, positive predictive accuracy and negative predictive accuracy of the screening tests were calculated using the formula:

Sensitivity = a/ a + c * 100

Specificity = d/ b + d * 100

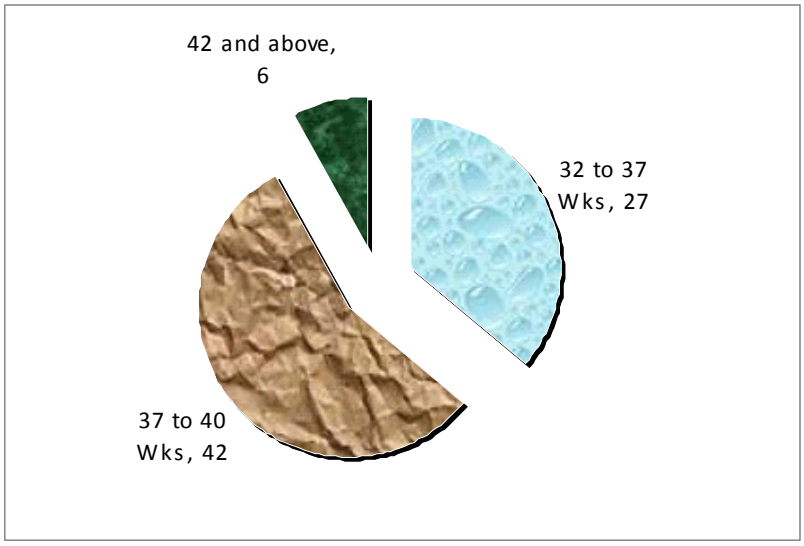
Positive predictive value = a/ a + b * 100

Negative predictive value = d/ c +d * 100

OBSERVATION AND RESULTS

Seventy five babies who fulfilled the inclusion criteria were included in the study.

Gestational age and Sex distribution of the babies who were included in the study:



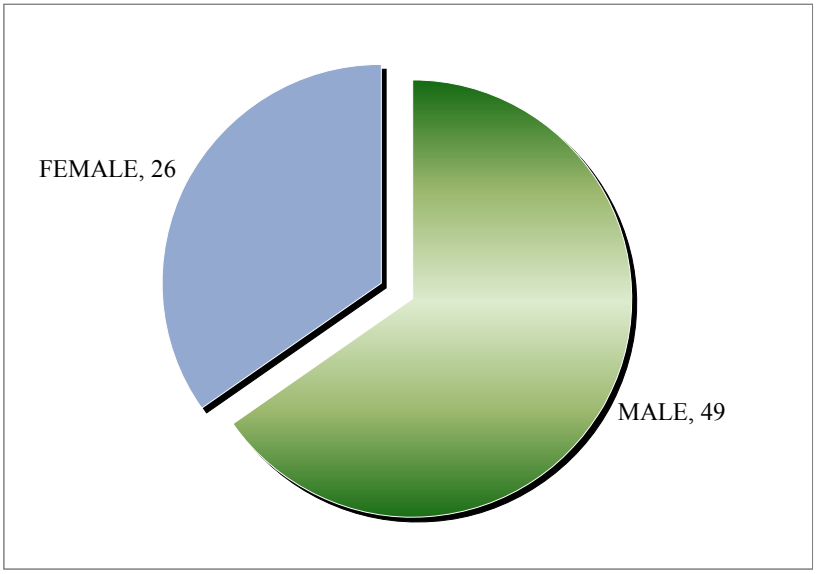
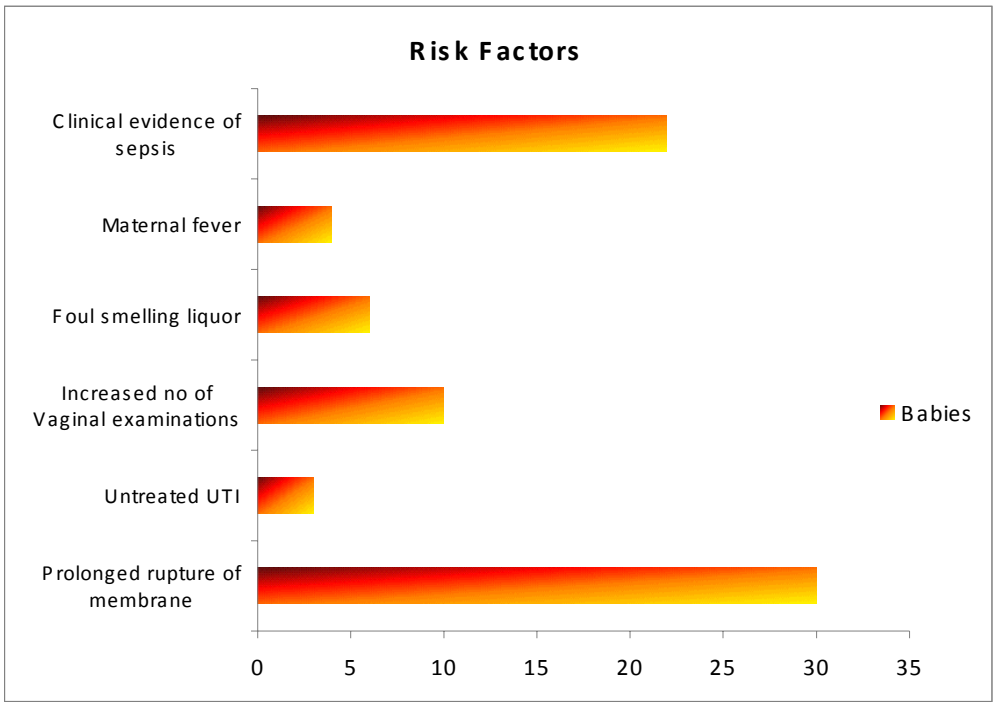
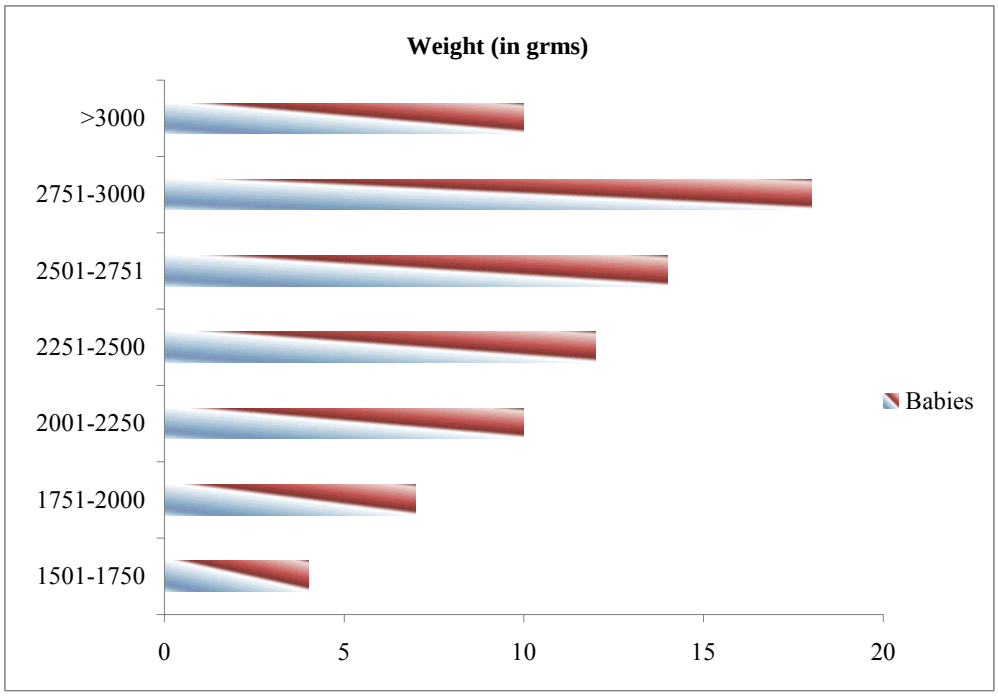


