“ROLE OF HEMATOLOGICAL SCORING SYSTEM IN DIAGNOSIS OF NEONATAL SEPSIS.”

By

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A Dissertation submitted to the

BLDE University, Bijapur, Karnataka

In partial fulfillment of the requirements for the award of the degree of

DOCTOR OF MEDICINE

IN

PATHOLOGY

Under the Guidance of

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2015

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I hereby declare that this dissertation entitled **"ROLE OF HEMATOLOGICAL SCORING SYSTEM IN DIAGNOSIS OF NEONATAL SEPSIS"** is a bonafide and genuine research work carried out by me under the guidance of **Dr. Surekha. B. Hippargi, Professor Department of Pathology.**

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ABSTRACT

BACKGROUND

Neonatal sepsis is one of the major cause of morbidity and mortality in the newborn, more so in the developing countries. It is responsible for about 30-50% of total neonatal deaths in the developing countries. Although blood culture is considered to be the gold standard for diagnosis of septicemia, this technique is time consuming.

AIMS

To analyse the diagnostic utility of hematological scoring system (HSS) and its correlation with C-reactive protein (CRP) and blood culture in neonatal sepsis.

MATERIAL AND METHODS

This study included 150 neonates admitted in Neonatal Intensive Care Unit (NICU) with clinical suspicion of neonatal sepsis from November 2012 to April 2014 considering the inclusion and exclusion criteria**.**

RESULTS

Hematological scoring system (HSS) had the highest sensitivity (93.7%) and identified > 90% of neonates with clinical suspicion of sepsis. Also total leukocyte count showed high specificity but least sensitivity, I:T ratio and I:M ratio showed high specificity and high sensitivity, platelet count showed high negative predictive value and least positive predictive value.

CONCLUSION

Hematological scoring system is a simple, easy, cheap and rapid adjunct for the diagnosis of clinically suspected cases of neonatal sepsis.

Key words

Neonatal sepsis, immature to total neutrophils ratio (I:T), immature to mature neutrophils ratio (I:M), hematological scoring system (HSS).

LIST OF ABBREVIATIONS USED

ANC	Absolute neutrophil count
CRP	C-Reactive protein
HSS	Hematological scoring system
I:M	Immature to mature neutrophil ratio
I:T	Immature to total neutrophil ratio
LBW	Low birth weight
NICU	Neonatal intensive care unit
NNPD	National neonatal perinatal database
NPV	Negative predictive value
PPV	Positive predictive value
SEN	Sensitivity
SP	Specificity
TLC	Total leukocyte count
TNF	Tumour necrosis factor
VLBW	Very low birth weight
WBC	White blood cell

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INTRODUCTION

Neonatal sepsis is a clinical syndrome resulting from pathophysiologic effects of local and systemic infection in the first month of life. Septicemia usually consists of bacteraemia with a constellation of signs & symptoms caused by microorganisms or their toxic products in the circulation. The presence of signs & symptoms distinguishes this condition from transient bacteraemia observed in some healthy neonates.[1]

Neonatal sepsis has some unique features:

- Infection can be transmitted from mother to fetus.
- New born infants are less capable of responding to infection because of one or more immunologic deficiencies.
- Coexisting condition often complicates the diagnosis & management of neonatal infection.
- Clinical manifestation of neonatal sepsis vary & include sub clinical infection, mild to severe manifestation of local or systemic infection & rarely congenital malformation resulting from infection in the first trimester.
- Maternal infection is often undiagnosed during pregnancy as patient will be asymptomatic or show nonspecific signs & symptoms at the time of acute infection. Very low birth weight neonates (<1500 gms) are at a higher risk for hospital acquired infection.[2]

Neonatal sepsis is an important cause of neonatal mortality & morbidity. Incidence of neonatal sepsis has been reported to be 30 per 1000 live births according to National Neonatal Perinatal Database (NNPD) 2002-03. This recent data is collected from 18 centers of various parts of India.

Neonatal sepsis was one of the common cause of neonatal mortality, contributing to 16% of all intramural deaths.[3] Early onset sepsis contributed 67% of all sepsis.

Early diagnosis of sepsis is difficult due to its non-specific clinical presentation. Although blood culture is considered to be the gold standard for diagnosis of septicaemia, the technique is time consuming and demands a well equipped laboratory, which is not available in most of the community hospitals. In recent years various investigators have evaluated some highly sensitive and specific inflammatory markers (ELIZA, Haptoglobin, Interleukin) to diagnose neonatal sepsis, but are sophisticated and expensive ,so impractical for developing countries.[4,5,6] Therefore, the need is a test that is cheap, easily performed with quick availability of reports with maximum sensitivity and specificity.

Various studies have shown that hematological parameters are simple, quick and cost effective tool in the early diagnosis of neonatal sepsis. When these were studied together as combination of tests, it had proved that they increased both sensitivity and specificity. They are also useful early predictors of neonatal septicemia. Thus helping to initiate early treatment with appropriate antibiotics.

The combination of tests used by various workers mainly consist of total leukocyte count, absolute neutrophil count, immature: total neutrophil ratio (I:T), C- reactive protein (CRP), platelet count. Two or more positive tests have a good sensitivity and specificity [5] and recommended it as a screening procedure even in the absence of C-reactive protein.[6]

The early diagnosis of neonatal septicemia is still a challenge for paediatricians and pathologists. So, present study is undertaken to evaluate the utility of the hematological scoring system (HSS) in early diagnosis of neonatal sepsis.

AIMS & OBJECTIVES:

- To analyse the diagnostic utility of hematological scoring system (HSS) and its correlation with C-reactive protein (CRP) and blood culture in neonatal sepsis.

REVIEW OF LITERATURE

NEONATAL SEPSIS:

Infections are frequent and an important cause of morbidity and mortality in the neonatal period. Neonatal sepsis is one of the leading cause of morbidity and mortality in India. It is probably responsible for 30-50% of the total neonatal deaths each year in developing countries.[1]

Neonatal sepsis is a clinical syndrome of bacteraemia characterized by systemic signs and symptoms of infection in the first month of life. [1,2]

Probable sepsis is defined as presence of clinical signs of bacterial infection associated with positive laboratory tests in the absence of blood culture positivity.[1,2]

HEMATOLOGICAL SCORING SYSTEM (HSS):

The inability of any single laboratory test to provide rapid, reliable and early identification of neonates with bacterial sepsis has led to efforts to devise a panel of tests combining data from several different determinants, as a means of increasing positive predictive accuracy compared with most of the individual tests. Prerequisite of any such kind of screening panels would be that the result should be available in a short period of time. Many authors have evaluated the efficacy of the "Hematological scoring system" using various parameters.

Rodwell et al published a complete blood cell count criteria for evaluating screening tests for neonatal sepsis. From the data obtained, a hematologic scoring system was formulated that assigned a score of 1 for each of seven findings: abnormal total leukocyte count, abnormal total neutrophil (PMN) count, elevated immature PMN count, elevated immature to total PMN ratio, Immature to mature PMN ratio ≥0.3, platelet count ≤150,000/mm^3 and pronounced degenerative changes in PMNs. There were 298 neonates evaluated for sepsis. Twenty-six of 27 (96%) neonates with sepsis and all 23 neonates with probable infection had scored ≥3, compared with 35 of 248 (14%) non-infected infants. The likelihood of sepsis with score ≥3 was 31%. The higher the score, the greater was the likelihood of sepsis. With score ≤ 2 the likelihood that sepsis was absent was 99%. He concluded that hematologic scoring system should improve the diagnostic accuracy of the complete blood cell count as a screening test for sepsis and could simplify and standardize the interpretation of this global test.[7]

Ghosh S et al studied 103 peripheral blood smears of neonates using hematological scoring system of Rodwell et al. for early detection of sepsis. They found that an abnormal immature to total neutrophil (I:T) ratio followed by an abnormal immature to mature neutrophil (I:M) ratio were the most sensitive indicators in identifying neonates with sepsis. Along with thrombocytopenia had a high negative predictive value over 94%. Also found that higher the score ,the greater the certainty of sepsis being present.[8]

Shirazi Haider et al studied on role of hematological profile in early diagnosis of neonatal sepsis. They found sensitivities of the parameters studied were below 60%. However , the specificities were more than 70%. In their tests, individual parameters had not desired specificities but if put together can be a good tool in ruling out the possibility of the neonatal sepsis.[4]

Khair et al studied the hematological scoring system for 100 neonates by Rodwell et al. criteria for early detection of sepsis in neonates. They found score ≥ 4 had a sensitivity of 100%, specificity of 60%, with positive predictive value (PPV) 26% and negative predictive value (NPV) 100%. Thus, is a more reliable screening tool for sepsis.[9]

Narasimha A et al analysed 50 peripheral blood smears of newborns for neonatal sepsis using HSS of Rodwell et al. criteria and used white blood cell and platelet count, White blood differential count, Nucleated red blood cell count and assessment of neutrophil morphology for degenerative changes. An abnormal I:M ratio was highly sensitive in their study. Thrombocytopenia was consistently associated with poor prognosis in their study.[10]

Sriram R et al. correlated blood culture results with sepsis score and sepsis screen using 6 criteria. She concluded that value of sepsis screen was more for excluding the diagnosis of neonatal septicemia which could be done reasonably with two screens 12-24 hours apart. In a neonate who is stable otherwise or suspected of sepsis because of maternal risk factors, it is desirable to wait the results of sepsis screen

before initiation of antibiotics. Since symptoms suggestive of sepsis may be caused by variety of illness, confirmation of sepsis by the sepsis screen tests may help in avoiding unnecessary antibiotic therapy.[11]

Manisha Makkar et al. did performance evaluation of hematological scoring system in early diagnosis of neonatal sepsis. All smears were analysed by using Rodwell criteria. Assigned score of 1 for each 7criteria. Hematological scoring system had higher sensitivity and specificity in preterm than in term neonates. Positive predictive value and negative predictive value were higher in preterm than term for HSS. It was also seen that with increasing scores, the likelihood of sepsis also increased.[12]

Namdeo et al studied the usefulness of a combination of various Hematological indices in the early diagnosis of neonatal septicemia and reported that leukopenia, Band: Total neutrophil count ratio of >0.3, neutrophils with toxic granules in more than 40% and mini-ESR of >8 mm were particularly predictive of septicemia with a specificity of 98% and positive predictive accuracy of 89%. If more than one of the four tests were positive, they became more sensitive and sufficiently specific. They also observed an elevated I: T ratio in 84% of the culture positive cases studied by them.[13]