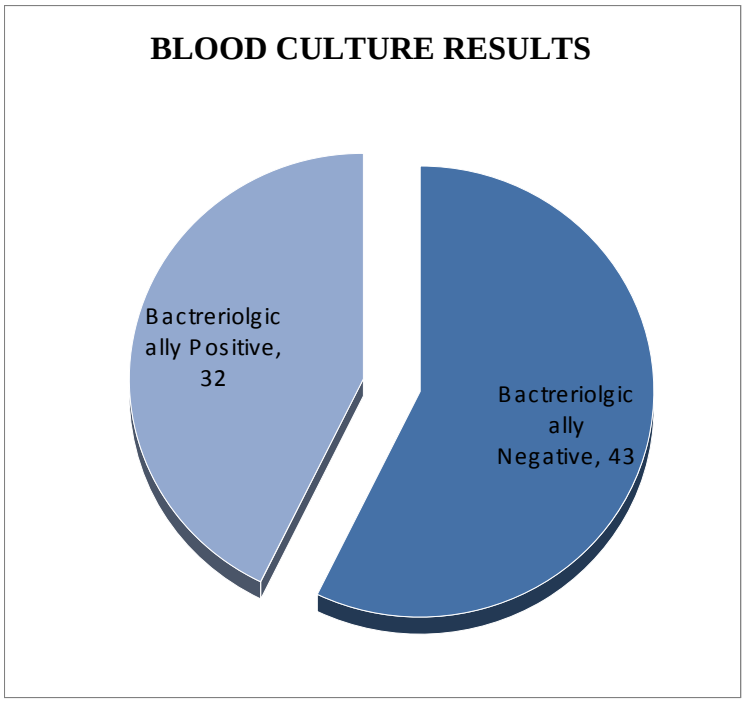
TABLE IV

Risk factors	No. of Babies
Prolonged rupture of membrane >18 hrs	30
Untreated UTI at the time of delivery	3
Vaginal examination >3 in labour	10
Foul smelling liquor	6
Maternal fever at the time of delivery	4
Clinical evidence of sepsis the baby	22



Weight in grams	No. of Babies
1501 -1750	4
1751 -2000	7
2001—2250	10
2251—2500	12
2501—2750	14
2751—3000	18
>3000	10

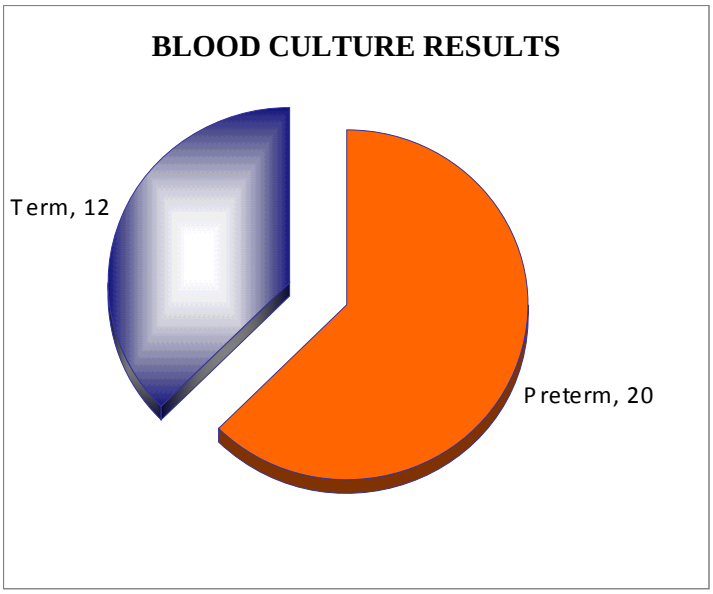




Culture	Bacteriologically Positive	Bacteriologically Negative	Total
No. of cases	32	43	75

Observations:

- Blood culture was bacteriologically positive in 42.6% of cases (32).
- Blood culture was bacteriologically negative in57.33% cases (43).



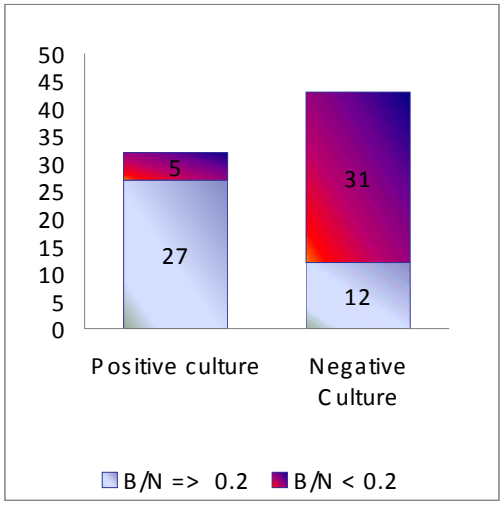
Bacteriologically Positive	Preterm	Term	Total
No. of cases	20	12	32

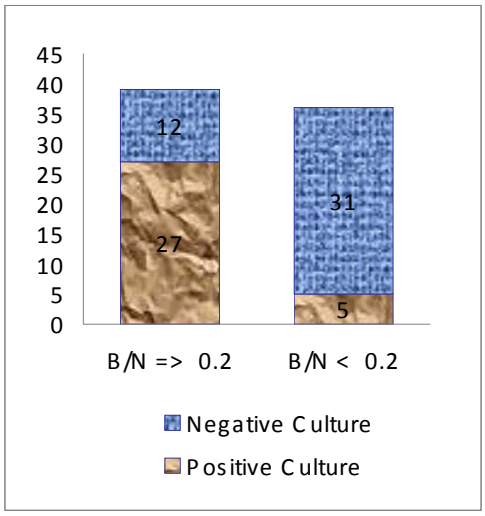
Observations :

- 20 preterm babies (62.5%) were affected by septicemia.
- 12 full term babies (37.5%) were affected by septicemia.
- Preterm babies were more affected by septicemia than full term babies.

Band Neutrophil Ratio:

B/N	Blood Culture		Total
	Positive	Negative	
B/N ≥ 0.2	27(84.37%)	12(27.9%)	39
B/N < 0.2	5 (15.62%)	31(72.09%)	36
Total	32	43	75

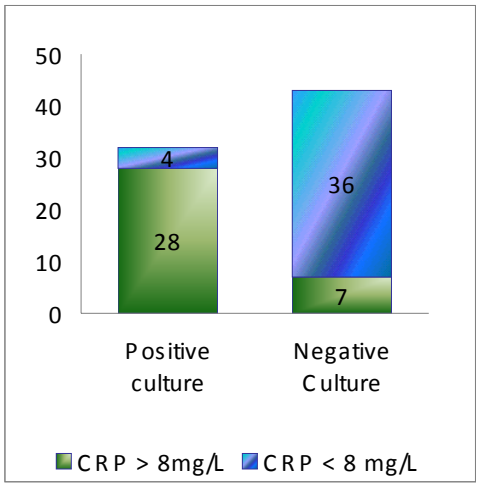


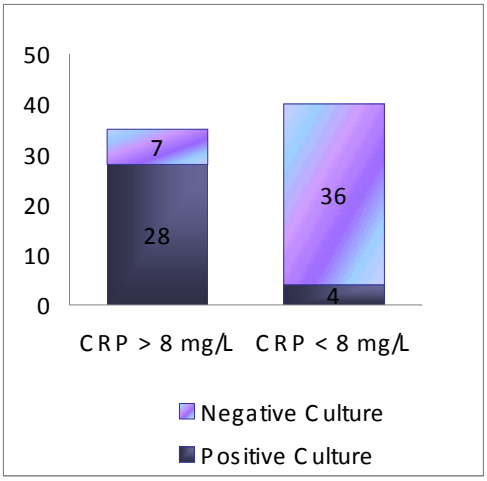


Band Neutrophil Ratio			
Sensitivity	Specificity	PPV	NPV
84.37%	72.09%	69.23%	86.11%

C – Reactive Protein:

CRP	Blood Culture		Total
	Positive	Negative	
CRP > 8 mg/L	28(87.5%)	7(16.28%)	35
CRP < 8 mg/L	4 (12.5%	36(83.72%)	40
Total	32	43	75

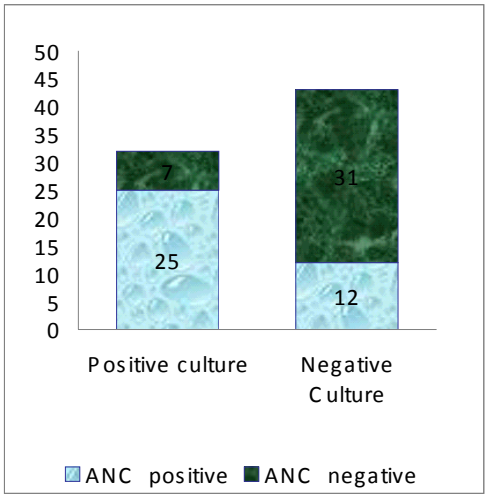


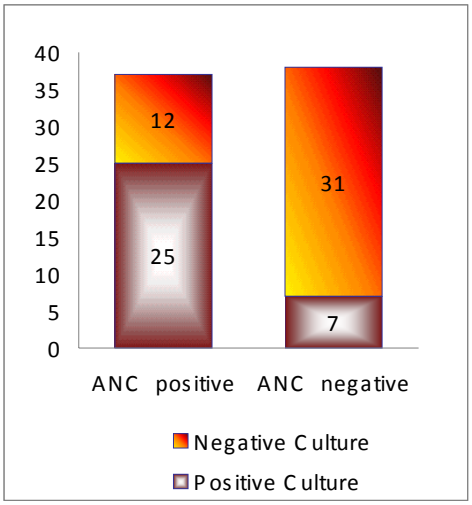


C – Reactive Protein			
Sensitivity	Specificity	PPV	NPV
87.5%	83.72%	80%	90%

Absolute Neutrophil count:

ANC	Blood Culture		Total
	Positive	Negative	
ANC positive	25(78.12%)	12(27.9%)	37
ANC negative	7(21.88%)	31(72.09%)	38
Total	32	43	75