Mishra et al formed sepsis screen using following parameters: band cell: neutrophil count ratio of > 0.2, micro ESR >8 mm at the end of one hour, leukopenia <5000 cells/cumm, thrombocytopenia <150,000cells/cumm. He found that when two

positive tests combinations were analyzed for detection of sepsis, it was observed that the best combination was band cell: neutrophil count ratio and micro ESR which had a high sensitivity of 75% followed by band cell: neutrophil count ratio and leukopenia. On analyzing three tests combinations, the best on was that of band cell: neutrophil count ratio with microESR and leukopenia which had highest predictive accuracy of 94% and also high specificity of 96%. [14]

Sharma et al evaluated the use of sepsis screen in the early diagnosis of septicemia. The parameters and their cut off values used by them were: Total leucocyte count < 5,000/cmm or >20,000/cmm, band cell: neutrophil count ratio of > 0.2, Gastric aspiration cytology >5 polymorphs/hpf, microESR >10 mm at the end of one hour and CRP > 6 µgm/ml Among the individual tests, CRP had the maximum sensitivity (80%) and specificity (93.8%). Toxic granules were seen in 60% of the cases.[15]

Varsha et al (2003) evaluated validity of hematological parameters in identification of early and late onset neonatal sepsis. Criteria used by them were total leucocyte count, TNC, I:T ratio, I:M ratio and CRP. They concluded that CRP elevation, leucopenia, neutropenia and elevated I/T ratio are comparably good diagnostic aids, while after 3 days life, CRP is the best single test.[16]

TOTAL LEUKOCYTE COUNTS:

Neonatal sepsis is suspected when TLC < 5,000 cells/cumm or > 20,000 cells /cumm. **Jeevasankar M et al** showed leukopenia as a good parameter to detect sepsis during the first 3 days of life.[3]

Zawar MP et al observed leukopenia in 83% of culture positive cases and it had 82% sensitivity and 70% specificity.[17]

Bhat R Y et al observed total leukocyte count of <5,000 and >20,000 in 29.7% of cases. It had significantly more positivity among the symptomatic than the asymptomatic neonates.[18]

Buch et al considered total count of < 5,000cells/cumm or > 20,000 cells/cumm as abnormal. They observed 50.77% sensitivity and 63.4% specificity. They concluded that total leucocyte count alone is not significant in the diagnosis of neonatal sepsticemia.[19]

Leukopenia is considered a more sensitive index than leukocytosis, especially in severe ill neonates.

ABSOLUTE NEUTROPHIL COUNT (ANC):

Normal ranges for neonatal absolute neutrophil count are different from those of infants and children.

Manroe et al observed that The reference values of neutrophils in neonates between 0 and 60 hours, the minimum polymorphonuclear cell count was 7,800cells/cumm and maximum was 14,500 cells/cumm with a peak at 12 to 14 hours. By 72 hours, minimum value was 1,750cells/cumm. At five days, maximum was 5,400 cells/cumm, values remaining unchanged from 5 to 28 days. Also observed that there was a significant increase in absolute neutrophil counts in mild or early onset infections with values reaching as high as 17,500 cells/cumm. So, neutropenia might be the indicator of very severe infection.[20]

Xanthou M et al. observed that in neonates the differential leukocyte count follows a predictable course. At birth, the polymorphonuclear neutrophils are the predominant cells found in the blood with a mean of 8,000cells/cumm. There is marked increase in the absolute value in the first 24 hours reaching a peak of 13,000cells/cumm at 12 hours and dropping to a mean of 4,000cells/cumm by 72 hours of age, thereafter remaining stable.[21]

Gregory J et al. observed that more than 98% of healthy neonates had a neutrophil count within the range of 1,350 to 8,840 cells/cumm after the first four days of life; counts outside this range nearly always occurred during serious bacterial infection. Most neonates with infection had a neutrophil count more than 9,000 cells/cumm. So, neutrophil count could be taken as an indicator of early sepsis.[22]

IMMATURE TO TOTAL NEUTROPHIL (I:T) RATIO:

The effectiveness of immature counts and the ratio of immature forms to mature polymorphs proved by Xanthou Rogatz had its roots in 1929 -1930.The reference values for maximum immature: total polymorphonuclear cell ratio (I:T ratio). The maximum value for first 24 hours was 0.16 gradually falling to 0.13 by 60 hours, and thereafter, remaining stable till 120 hours of age. From 5 to 28, the value remained unchanged at 0.12. In 82% of the cases, increased I: T ratio was associated with infection.[20]

Christensen RD et al observed that in many infants, in spite of a shift to left in the neutrophil count due to the exhaustion of marrow reserves, there was no rise in the absolute number of band cells in circulation. However, in spite of its relative insensitivity, the immature neutrophil count is quite specific for diagnosis of neonatal sepsis and elevated counts are unusual in the uninfected infant. Immature: mature neutrophil ratio of greater than 0.3 was present in 93% of neonates with sepsis as compared to normal newborns.[23]

Philip AG et al observed that Immature: Total neutrophil (I:T) ratio > 0.2 had sensitivity for detecting septicemia. They reported an elevated Immature: Total neutrophil (I:T) ratio and Immature: Mature neutrophil (I:M) ratio in 85% and 87% of the cases of neonatal sepsis, respectively. Thus, they suggested that an elevated I: T ratio can aid in the early diagnosis of bacterial infection in the newborn and the degree of elevation may help in detecting subject at high risk for death from sepsis.[5]

MORPHOLOGY:

Degenerative changes like degranulation, swelling pyknosis, toxic granulations, cytoplasmic vacuolization, Dohle bodies and ingested micro-organisms are frequently seen within the neutrophils in the blood of patients with infection. Toxic granules are primary granules (azurophilic granules) present within the cytoplasm of neutrophils. They are deeply eosinophilic, peroxidase positive granules and stain more deeply than the granules within normal cells.

Buch AC et al demonstrated that digestion of the engulfed bacteria by the neutrophils depends upon lytic enzymes released from granules (lysosomes) present within the white blood cells. The products of digestion are then enclosed within vacuoles formed by the invagination of cell membrane and fusion of this membrane with the lysosomal membrane.[19]

PLATELET COUNT:

Platelets first appear in the human fetus at 5 weeks after conception and increase in number during fetal life, reaching a mean of $150x10^9/L$ by the end of the first trimester of pregnancy and values within the normal adult range by 2 weeks of gestation.

Torkman M et al did a study on platelet count and neonatal sepsis. In their study thrombocytopenia was found in 56.6% of neonatal population. They observed that patients with enterobacter specific sepsis, 85.7% had thrombocytopenia. There was also a significant difference between gestational age and severity of thrombocytopenia.[24]

C- REACTIVE PROTEIN:

C - reactive protein is the long established marker of sepsis. Like many other acute phase proteins, CRP is predominantly synthesized by the liver, mainly in response to interleukin 6 (IL-6). CRP rises whenever an inflammatory process is present; its serum concentration only depends on the intensity of the stimulus and on the rate of synthesis.

Singh M et al. observed a sensitivity and specificity of CRP as 80% and 91% respectively. CRP has been found to highly correlate with infection positivity. It has been used to decide the duration of antibiotic therapy.[8]

Mishra UK et al. took serum CRP levels of > 0.8 mg as a positive result and observed CRP positivity in 69.7% of the cases of proved sepsis and the sensitivity and specificity reported by them were 70% and 64%, respectively. [14]

Hindocha P et al. evaluated the usefulness of a serial study of CRP in the early detection of neonatal septicemia using commercially available latex agglutination slide test. The CRP response is non-specific, as also seen in non-infected infant who showed signs of birth asphyxia with meconium aspiration. The test has the advantage of being performed easily, quickly and cheaply. [25]

Póvoa P et al reported that the immediate decrease in CRP reflects the effect of treatment. Thus, the determination of these proteins can help to guide the treatment of infection in neonates.[26]

ETIOPATHOGENESIS OF NEONATAL SEPSIS:

Maternal, environmental and host factors determine which neonates exposed to a potentially pathogenic organisms will develop sepsis.

Maternal factors

• Age and Parity

- These determine the development of obstetric complications like premature labour or prolonged rupture of fetal membranes.

- A primiparous teenage mother from a low socio-economic group often forms the background of a neonate presenting with sepsis.
- A grand multipara with obstetrical hazards of precipitate delivery, failure of secondary forces with traumatic delivery or birth asphyxia forms another category.

Pathways for intra uterine infection-

- Ascending from vagina and cervix.
- Haematogenous dissemination through the placenta during the stage of blood borne maternal infection.
- Retrograde seeding from the peritoneal cavity through the fallopian tubes.
- Accidental introduction at the time of intra uterine procedures such as amniocentesis, percutaneous umbilical cord sampling, chorionic villous sampling or shunting procedures.

The most common pathway of intra uterine infection is the ascending route. Once microorganisms gain access to intra uterine cavity, they reside in the decidua. A localized inflammatory reaction leads to deciduitis and future extension to chorionitis. The organisms may invade the fetal vessels (choriovasculitis) and proceed through the amnion (amnionitis) into the amniotic cavity leading to intra-amniotic infection. Rupture of membrane is not a prerequisite for intra-amniotic infection because bacteria are capable of crossing intact membranes. Once in the amniotic cavity the bacteria may invade the fetus through different ports of entry. The fetus could aspirate or swallow the infected fluid leading to congenital pneumonia and gastroenteritis.

These could be E. coli, streptococcus faecalis, proteus, klebsiella, pneumococcus, listeria and candida.[27,28]

Neonatal factors:

• Low APGAR scores are associated with increased risk of bacterial infection.

• Premature rupture of membranes has been documented to show a direct correlation between duration of leaking and frequency of early neonatal sepsis.