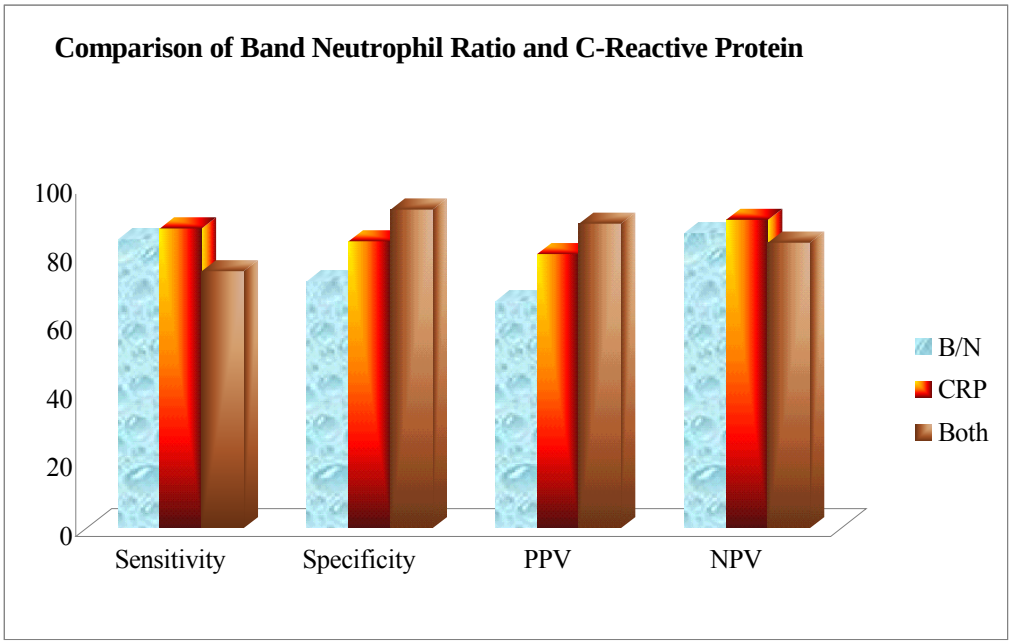




Absolute Neutrophil Count			
Sensitivity	Specificity	PPV	NPV
78.12%	72.09%	67.56%	87.51%

Band Neutrophil Ratio and C-Reactive Protein:

B/N CRP	Blood Culture		Total
	Positive	Negative	
Both Positive	24(75%)	3(7%)	27
Negative	8 (25%)	40 (93%)	48
Total	32	43	75

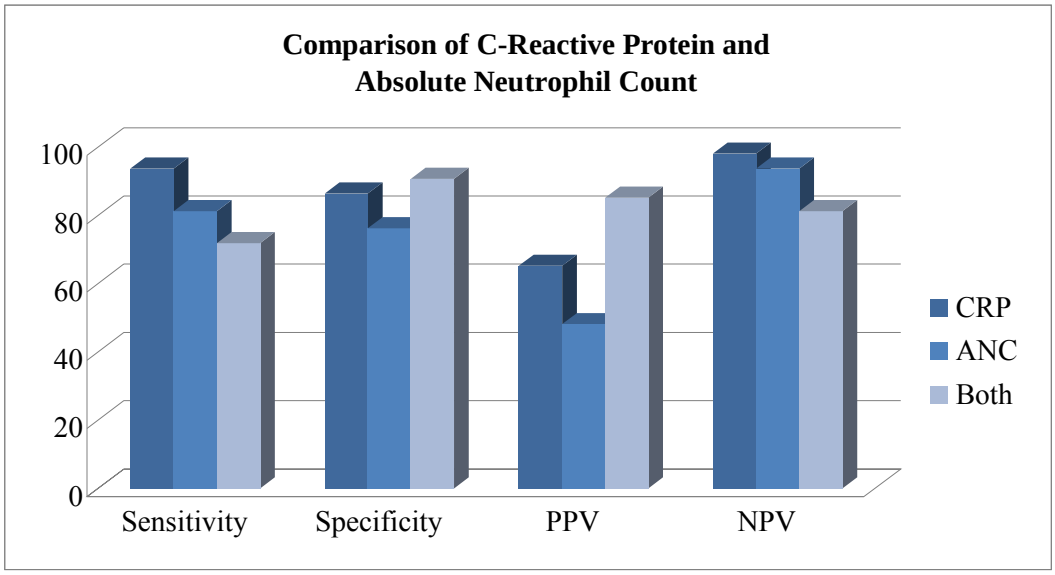


Band Neutrophil Ratio and C-Reactive Protein			
Sensitivity	Specificity	PPV	NPV
75%	93%	88.88%	83.33%

C-Reactive Protein and Absolute Neutrophil Count:

CRP ANC	Blood Culture		Total
	Positive	Negative	
Both Positive	23(71.87%)	4(9.3%)	27
Negative	9 (28.15%)	34(81.25%)	48
Total	32	43	75

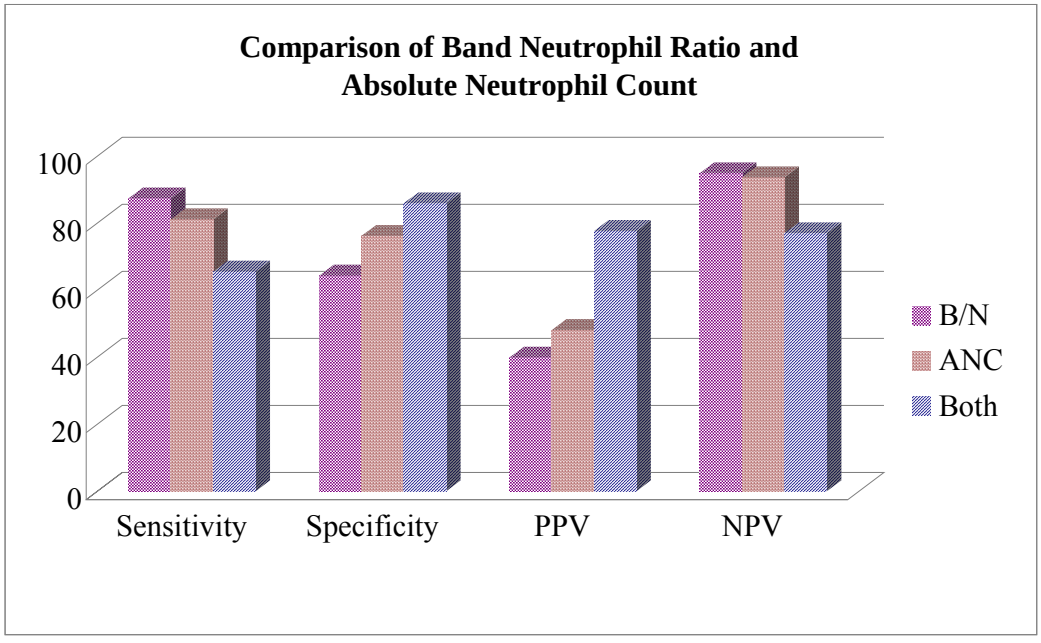
C-Reactive Protein and Absolute Neutrophil Count			
Sensitivity	Specificity	PPV	NPV
71.87%	90.7%	85.18%	81.25%



Band Neutrophil Ratio and Absolute Neutrophil Count:

B/N ANC	Blood Culture		Total
	Positive	Negative	
Both Positive	21(65.62%)	6 (16.95%)	27
Negative	11(34.37%)	37(86.04%)	48
Total	32	43	75

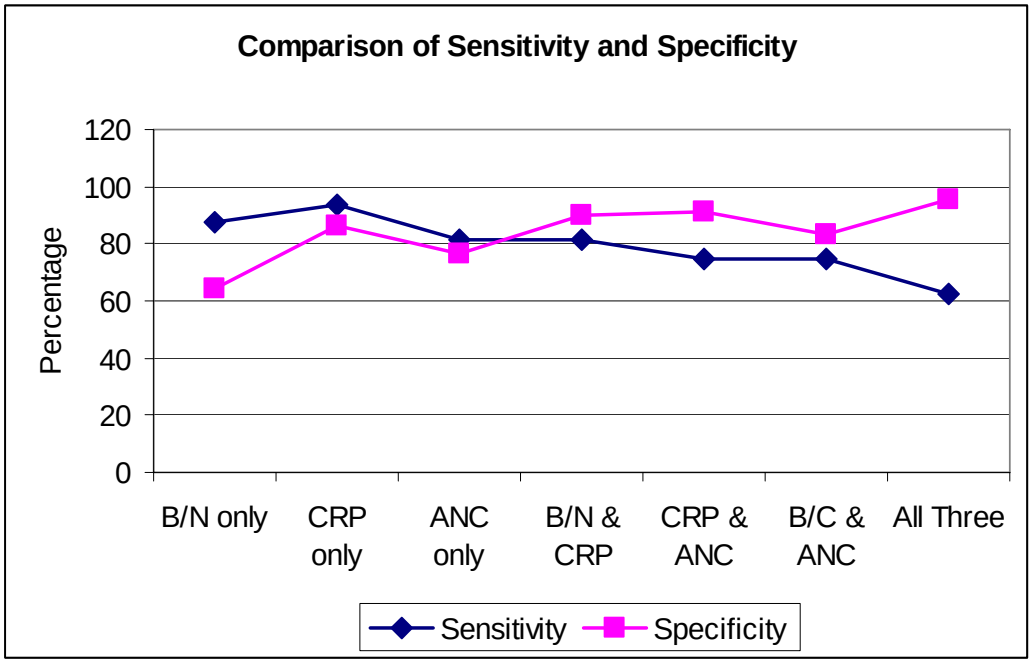
Band Neutrophil Ratio and Absolute Neutrophil Count			
Sensitivity	Specificity	PPV	NPV
65.62%	86.04%	77.77%	77.08%



Band Neutrophil Ratio, C-Reactive Protein and Absolute Neutrophil Count:

B/N CRP ANC	Blood Culture		Total
	Positive	Negative	
ALL Positive	20(62.5%)	2(4.6%)	22
Negative	12(37.5%)	41(95.34%)	53
Total	32	43	75

Band Neutrophil Ratio, C-Reactive Protein and Absolute Neutrophil Count			
Sensitivity	Specificity	PPV	NPV
62.5%	95.34%	90.90%	77.35%



DISCUSSION

This prospective study was done to assess the diagnostic value of a panel investigations in early diagnosis of early onset septicemia. The study was conducted in the new born ward of Government Mohan Kumaramangalam Medical college Hospital during the period of March to august, 2010. 75 babies with age less than 3 days of age with risk factor for septicemia or with signs and symptoms of sepsis were included in the study.

The panel consists of three Screening tests namely Absolute neutrophil count, (Band form) Immature neutrophil count to mature cell count ratio and C- reactive protein. All the 75 babies were subjected to these three investigations along with blood culture. Positive blood culture was taken as gold standard. The diagnostic value of these three tests individually and in combinations were analysed against blood culture. The following results were observed.

The incidence of septicemia among male babies was 65.33%. Piyush Gupta et al.[74] observed male predominance (64.7%) in neonatal septicemia.. N. Somu et al.[24], Philip et al.[55], Khatua et al.[72], N. Sinha et al.[68] and so many other authors observed similar results.

Prematurity and low birth weights are the two important risk factors for septicemia. In our study it was observed that Preterm and low birth weight babies had high incidence of septicemia 62.5%. Philip et al[55].

observed that low birth-weight was present in 18 cases out of 30 proven cases of sepsis is 60%. Moreno et al.[8] observed that 58% patients were of pre term , low birth-weight. Anand et al.[81] found that more than 2/3 cases of neonatal sepsis were preterm and low birth- weight.

Lethargy, refusal of feed, fast breathing were the common clinical manifestation observed in our study.

- Khatua et al[69] observed that refusal of feeds, lethargy, diarrhea, temperature abnormality, abdominal distension, jaundice and vomiting were most common presenting features.
- Agarwal et al.[82] observed that refusal to suck, sluggish activity, fever, jaundice were common clinical features.
- Other authors like Mishra et al.[67] ,Somu et al.[24], Anand et al.[81] had found more similar results.
- Our study and many other studies like these show that clinical features of neonatal septicemia are highly non specific and can mimic various common conditions that may occur in newborn period.

Prolonged rupture of membrane was the common maternal risk factor observed

- Anand et al.[81] observed prolonged rupture of membranes in 29.3% of cases.

- N. Mehrotra[11] noted threefold increase in the incidence of sepsis after prolonged rupture of membranes.
- Table No. IX shows our blood culture results.
- Out of 75 cases 32(42.66%) cases were bacteriologically positive.
- Gupta et al.[59] ,Sharma et al.[70], Khatua et al.[69], Namedo et al.[71], Bhatia et al.[83] and Chaturvedi et al.[84] observed culture positivity rate of 33%, 56%, 59.8%, 50%, 66.7%, and 73% and respectively.
- Sugandhi et al.[85] observed culture positivity rate of 42.5 %

Although blood culture is normally the basis for a diagnosis of bacterial infection, the bacteremic phase of the illness may be missed by poor timing, inadequate blood sample size will decrease the sensitivity and also before drawing blood sample for culture the patient may be treated with some parenteral antibiotic by private practioners or by other hospital. Due to these reasons the blood cultures, most of the time has low sensitivity.

The ratio of immature to mature neutrophils is a sensitive indicator of sepsis, as observed in our study has also been documented by other authors. The observation was as follows. Sensitivity was 84.37%, specificity was 72.09%, positive predictive value and negative predictive value were 69.23% and 86.11% respectively.

It is apparent that while investigating septic neonates, increase in percentage of band forms in peripheral blood smear has a good predictive value and the sensitivity as was observed in our study. M.singh et al.[30] and Namedo et al.[71] observed sensitivity of B/N ratio 62% and 82% respectively.