Foul smelling liquor or abnormal gastric aspirates are indication of chorioamnionitis. Respiratory distress in the newborn can be due to congenital pneumonia or due to super imposed pneumonia. Low birth weight (< 2.5kgs) neonates comprise 7-16% of hospital births but account for 75% of neonatal deaths, half of which can be related to perinatal infections. Similarly pre-term neonates are at an increased risk due to infection, as it is believed that pre-term labour is triggered by amniotic infection.[29,30]

CLINICAL MANIFESTATIONS:

The clinical expression of illness is usually non-specific so that antemortem diagnosis depends on a high index of suspicion. The maternal history is very important in suspecting the onset of neonatal sepsis. Sepsis in the neonates takes into account, the role of prenatal of the newborn capacity to resist infection. The onset of sepsis in the neonates is difficult to determine but may occur at any time during the first month of life.

Early signs and symptoms: [27,31,32]

1. General – Does not look well, off colour, poor temperature regulation.

2. Central nervous system – Apnoea / irritable / high pitched cry, jitteriness / Hypotonic, coma, convulsions.

3. Respiratory system – Apnoea / tachypnoea, cyanosis / grunting, intercostal recession.

4. Gastro intestinal system – Drink poorly, vomiting / increased aspirates, diarrhoea. Abdominal distension and tenderness, hepatomegaly / splenomegaly / enlarged kidneys.

5. Cardio vascular system – Pallor / cyanosis / cutis marmorata. Decreased capillary refill tachycardia / bradycardia / Arrhythmia.

6. Skin - Spots / erythema , Petechiae / Purpura, Pustules / paronychea, Omphalitis, Sclerema.

7. Haemolytic – Jaundice, bleeding, purpura.

8. Musculoskeletal – Pseudo paralysis, Odd limb position and pain on movements

9. Miscellaneous – UTI, conjunctivitis, ethmoiditis, endophthalmitis and otitis media.

DIAGNOSIS:

The definitive diagnosis depends on the positivity of recovering the responsible organism from the blood culture. D V Eltzman and Richard T. Smith in 1957 have reported the reliability of a single pre treatment blood culture in the suspected newborn.[33,34]

Several observations in works done to define the quality of blood necessary to detect bacteremia in infants have been made. In most instances, bacteria may be present in small numbers and it is important that a sufficient volume is drawn for culture. The proportion of blood to liquid medium should be 1: 10. Adequate results can be obtained with 1 ml of venous blood (Flinn American Journal of Applied Medicine 1986). Once blood is drawn and inoculated in to the appropriate media, it should be immediately sent to the microbiology laboratory for incubation.

Urine cultures are frequently positive in septicemic neonates. A suprapubic needle aspiration of urine is the preferred mode of collection. All infected infants will have counts more than 10,000 to 1,00,000 colonies /cumm. Counts more than 1,00,000/cumm must be present if urine is collected by other methods.[35]

Cerebrospinal fluid is to be examined and cultured mandatorily, as 1/3 of septicemia neonates have associated meningitis. Interpretation of CSF counts in the neonate may be difficult. Cell counts in the range of 0-10 cells/cumm are observed at one month of age. Thus, it is apparent that the total evaluation of the CSF is essential to make an early diagnosis of neonatal meningitis.[36]

MATERIALS AND METHODS

The present prospective study was conducted in department of pathology, Shri B. M. Patil Medical College and Hospital, Bijapur from November 2012 to April 2014. A total number of 150 neonates were included in this study with clinical suspicion of sepsis. Blood samples of these neonates sent to laboratory were taken to study the hematological parameters. Data including clinical history, physical findings and probable diagnosis were noted in pretested proforma.

Total leukocyte and differential counts, absolute neutrophil counts and platelet counts were measured using Sysmex XN- 1000 automated analyzer.

Leishman's stained peripheral smears were examined for immature neutrophils and degenerative changes in neutrophils.

Reports of blood culture and c-reactive protein of same samples were collected and evaluated statistically based on the standard reference values. **Culture positivity was taken as criteria for definitive diagnosis.**

INCLUSION CRITERIA:

- All clinically suspected cases of neonatal sepsis.

EXCLUSION CRITERIA

- Neonates of mothers with pregnancy induced hypertension.
- Neonates undergone surgeries.

The Hematological Scoring System (HSS) in this study are as follows:[7, 10]

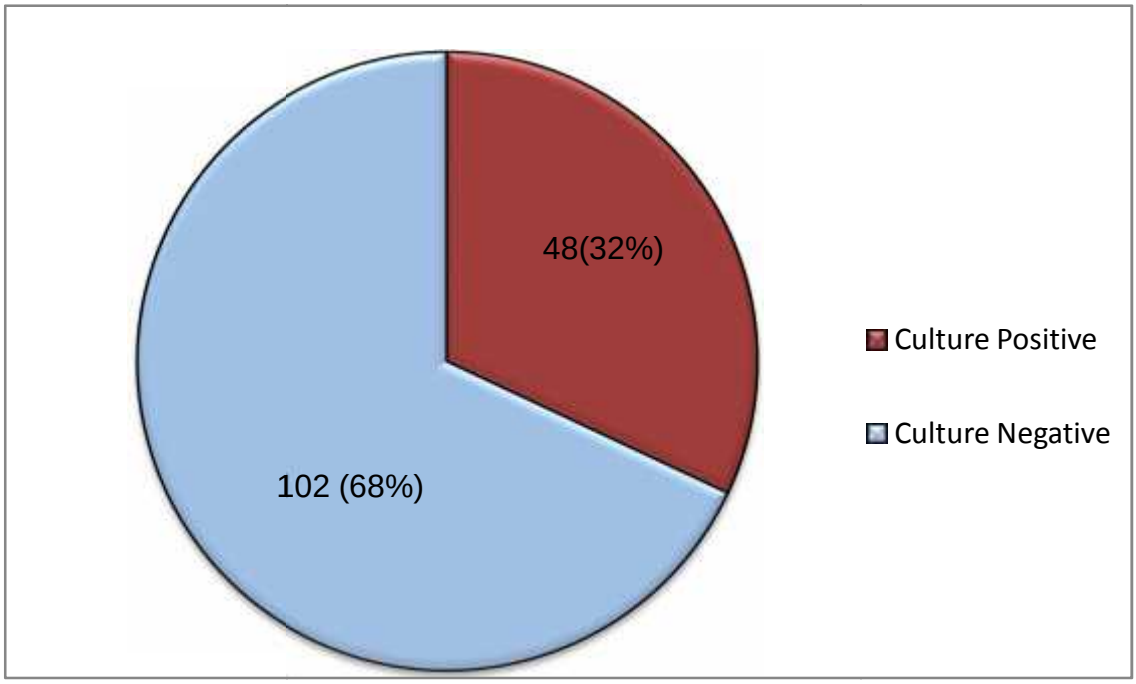
Criteria	Abnormality	Score
1) Total leukocyte count:	< 5000cells/cumm >20000cells/cumm	1
2) Absolute neutrophil count:	< 1800cells/cumm	1
3) Immature neutrophil count :	< 1200cells/cumm	1
4) Immature: total neutrophil count ratio (I/T ratio):	≥0.2	1
5) Immature: mature neutrophil count ratio (I/M ratio):	≥ 0.3	1
6) Platelet count:	< 150000 cells/cumm	1
7) Degenerative changes in neutrophils:	Toxic granules / Cytoplasmic vacuoles	1

RESULTS

Table 1: Showing distribution of cases according to culture result

Total number of cases	Culture Positive	Culture Negative
150	48(32%)	102(68%)

Figure 1: Pie chart showing distribution of cases according to culture result



Out of 150 cases, 48 cases (32%) were culture positive. 102 cases (68%) were culture negative.

HEMATOLOGICAL SCORING SYSTEM:

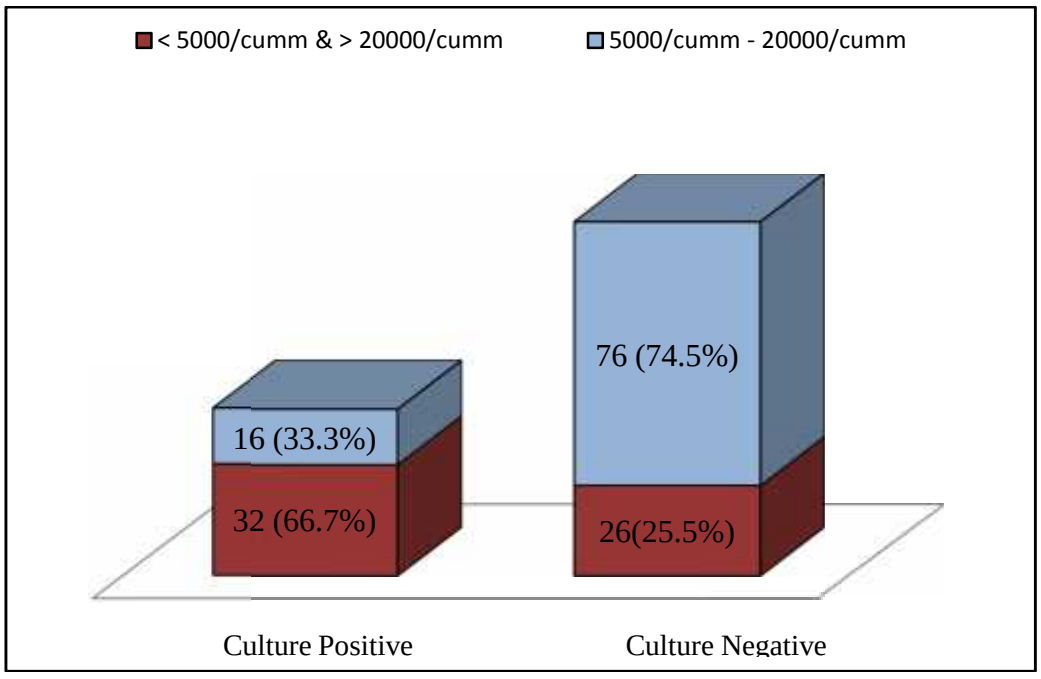
Results of individual diagnostic tests:

1) Total leukocyte count-

Table 2: Showing total leukocyte count in comparision with culture result

Total Count	Culture Positive	Culture Negative	Total
< 5000cells/cumm & >20000cells/cumm	32(66.7%)	26(25.5%)	58
5000cells/cumm – 20000cells/cumm	16(33.3%)	76(74.5%)	92
Total	48	102	150