Xanthou[41] has studied the marked changes in band forms more precisely in health and diseased neonates and established its usefulness as a supportive test for the diagnosis of neonatal sepsis. Zipusky et al.[86], Gragory et al[87], Monroe et al .[45] has also shown the similar results.

The absolute neutrophil count varies considerably in the immediate neonatal period .The work of Monroe et al[45]. has increased the utility of this test through establishment of normal reference ranges for absolute neutrophil count and indices for immature neutrophils. The values were plotted in the graph. Those babies who were falling outside these values were taken as positive. The sensitivity, specificity, positive predictive value, negative predictive value were 78.12%, 72.09%, 67.56%, 81.51% respectively.

Monroe *et al.*[45] observed a 100% negative predictive value if the total neutrophil count, immature neutrophil count, and I/T were all normal. In a subsequent study by Benuck I et al.[88] however, these indices identified only 94% of septic patients .Our study is consistent with these studies.

CRP level were found to be more useful in screening of neonatal septicemia through various studies. Philip[2] and later others suggested that

serial normal levels may be useful for identification of infants who do not have bacterial infection. Philip and Hewitt[55] reported no recurrence of infection within 7 days of discontinuation of antibiotics based on three normal CRP determinations within 48 hours and negative cultures in 147 low birth infants at risk for early-onset infection. In early onset sepsis a single CRP 24 hours into illness has a 93% sensitivity in detecting sepsis. (Benitz, W.E. et al.[89])

In a series of 218 infants (13 with sepsis), Gerdes and Polin[90]reported high sensitivity (93%) and NPV (99%) for CRP levels determined by a latex agglutination method at the time of evaluation and 12 to 24 hours later. Our study results were consistent with other studies.

The specificity of the screening test was increased significantly by combining these three tests. But there was little acceptable compromise in sensitivity.

SUMMARY

Neonatal sepsis is the single most important cause of neonatal deaths globally and the magnitude of the problem is more immense particularly in our country. Given the fulminating course of the disease and the high mortality and morbidity associated with it early diagnosis and aggressive management is the key to successful outcome. Since the clinical manifestation of septicemia in newborn is highly non specific, vague and mimics numerous others non infectious condition the early diagnosis remains a difficult task.

Even though blood culture is the gold standard for diagnosis of septicemia, the technique of blood culture is time consuming (It takes 48-72 hrs for the results to be available) and success rate is only around 40%.The culture result may be influenced by previous antibiotic exposure and also the bacteremic phase will be missed by poor timing and blood sample size. So certain indirect markers of sepsis are being used for screening the neonates with sepsis. Among them the well known, most useful and widely used tests include total WBC count, absolute neutrophil count ,Immature to total WBC ratio(I:T ratio),ESR and CRP.

Among these, three parameters which were proven to have good sensitivity and specificity individually were chosen and the diagnostic value and reliability of this panel was studied. 75 babies with age less than 3 days and with well defined maternal risk factor or with clinical suspicion of sepsis were included in the study.

All these babies were subjected to our three tests namely absolute neutrophil count, band form to total leucocyte ratio, and CRP along with blood culture. Blood culture positivity was taken as gold standard for sepsis and the sensitivity, specificity, positive predictive accuracy and negative predictive accuracy for the individual tests and their combinations were analysed .The following results were observed. The incidence of sepsis was high among preterm low birth weight babies 62.5%.Male babies were more prone for septicemia .Male to female ratio observed was 1.88:1.Band form to total neutrophil ratio had a sensitivity, specificity, positive predictive accuracy and negative predictive accuracy of 84.37%,72.09%,69.23%, and 86.11% respectively. CRP had the highest sensitivity of 87,5%, specificity, positive predictive accuracy and negative predictive accuracy of 83.72% ,80%, and 90% respectively. Absolute neutrophil count had a sensitivity, specificity ,positive predictive accuracy and negative predictive accuracy of 78.12%,72.09%,67.56%, and 81.57% respectively.

CONCLUSION

1. The incidence of sepsis was high among preterm babies and low birth weight babies 62.5%.
2. The incidence was more in male babies 65.33%.
3. Among the maternal risk factors, prolonged rupture of membrane was the commonest observed.
4. The blood culture positivity was 42.66% in our study group.
5. This panel of sepsis screening tests provides a reliable, cheap and rapid screening tool for screening neonatal septicemia.
6. Absolute neutrophil count test done using Monroe's reference chart has increased its sensitivity and specificity compared to similar studies.
7. Band form to mature neutrophil ratio has got more sensitivity and negative predictive value than absolute neutrophil count.
8. CRP as an individual test has highest sensitivity, specificity and positive predictive accuracy and is a sensitive and responsive indicator of neonatal sepsis.
9. When both CRP and band form to mature neutrophil counts were positive the sensitivity and the negative predictive value were high compared to other combinations of two.
10. The combination of all these three tests gave the highest specificity 94%.

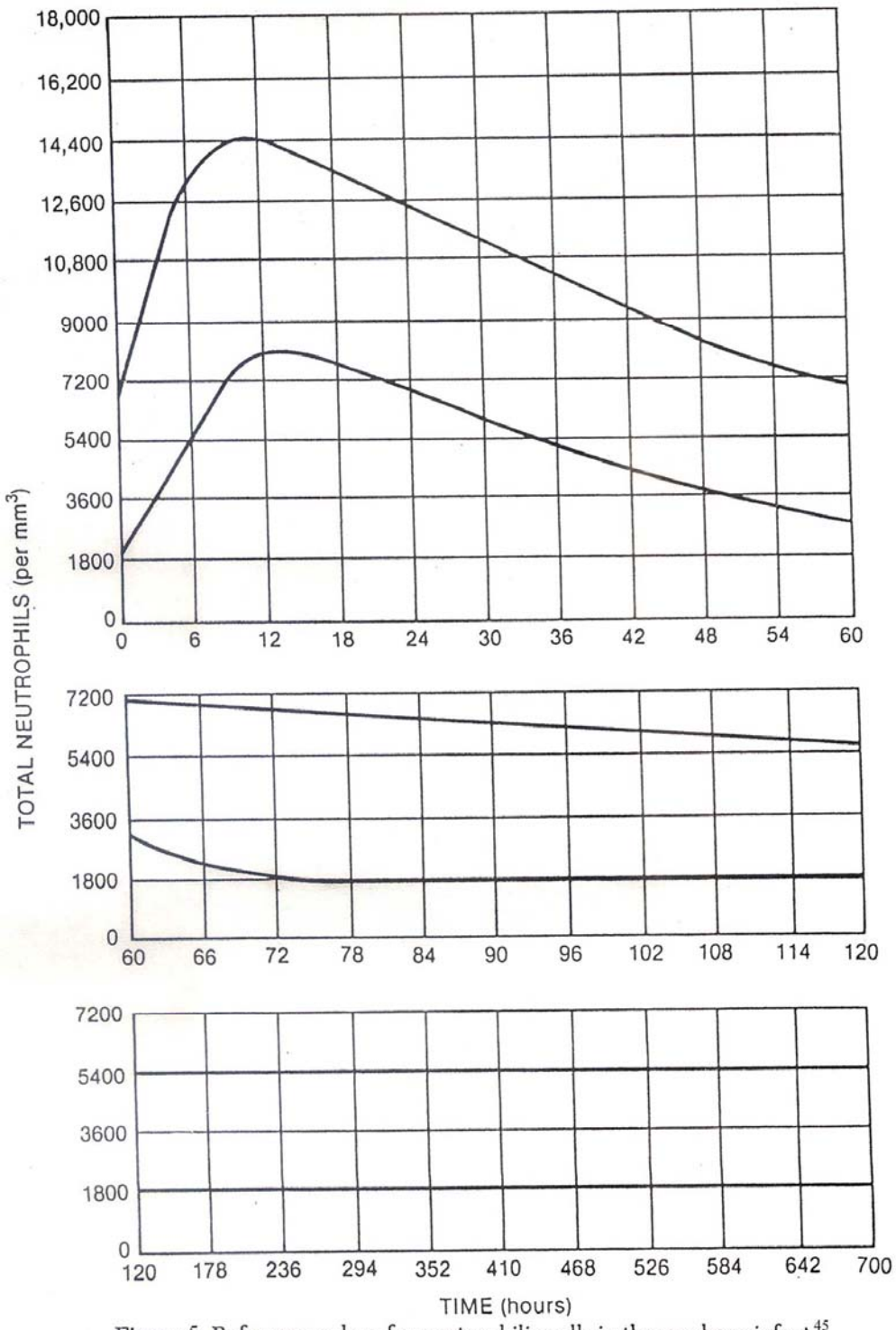


Figure.5 Reference values for neutrophilic cells in the newborn infant.[45]

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PROFORMA

Neonatal septicemia

Name:.....

Date of Admission:..…/……../2010

Age/Sex:……/……Hosp. No.:……

Date of Discharge:…../……/2010.

Mode of delivery

☐ NVD ☐ Instrumental ☐ LSCS

Place of delivery

☐ GMKMCH (Referred from :…….)

☐ Outside (Specify place :…….)

Maturity:

☐ Term ☐ Preterm: Gestational age in weeks:……..

☐SGA ☐AGA ☐ LGA ☐SGA ☐AGA ☐ LGA

Maternal risk factors:

☐PROM ≥18hrs

☐Foul smelling liquor

☐Handled Outside (>3PV)

☐Maternal fever during delivery

☐Maternal untreated UTI.

Signs and symptoms in newborn:

☐Lethargy	☐refusal to feed
☐Tachyapnea.	☐Apnea
☐Bradycardia.	☐Tachycardia.
☐Hypothermia.	☐ fever
☐abdominal distention	☐pallor
☐Jaundice.	

General examination:

Abnormal facies : (Yes/No)

Congenital anomalies : (Yes/No)

Cry / activity: (Nil/Minimal/Weak/Fair/Good)

Colour (pallor/Plethoric/Cynosis/Mottling/Skin rashes)

Edema (Nil/Generalised/Pedal/Presacral/Facial)

Head circumference:……..cm Temperature…….

Respiratory rate:……..…./min Heart rate:……../min

Pulse (central……..…../Peripheral……)

CVS:……

RS:……

Abdomen:……

CNS:

Tone: NNR: AF: Spine/cranium:

……..……

Investigations:

Total WBC Count : ...……

Absolute neutrophil count : ...……

Band forms count : ...……

CRP : ...……

Blood culture : ...……

Management:

……..……

……..……

Outcome:

……..……

……..……

Assessment of results:

……..……

……..……

MASTER CHART

Sl. No.	AGE (In hours)	SEX	MATURITY	WEIGHT g.	Cells/mm3 TC	ANC	B/N RATIO	CRP mg/l	BLOOD CULTURE	MATERNAL RISK FACTORS
1	70	M	T	2650	4200	1680/P	0.26	P	E.Coli	PROM>18 Hrs
2	58	F	T	2750	9500	3990/N	0.13	N	sterile	Foul smelling liquor
3	1	M	PT	1880	9990	1650/P	0.12	N	sterile	PROM>18hrs
4	23	M	PT	1900	3000	1900/P	0.21	P	E.coli	Untreated UTI
5	54	F	T	2350	17700	6200/N	0.08	P	sterile	>3PV
6	42	F	T	2650	16500	8800/N	0.24	N	sterile	PROM>18Hrs
7	40	M	T	3000	12840	3853/P	0.31	N	S.aureus	Maternal fever
8	28	M	T	2950	9850	25910/N	0.11	N	sterile	PROM>18Hrs
9	10	F	T	2700	20	10100/N	0.17	N	sterile	>3PV
10	4	M	T	2400	4600	1656/P	0.34	N	enterococus	Clinical evidence of sepsis
11	45	F	T	2450	13500	5400/N	0.19	N	sterile	PROM>18hrs
12	2	M	PT	1950	9850	5910/P	0.22	N	sterile	Clinical evidence of sepsis
13	28	F	T	2300	10880	5440/P	0.24	P	Klebsiella	PROM>18 Hrs
14	1	M	T	2200	28400	7100/N	21	N	sterile	Clinical evidence of sepsis
15	71	M	T	2600	5600	1680/P	0.12	N	sterile	PROM>18Hrs
16	34	F	PT	2800	14340	4445/P	0.28	P	proteus	Maternal fever
17	18	F	PT	2250	17650	5300/P	0.17	N	CONS	>3PV
18	49	M	POT	3600	21660	7800/N	0.08	N	Sterile	PROM>18hrs
19	6	M	T	3450	15600	4992/P	0.3	N	sterile	PROM>18 Hrs
20	52	M	PT	2100	16240	9744/P	0.34	P	CONS	> 3 PV
21	21	F	PT	2650	18900	9639/N	0.29	P	S.aureus	>3PV
22	64	M	PT	1800	5300	1696/P	0.22	P	acinetobcater	Clinical evidence of sepsis
23	1	M	T	3700	10750	4300/N	0.09	N	sterile	PROM>18hrs
24	2	F	PT	1550	3125	1250/P	0.1	P	sterile	>3PV
25	46	M	T	2800	17000	5100/N	0.19	N	sterile	PROM>18 Hrs