Figure2: Showing total leukocyte count in comparision with culture results



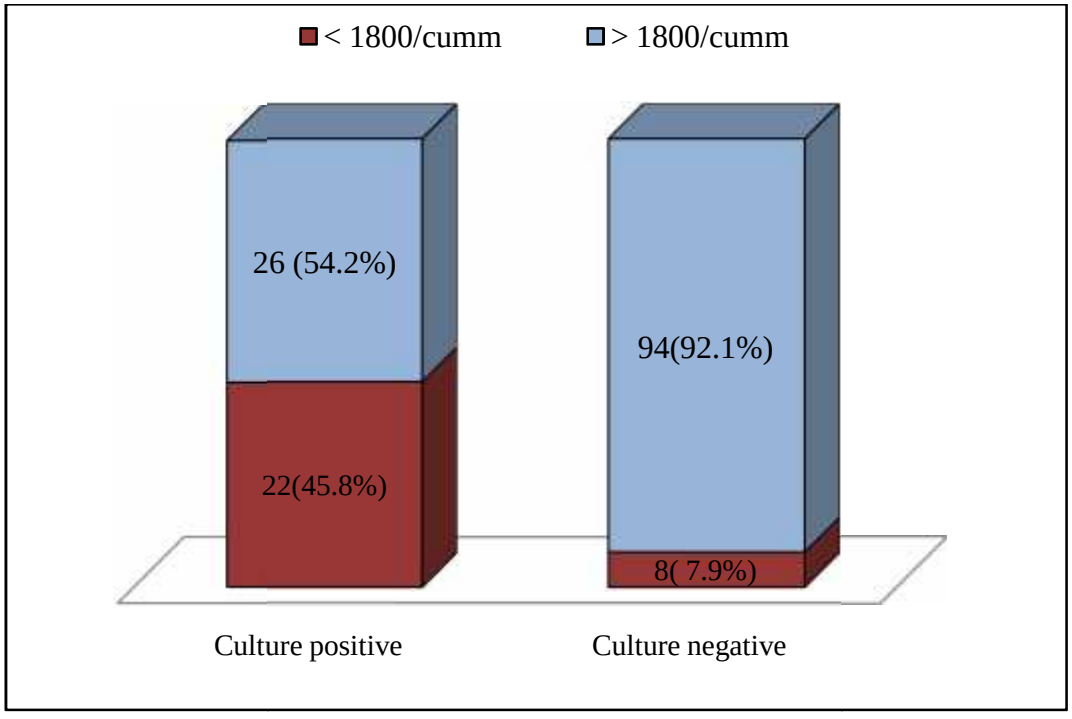
Total leukocyte count < 5000 cells/cumm and > 20000 cells/cumm were seen in 66.7% (32cases) of culture positive neonates, which found to be statistically significant. (P value: <0.0001)

2) Absolute neutrophil count:

Table 3: Showing absolute neutrophil count in comparision with culture results

ANC	Culture Positive	Culture Negative	Total
<1800cells/cumm	22(45.8%)	08(7.9%)	30
≥1800cells/cumm	26(54.2%)	94(92.1%)	120
Total	48	102	150

Figure 3: Showing absolute neutrophil count in comparision with culture result



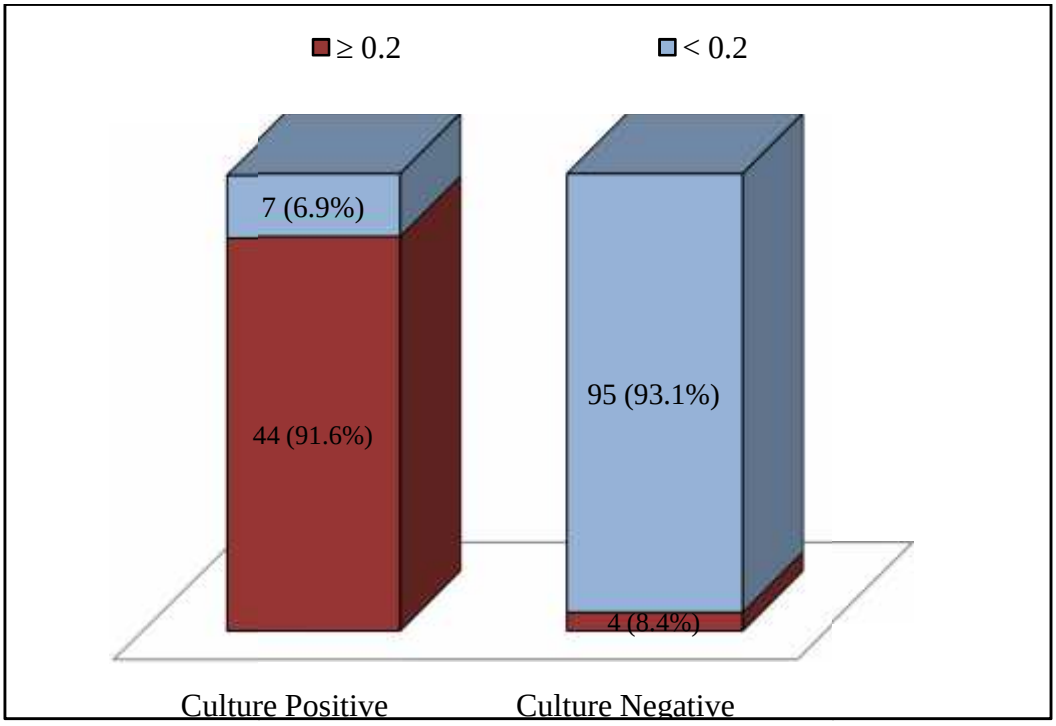
Among culture positive neonates, 45.8% (22cases) showed absolute neutrophil count < 1800 cells/cumm. Among culture negative cases, only 7.9% showed absolute neutrophil count <1800/cumm. This result was statistically significant. (P value: < 0.001)

3) Immature:Total (I:T) neutrophil ratio :

Table 4: Showing I:T ratio in comparison with culture results

I/T Ratio	Culture Positive	Culture Negative	Total
≥0.2	44(91.6%)	07(6.9%)	51
< 0.2	04(8.4%)	95(93.1%)	99
Total	48	102	150

Figure 4: Showing I:T ratio in comparison with culture results



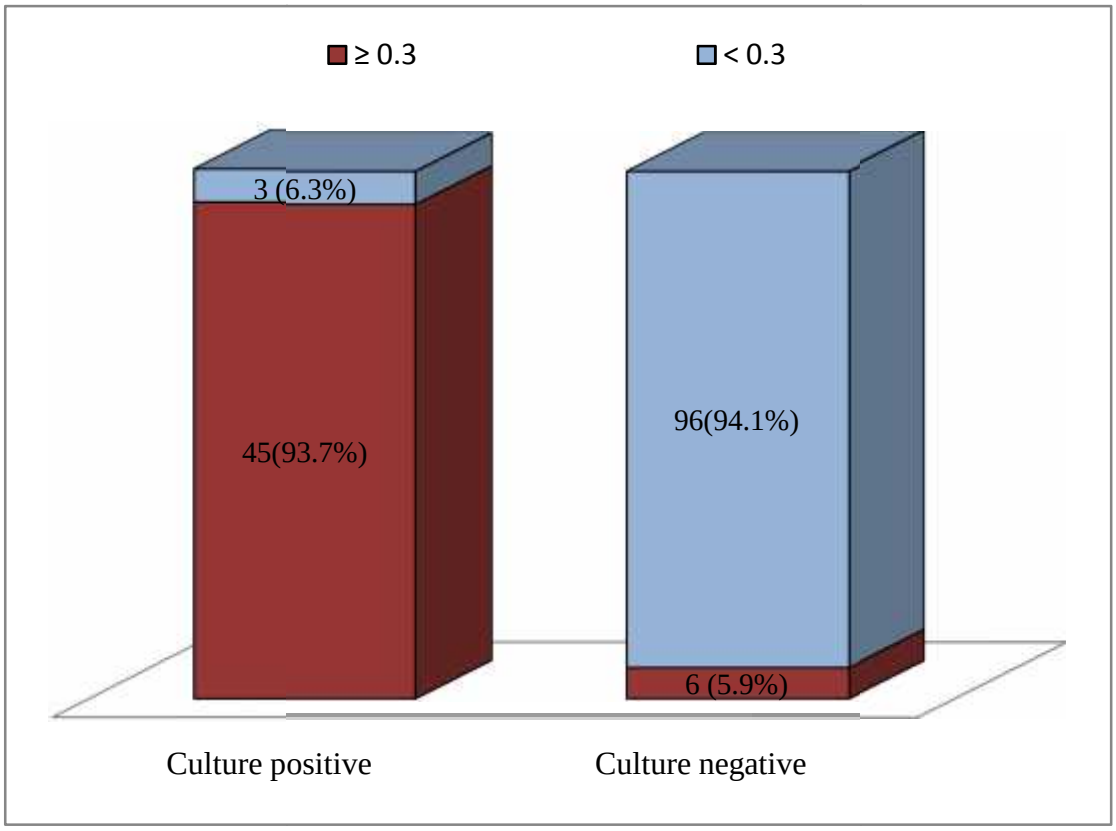
Majority of culture positive cases show I:T ratio of more than or equal to 0.2. Among culture negative cases only 6.9% (7 cases) showed I:T ratio more than equal to 0.2. This result was statistically significant.(P value < 0.001)

4) Immature: mature (I:M) neutrophils ratio:

Table 5: Showing I:M ratio in comparison with culture results

I/M Ratio	Culture Positive	Culture Negative	Total
≥0.3	45(93.7%)	06(5.9%)	51
< 0.3	03(6.3%)	96(94.1%)	99
Total	48	102	150