Sl. No.	AGE (In hours)	SEX	MATURITY	WEIGHT g.	Cells/mm3 TC	ANC	B/N RATIO	CRP mg/l	BLOOD CULTURE	MATERNAL RISK FACTORS
26	34	M	PT	2400	4636	2800/P	0.21	P	E.coli	Untreated UTI
27	70	M	POT	3650	14500	5800N	0.18	N	sterile	PROM>18hrs
28	61	F	PT	1950	11390	6380/N	0.14	N	sterile	>3PV
29	84	M	PT	3400	12680	8870/P	o.34	P	klebsiella	PROM>18hrs
30	58	M	T	2725	17350	5200N	0.05	N	sterile	Clinical evidence of sepsis
31	24	M	T	2600	9400	5640/P	0.23	P	proteus	Maternal fever
32	72	M	PT	2600	9360	20800/P	0.19	N	S.aureus	>3PV
33	12	F	PT	2875	3600	4500/P	o.06	P	E.coli	Untreated UTI
34	8	M	T	3400	8600	19560/N	0.15	N	sterile	PROM>18hrs
35	16	M	PT	1700	22000	15840/P	.4O	P	CONS	Foul smelling liquor
36	1	F	PT	2900	3540	1628/P	0.27	N	sterile	>3PV
37	26	M	T	2600	4200	16800/N	0.24	P	klebsiella	Clinical evidence of sepsis
38	1	M	PT	2450	11400	22450/N	0.18	N	sterile	PROM>18hrs
39	90	F	T	2400	6240	1560/P	0.26	P	E.coli	Clinical evidence of sepsis
40	6	M	PT	1950	19630	6300/N	0.33	P	sterile	PROM>18hrs
41	9	F	POT	2400	19900	9560/N	0.16	P	sterile	PROM>18hrs
42	70	M	PT	2100	21650	11474/P	O.3	P	Klebsiella	Maternal fever
43	2	M	T	3700	12280	4300/N	0.23	N	sterile	Clinical evidence of sepsis
44	66	F	T	2950	17600	11792/P	0.29	P	E.coli	PROM> 18 HRS
45	12	M	PT	2300	21100	9500/N	0.13	N	sterile	>3PV
46	1	M	T	2600	18300	1189/P	0.21	N	sterile	Clinical evidence of sepsis
47	45	M	PT	1900	3650	2774/P	0.31	P	S.aureus	Clinical evidence of sepsis
48	56	M	T	3900	19760	8300/N	0.17	N	sterile	PROM>18hrs
49	70	M	POT	2340	7800	3200/N	0.07	N	sterile	Clinical evidence of sepsis
50	69	M	T	2700	8040	1688/P	0.27	P	E.coli	Clinical evidence of sepsis
51	42	F	PT	1875	18830	5650/N	0.19	N	sterile	PROM>18hrs
52	60	M	T	2750	23300	9300/N	0.35	P	E.coli	Foul smelling liquor

Sl. No.	AGE (In hours)	SEX	MATURITY	WEIGHT g.	Cells/mm3 TC	ANC	B/N RATIO	CRP mg/l	BLOOD CULTURE	MATERNAL RISK FACTORS
53	58	F	PT	1800	223400	13404/PP	0.4	P	enterococuss	PROM>18 HRS
54	71	M	T	2400	5365	2,200/N	8	N	sterile	Clinical evidence of sepsis
55	36	M	T	2750	9000	3600/P	0.16	N	sterile	Clinical evidence of sepsis
56	26	M	PT	2400	10440	5428/P	0.24	P	klebsiella	PROM>18 HRS
57	45	M	T	3000	18930	5680/N	0.09	N	sterile	Clinical evidence of sepsis
58	16	F	T	2200	24200	9680/N	0.24	P	sterile	PROM>18hrs
59	38	M	PT	2150	14700	6027/N	0.11	N	sterile	Clinical evidence of sepsis
60	1	F	POT	27750	3560	2848/P	0.11	P	S.aureus	Foul smelling iquior
61	26	M	PT	1950	12300	4920/N	0.12	N	sterile	Clinical evidence of sepsis
62	50	T	PT	1650	3900	1716/P	0.22	P	enterococcus	Clinical evidence of sepsis
63	61	M	T	2750	16300	6520/N	0.07	N	sterile	Clinical evidence of sepsis
64	1	F	PT	2250	10500	4200/N	0.28	N	E.coli	PROM>18 hRS
65	68	M	T	2850	14900	5215/N	0.11	N	sterile	Clinical evidence of sepsis
66	71	M	T	2600	23600	13688/P	0.36	P	s.aureus	PROM>18hrs
67	1	F	T	2450	20400	9600/N	0.16	N	sterile	PROM>18hrs
68	2	M	PT	2795	18570	13,000/P	0.09	P	klebsiella	PROM>18 hRS
69	70	M	PT	2300	12800	9088/P	0.3	P	E.coli	Clinical evidence of sepsis
70	12	M	POT	2850	26200	10400/N	0.19	N	sterile	PROM>18hrs
71	42	F	T	3100	4450	3120/P	0.13	N	sterile	Foul smelling liquor
72	36	M	PT	1700	15280	5500/N	0.8	N	sterile	PROM>18hrs
73	59	F	T	2750	10130	4560/N	0.15	P	entero	Clinical evidence of sepsis
74	4	M	T	2050	8800	3200/N	0.28	P	Pseudomonasas	Foul smelling liquor
75	64	F	T	2900	15420	5,400/N	0.31	P	sterile	PROM>18hrs

M - Male
F - Female
T - Term
PT - Pre Term

P - Positive
N - Negative
PV - Pervaginal Examination
PROM - Premature rupture of Membrane

Sl. No.	AGE (In hours)	SEX	MATURITY	WEIGHT g.	Cells/mm3 TC	ANC	B/N RATIO	CRP mg/l	BLOOD CULTURE	MATERNAL RISK FACTORS

POT - Post Term
ANC - Absolute Neutrophil Count
B/N - Bandform Total Neutrophill Ratio
TC - Total Count
CRP - C-Reactive Protein