Figure 5: Showing I: M ratio in comparison with culture results



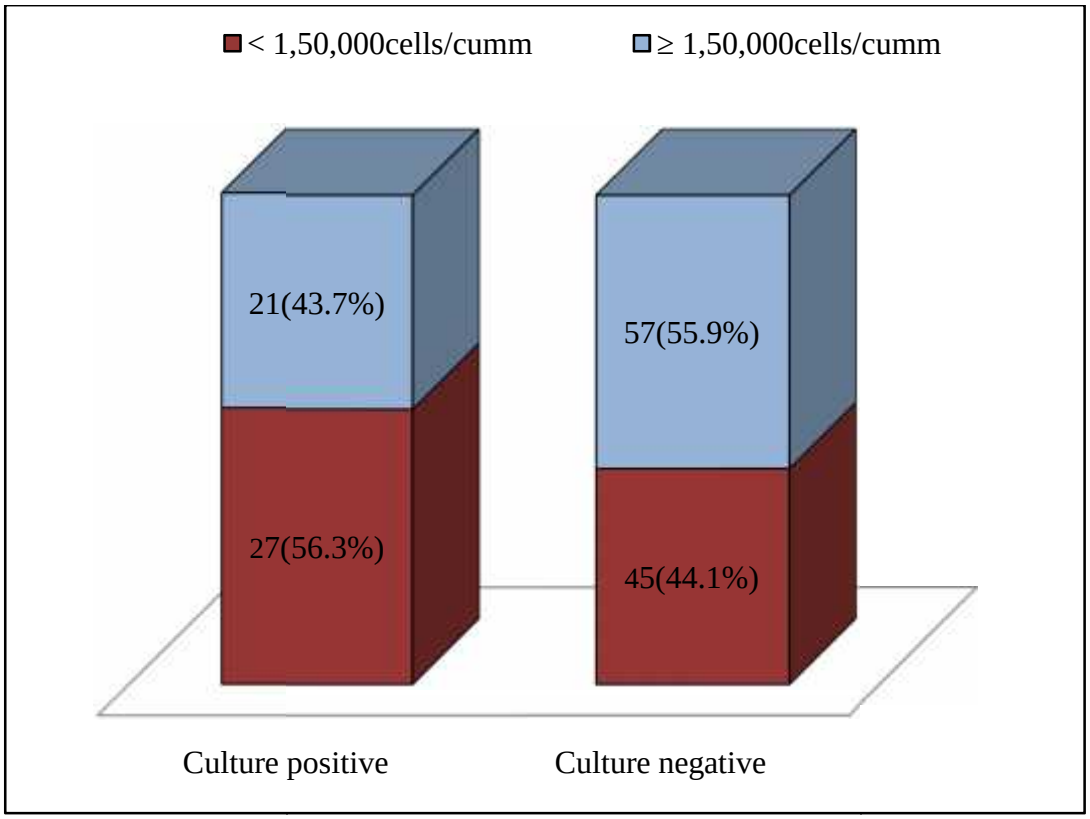
Majority of culture positive cases show I:M ratio ≥ 0.3. Among culture negative cases only 5.9 % (6 cases) showed I:M ratio ≥ 0.3. This result was statistically significant. (P value < 0.001)

5) Platelet count in comparision with culture:

Table 6: Showing platelet count in comparision with culture results

Platelet count	Culture positive	Culture Negative	Total
<150000cells/cumm	27(56.3%)	45(44.1%)	72
≥ 150000cells/cumm	21(43.7%)	57(55.9%)	78
Total	48	102	150

Figure 6: Showing platelet count in comparision with culture results



Here 56.3% of culture positive cases showed platelet count of <150000 cells/cumm. This result was statistically significant.(P value < 0.0001)

6) Peripheral smear findings:

Table 7: Showing presence of toxic granules in neutrophils on peripheral smear in culture positive and negative cases.

Toxic Granules	Culture Positive	Culture Negative	Total
Present	37(77%)	53(51.9%)	90
Negative	11(23%)	49(48.1%)	60
Total	48	102	150

Peripheral smear showed toxic granules and degenerated neutrophils in the form of vacuolisation in many cases. Out of all culture positive cases 77% (37 cases) showed toxic granules.

Presence of toxic granules on peripheral smear showed 77% of sensitivity, 48.1% specificity, 36% and 74.7% of positive and negative predictive value respectively.

Figure 7 : Peripheral smear showing toxic granules in neutrophil (leishman stain,100x)

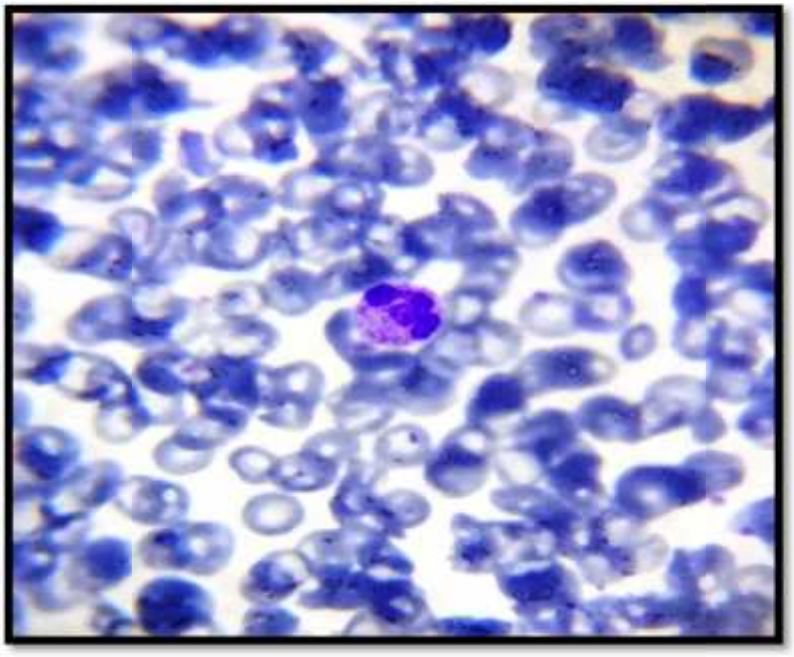
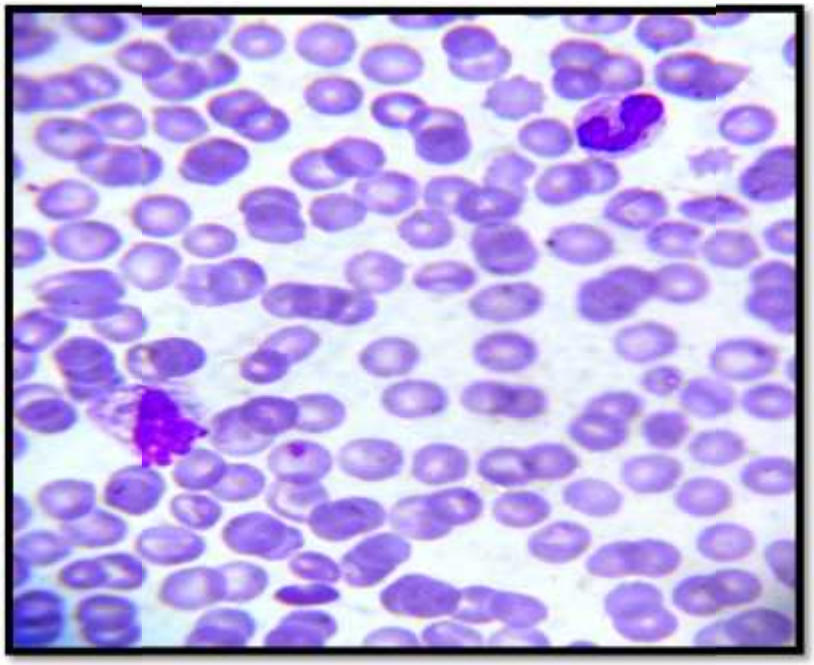


Figure 8 : Peripheral smear showing degenerative neutrophil (leishman stain,100x)

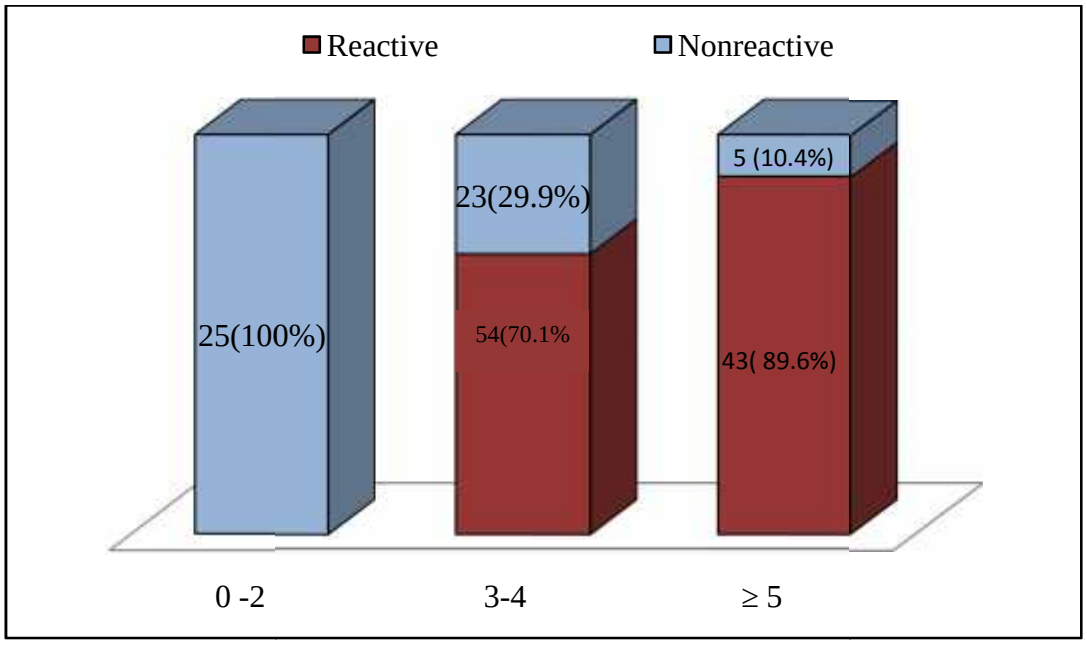


Hematological score comparision with C-Reactive protein:

Table 8: Showing hematological scoring system (HSS) comparision with C-Reactive protein:

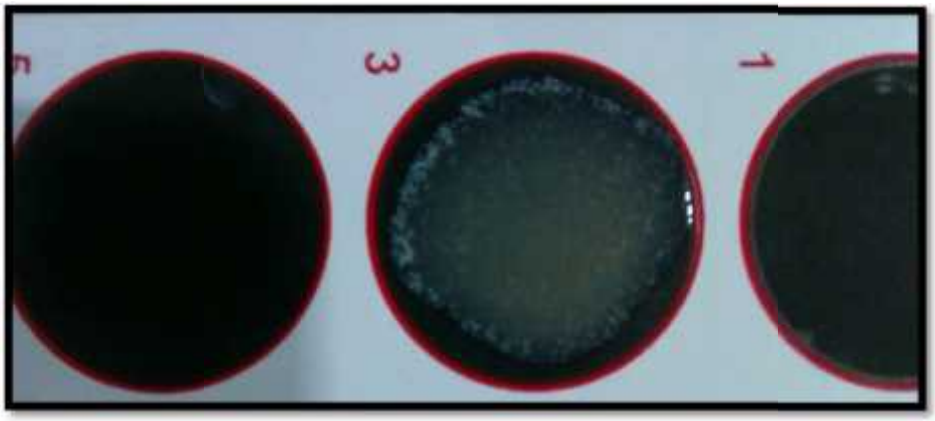
HSS	CRP-Reactive	CRP-Nonreactive	Total
0-2	00	25(100%)	25
3-4	54(70.1%)	23(29.9%)	77
≥5	43(89.6%)	05(10.4%)	48
	97	53	150

Figure 9: Showing hematological scoring system comparision with C-Reactive protein:



Hematological score ≥5 showed 89.6% (43 cases) CRP test reactive. Score 3-4 showed 70.1% (54 cases) CRP test reactive. Score 0-2 (25 cases) showed CRP test nonreactive. This result was statistically significant. (P value < 0.001)

Figure 10: Showing reactive CRP

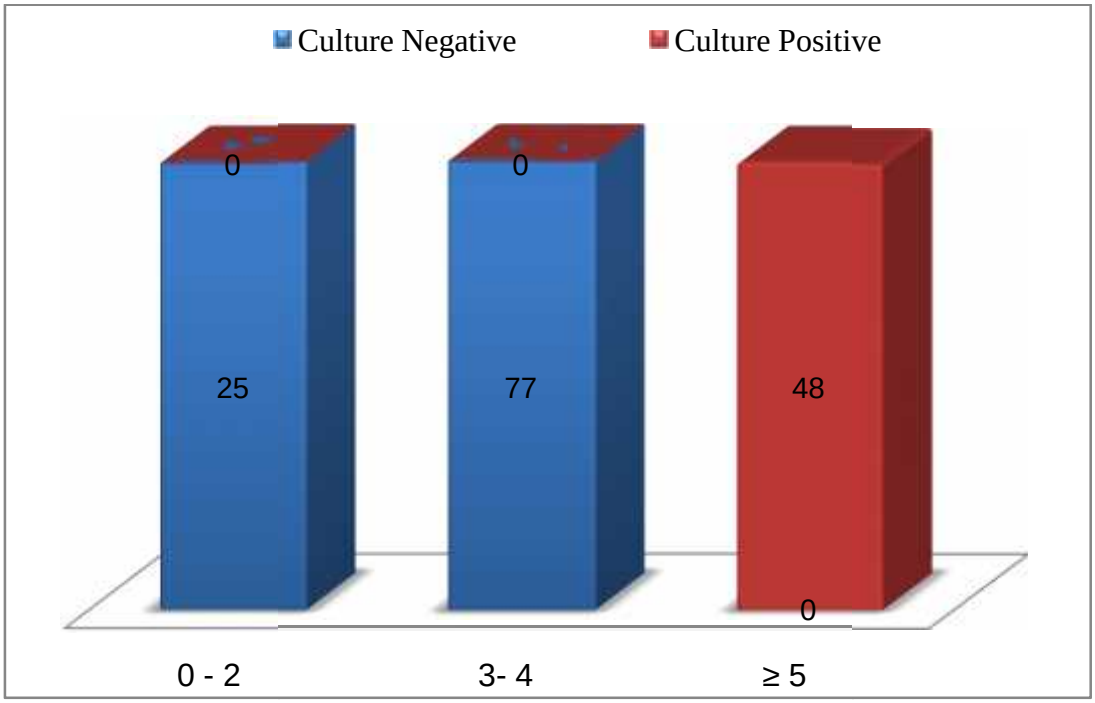


Hematological score comparision with culture:

Table 9 : Showing hematological scoring system (HSS) comparision with culture results:

HSS	Culture Positive	Culture Negative	Total
0 - 2	00	25	25
3 - 4	00	77	77
≥ 5	48	00	48
	48	102	150

Figure 11: Showing haematological scoring system (HSS) comparision with culture results



All the culture positive cases showed hematological score ≥5. Among culture negative cases 25 cases with score 0-2 and 77 cases with score 3 – 4. This result was statistically significant. (P value < 0.001)

Sensitivity, specificity, positive predictive value, negative predictive value of each test.

Total leukocyte count showed high specificity but least sensitivity

Absolute neutrophil count showed high specificity but least sensitivity

I:T ratio showed high specificity and high sensitivity

I: M ratio showed high specificity and high sensitivity

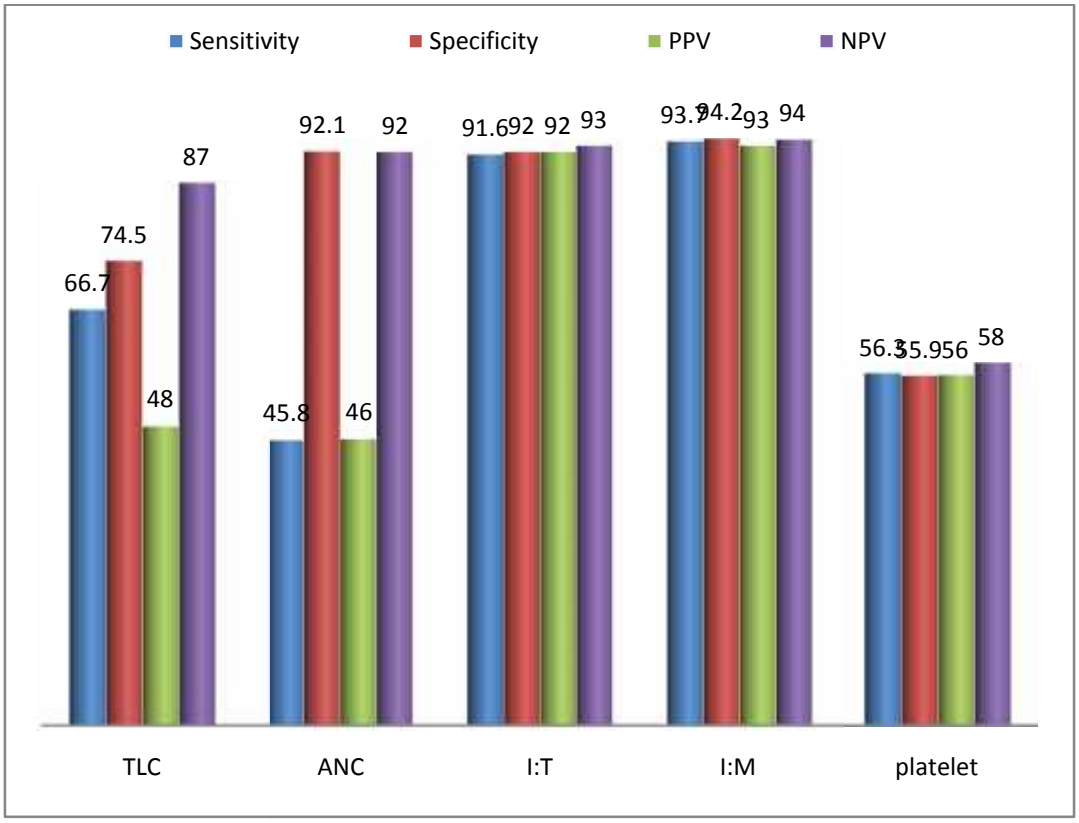
Platelet count showed high negative predictive value and least positive predictive value

Table 10: Showing sensitivity, specificity, positive predictive value and negative predictive value of each test.

	SENSITIVITY	**SPECIFICITY**	**PPV**	**NPV**
TLC	66.7%	74.5%	48%	87%
ANC	45.8%	92.1%	46%	92%
I:T	91.6%	92.1%	92%	93%
I:M	93.7%	94.2%	93%	94%
Platelet	56.3%	55.9%	56%	58%

TLC: Total leucocyte count, ANC: Absolute neutrophil count, I:T: Immature to total neutrophil ratio, I:M Immature to mature neutrophil ratio, PPV-Positive predictive value, NPV-Negative predictive value.

Figure 12: Showing sensitivity, specificity, positive predictive value and negative predictive value of each test.



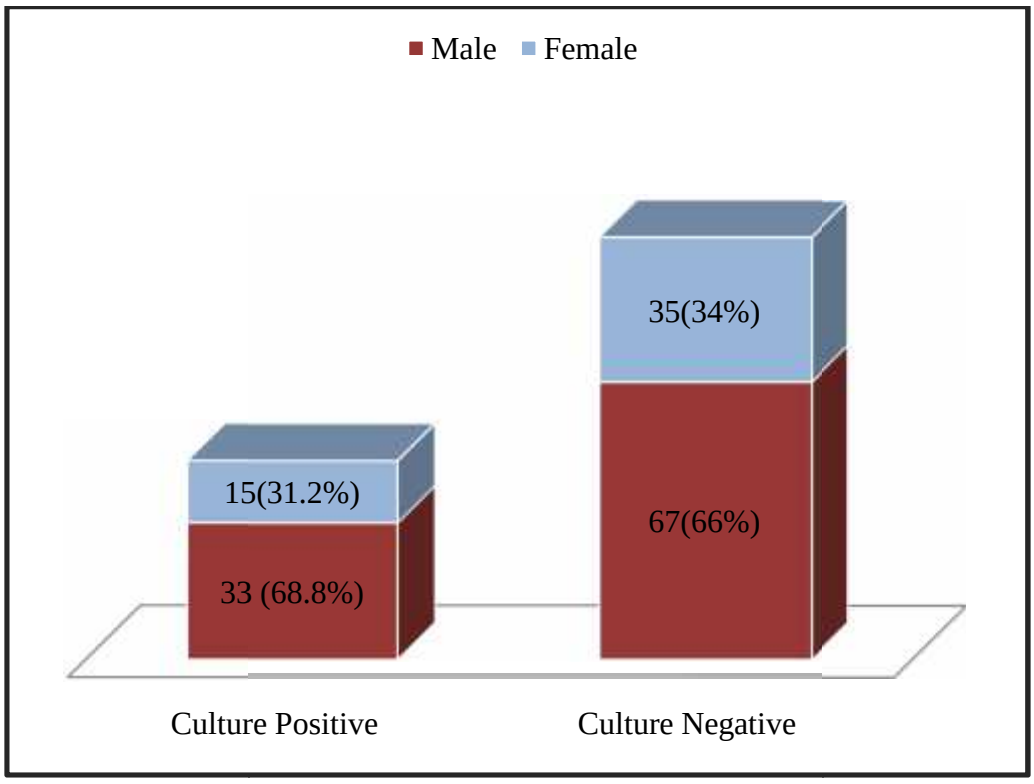
TLC: Total leucocyte count, ANC: Absolute neutrophil count, I:T ratio: Immature to total neutrophil ratio, PPV-Positive predictive value, NPV-Negative predictive value.

Sex:

Table 11: Showing gender-wise distribution of cases of neonatal sepsis.

Blood Culture	Male	Female	Total
Positive	33(68.8%)	15(31.2%)	48
Negative	67(66%)	35(34%)	102
Total	100	50	150

Figure13: Showing gender-wise distribution of cases of neonatal sepsis



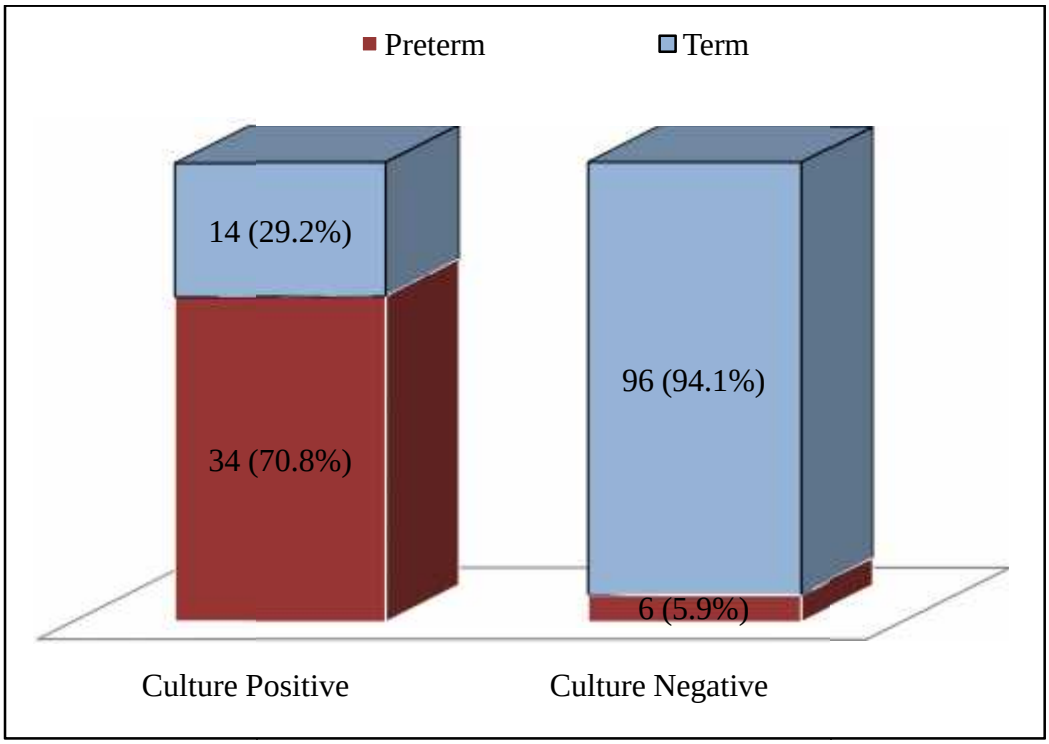
Males are more susceptible for sepsis. Among culture positive neonates 68.8% were males, 31.2% were females. Relation of gender and culture positivity was statistically insignificant. (P value: 0.71)

Maturity:

Table 12: Showing distribution of cases of neonatal sepsis based on maturity at birth

Blood Culture	Preterm	Term	Total
Positive	34 (70.8%)	14 (29.2%)	48
Negative	06 (5.9%)	96 (94.1%)	102
Total	40	110	150

Figure 14: Showing distribution of cases of neonatal sepsis based on maturity at birth



Preterms were accounting for 70.8% of cases among culture positive neonates, where as 29.2% were term neonates. Maturity and outcome of sepsis were statistically significant (P value- 0.02)

DISCUSSION

Neonatal sepsis is a serious illness with high morbidity and mortality. Though it is a life threatening condition, it is treatable due to advancement in antibiotic therapy.
Although blood culture is the most definitive test for the diagnosis of neonatal sepsis, it has low sensitivity and leads to delay in the diagnosis for at least 48 hours, if the reports are awaited.

The limitations in diagnosis of neonatal sepsis are frustrating for clinicians; at present there is no single test which meets the criteria of an ideal diagnostic test.
Therefore, in practice, pediatricians prescribe antibiotics empirically due to rapidity of clinical deterioration in septic newborn infants.[9] Now a days, prophylactic use of antibiotics came under vigorous scrutiny due to development of drug resistance, cost of unnecessary therapy and problems of drug toxicity. For example, long term use of amino-glycosides drug used during neonatal period is hazardous.

Therefore, quick diagnostic tests with greater sensitivity are desirable and , we need a useful screening protocol where a balance must be achieved between sensitivity and specificity.

one needs to be consider four characteristics of laboratory tests when evaluating an infant for possible sepsis i.e. sensitivity, specificity, positive predictive value and negative predictive value. A sensitive test will rarely miss an infant with sepsis and a specific test will rarely misclassify an infant who is healthy. Sensitivity is a desirable

characteristic when the condition is serious and treatable, which is certainly true of infants with sepsis. Given that the treatment of neonatal sepsis has a relatively low toxicity of the two sensitivity is clearly more important. Positive predictive value and negative predictive value tell clinicians how to interpret a laboratory test. Positive predictive value is the probability an infant is infected when the laboratory test is abnormal, while negative predictive value is the probability an infant is healthy when the test is normal. If the goal is to only treat infants with high probability of infection, then clinicians must use screening tests with a high negative predictive value.

Thus, realizing the importance of early and correct diagnosis of neonatal septicemia and unnecessary burden of antibiotics in these cases, many studies have made an attempt to diagnose septicemia by means of simple scoring called as Hematological Scoring System.

Individual tests

Total leukocyte count:

Neonatal septicaemia is associated with leukopenia. Gerdes et al,[37] Nandy et al,[38] Zaki et al,[39] Khair et al,[9] Buch et al,[19] Sriram[11] and Makkar et al,[12] observed low sensitivity for this test as in the present study. Gerdes et al[37] observed high specificity(91%). In the present study leukocyte count had low sensitivity and high specificity. Gerdes et al[37], Khair et al[9] and Sriram[11] study showed high negative predictive value while it was low in the study by zaki et al Nandy et al [38] observed relatively high positive predictive value.

Table 13: Showing comparison of diagnostic tests for total leucocyte count in the present study with other studies.

AUTHORS (YEAR)	SENSITIVITY	SPECIFICITY	PPV	NPV
Gerdes etal[37](1998)	29%	91%	27%	91%
Nandy etal[38](2007)	33.3%	66.6%	69.5%	31.4%
Zaki et al[39](2009)	48%	77%	67%	62%
Khair et al [9](2010)	50 %	91%	43%	93%
Buch et al [19](2011)	50.77%	63.64%	62.26%	52.24%
Sriram [11] (2011)	63.6%	51%	12.1%	93%
Makkar et al [12]	55%	63%	86.36%	56.89%
Present study	66.7%	74.5%	48%	87%

Absolute neutrophil count:

Absolute Neutrophil Count < 1800 cells/cumm is believed to be the best predictor of sepsis, while neutrophilia does not correlate well. Present study showed 45.8%, 92.1%, 46% and 92% of sensitivity, specificity, positive predictive value and negative predictive value respectively for absolute neutrophil count. Nandy et al,[38] Zaki et al,[39] Sriram[11] and present study showed low sensitivity for absolute neutrophil count but high specificity in the present study. But Buch et al[19] got comparatively high sensitivity. Specificity in the present study was higher as compared to other study.

Table 14: Showing comparison of diagnostic tests for absolute neutrophil count in the present study with other studies.

AUTHORS(YEAR)	SENSITIVITY	SPECIFICITY	PPV	NPV
Nandy et al[38](2007)	25%	84.8%	78%	34.1%
Zaki et al[39] (2009)	55%	74%	67%	64%
Buch et al[19](2011)	66.15%	90.91%	89.58%	69.44%
Sriram[11] (2011)	50%	49.6%	3.5%	96.5%
Present study	45.8%	92.1%	46%	92%

Immature to total neutrophil ratio (I:T ratio):

This has been one of the most extensively studied neutrophil index. Whereas some authors have calculated the I:T ratio taking into account all the immature neutrophils, many authors have calculated the Band neutrophil: neutrophil ratio wherein only the band neutrophils were considered in the numerator of the ratio. There is a wide range of reported sensitivity and specificity of the I:T ratio due to variation in methods, study design or the definition of infection. Factors which may account for the reported variability of the I:T ratio performance include: the I:T ratio is operator dependent, neutrophils are affected by non infective events and the I:T ratio is less sensitive after the first week of life. The definition of which constitutes an immature neutrophil also determines the outcome.[11]

Khair et al[9] observed 100% sensitivity and negative predictive value, but very low specificity(4%) and positive predictive value(13%). Nandy et al[38] also observed very low sensitivity in their study. Present study also showed high sensitivity, high specificity and negative predictive value. With the exception of negative predictive value, the present study correlates well with those by Sriram[11] and buch et al.[19] Sriram in her study observed very low negative predictive value, but present study showed comparatively high negative predictive value.

Table 15: Showing comparison of I:T ratio test among various studies

AUTHORS (Year)	Sensititvity	Specificity	PPV	NPV
Nandy et al[38](2007)	19.4%	78.8%	66.6%	83.8%
Zaki et al [39](2009)	76%	87%	85%	79%
Khair et al [9](2009)	100%	4%	13%	100%
Buch et al [19](2011)	89.29%	70.91%	78.38%	84.78%
Sriram et al [11](2011)	52.2%	56.5%	82.8%	22.8%
Present	91.6%	92.1%	92%	93%

Immature to mature neutrophil ratio (I: M ratio)

This is also one of the most extensively studied neutrophil index. Authors have calculated the I:M ratio taking into account all the immature neutrophils and mature neutrophils. Factors which may account for the reported variability of the I:M ratio performance include: the I:M ratio is operator dependent, neutrophils are affected by non infective events and the I:M ratio is less sensitive after the first week of life. But I:M ratio is more sensitive and more specific with compare of I:T ratio.[11]

Table 16: Showing comparison of I:M ratio test among various studies

AUTHORS (Year)	Sensititvity	Specificity	PPV	NPV
Nandy et al[38](2007)	21.4%	87.8%	59.6%	85.8%
Zaki et al [39](2009)	77%	89%	84.3%	79.2%
Khair et al [9](2009)	100%	7%	11%	100%
Buch et al[19](2011)	91%	70.91%	73.4%	85.9%
Sriram et al [11](2011)	54%	58.5%	83.8%	27.3%
Present	93.7%	94.2%	93%	94%

Platelet count:

Thrombocytopenia was frequently associated with sepsis and indicated poor prognosis. This is thought to be due to increase platelet destruction, sequestration secondary to infections, failure in platelet production due to reduce megakaryocytes or damaging effects of endotoxin.[12] Thrombocytopenia was a poor predictor of neonatal septicaemia in the present study. Present study observed 56.3% of sensitivity, 55.9% of specificity, 56% and 58% of positive and negative predictive value respectively. This is because platelet counts are significantly low in all neonates in the first week of life and only rise after this period.[11] Makkar et al[12] observed comparatively higher scores. Narasimha et al.[10] observed high positive predictive value for this test. She stated that platelet count was helpful in identifying neonates who really had sepsis.

Table 17: Showing comparision of diagnostic test for thrombocytopenia in the present study with other studies.

AUTHORS(YEAR)	Sensitivity	Specificity	PPV	NPV
Aprana Narasimha[10](2010)	47.36%	75%	85.71%	31%
Buch et al[19](2011)	46.15%	83.64%	76.92%	56.79%
Sriram et al[11](20110	57.5%	53.3%	39.7%	70.2%
Makkar et al[12](2013)	81.25%	94.44%	77.77%	82.92%
Present study	56.3%	55.9%	56%	58%

Hematological scoring system (HSS):

Table 18: Showing hematological scoring system (HSS) comparision with culture:

HSS	Culture Positive	Culture Negative	Total
0 - 2	00	25(100%)	25
3 - 4	00	77(100%)	77
≥ 5	48(100%)	00	48
	48	102	150

In our study HSS showed ≥ 5 score in 48 cases, which all cases were culture positive so, 100% sensitivity, specificity 68% with positive predictive value 30% and negative predictive value 100% which is similar to **Khair et al**.[9] study where they found score ≥ 4 had a sensitivity of 100%, specificity of 60%, with positive predictive value (PPV) 26% and negative predictive value (NPV) 100%.

Table 19: Showing hematological scoring system (HSS) comparision with C-Reactive protein:

HSS	CRP -Reactive	CRP-Nonreactive	Total
0 - 2	00	25(100%)	25
3 - 4	54 (70.1%)	23(29.9%)	77
≥5	43 (89.6%)	05(10.4%)	48
	97	53	150

In our study out of 97 CRP test reactive cases 43 cases had score more than 5 and 54 cases had score in between 3-4, indicating that, all the cases of HSS score more than 3 were CRP test reactive. All the cases of HSS score in between 0-2 were CRP test nonreactive, which is similar to **Sriram R et al**.[11] study where they found sensitivity and specificity of CRP as 80% and 91% respectively.

Sex:

Wilson et al (1974) observed male predominance because congenital anomalies of urinary tract are more in males, attributable to higher prevalence of congenital anomalies in them and hence risk of urinary tract infection.[41] In females, resistance occurs due to their heterozygosity for gene controlling immunoglobulin synthesis on X-chromosome.[29] Buch et al observed higher incidence of neonatal sepsis in males as compared to females. According to Buch et al ,it is because of the fact that the factors regulating the synthesis of gamma globulin are situated in X chromosome and male has only one X- chromosome.[19] In the present study 68.8% culture positive cases were males and 31.2% were females with male: female ratio being 2.2:1. In our study percentage of males affected by sepsis were more compared to females.

Table 20: Showing comparision of sex distribution in present study with different study.

AUTHORS(YEAR)	MALES(%)
Rodwell et al [7](1988)	57%
Haider et al [4](2010)	59%
Sriram et al [11] (2011)	60.3%
Buch et al [19](2011)	60%
Present study	68.8%

Maturity:

Mishra et al stated the higher incidence of sepsis in preterm and low birth weight neonates.[14] Our study showed higher percentage of culture positivity among preterm neonates compared to normal weight neonates. We found that 70.8% of culture positive cases were preterm neonates. This finding correlates with other studies, several other studies reported same. Findings from present study are consistent with other recent studies. Preterms are most susceptible to infections due to inherent deficiencies of both humoral and cellular defence mechanisms. It is suggested that the incidence of septicaemia increases with decreased gestational age of neonates.[29]

Table 21: Showing percentage of preterm neonates in the present study with different studies

Authors (year)	Preterm(%)
Haider et al[4](2010)	69%
Sriram et al[11] (2011)	37.4%
Buch et al [19](2011)	83.33%
Present study	70.8%

SUMMARY

This study was carried out in tertiary care centre to evaluate the role of hematological scoring system in early diagnosis of neonatal sepsis. Total number of 150 neonates with clinical suspicion of sepsis were studied. The clinical history of the patient was collected as indicated in the proforma. Out of 150 neonates studied, 48 cases were bacteriologically positive, 97 cases were CRP test reactive. Hematological scoring system (HSS) were correlated with CRP and culture results. All the 48 cases(100%) of culture positive cases showed HSS of ≥ 5. All the 97 cases (100%) of CRP test reactive showed HSS of ≥ 3. Diagnostic performances of individual tests were calculated statistically.

Among parameters studied, when single tests were considered, I:M ratio was the most sensitive(93.7%) and most specific(94.2%) test. I:M ratio had the highest positive and negative predictive value. I:T ratio and I:M ratio were best tests with good sensitivity, specificity, highest positive and negative predictive value. Degenerated neutrophil and toxic granules in neutrophils were important findings on peripheral smear examination.

CONCLUSION

Early clinical symptomatology of neonatal sepsis is mimicked by different other disorders affecting the newborn. Delay in the administration of antimicrobial therapy is fraught with dangers of several complications and increased mortality. Therapy cannot wait in a critically sick neonate till culture reports are available. On the other hand, indiscriminate over use of antibiotics on the basis of clinical suspicion alone is hazardous for any neonatal unit because this will lead to emergence of resistant organisms.

HSS is simple, quick and cost effective tool which includes very simple tests of total white blood cell counts, absolute neutrophil count, differential counts of mature and immature neutrophils ratios obtained from these values, platelet count and the degenerative changes of neutrophils. It can be inferred from the present study that the use of indirect indicators of infection, like hematological scoring system (HSS) of ≥ 5 and ≥ 3 in addition to the clinical judgement has greater sensitivity as it showed excellent correlation with blood culture and CRP test respectively. HSS of 0-4 and 0-2 score showed excellent specificity with blood culture and CRP test respectively. So HSS is valuable test and can be performed easily at the primary health care centre level also as routine screening of all clinically suspected cases of neonatal septicaemia , thus helps to provide early diagnosis and effective guidelines for the management of neonatal sepsis.

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ANNEXURE-I

B.L.D.E.UNIVERSITY'S
SHRI.B.M.PATIL MEDICAL COLLEGE, BIJAPUR-586 103
INSTITUTIONAL ETHICAL COMMITTEE

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Ethical Committee of this college met on 18-10-2012 *at* 3-30pm *to scrutinize the Synopsis of Postgraduate Students of this college from Ethical Clearance point of view. After scrutiny the following original/corrected & revised version synopsis of the Thesis has been accorded Ethical Clearance.*

Title Role of hematological scoring System in diagnosis of neonatal Sepsis

— x — x — x —

Name of P.G. student Dr. Omkar Bhalodia
Pathology

Name of Guide/Co-investigator Dr S. B. Hiddanagi
Prof. Pathology

DR.TEJASWINI. VALLABHA
CHAIRMAN
INSTITUTIONAL ETHICAL COMMITTEE
BLDEU'S, SHRI.B.M.PATIL
MEDICAL COLLEGE, BIJAPUR.

Following documents were placed before E.C. for Scrutinization
1) Copy of Synopsis/Research project.
2) Copy of informed consent form
3) Any other relevant documents.

ANNEXURE-II

PROFORMA FOR STUDY:

Demographic Details:

Name:

Age :

Sex: M/F

OPD / IPD no.:

Chief complaints:

History of present illness:

Past history:

Family history:

Maternal history:

General physical examination:

Systemic examination:

- **Cardiovascular system**
- **Respiratory system**
- **Central Nervous System**
- **Per Abdomen Examination**

Clinical diagnosis:

Hematological investigations: (Complete blood count)

Parameters	
WBC	
RBC	
HGB	
HCT	
MCV	
MCH	
MCHC	
PLATELETS	
LYMPHOCYTES(%)	
MIXED (%)	
NEUTROPHILS(%)	
RDW	
PDW	
MPV	

Peripheral Smear Examination:

RBC:

WBC:

Platelet count:

Hematological scoring system (HSS):

Total leukocyte count (TLC)

Absolute neutrophil count (ANC)

Immature neutrophil count

Immature to total neutrophil (I:T) ratio

Immature to mature neutrophil (I:M) ratio

Degenerative changes in neutrophils

Special investigations:

C- reactive protein:

Blood culture :

ANNEXURE-III

KEY TO MASTER CHART

S. No	Serial Number
M	Male
F	Female
TLC	Total leukocyte count
ANC	Absolute neutrophil count
I:T	Immature to total neutrophil ratio
I:M	Immature to mature neutrophil ratio
PLT	Platelet count
D.C.	Degenerative changes
CRP	C-Reactive protein
R	Reactive
NR	Non Reactive
BC	Blood Culture
P	Positive
N	Negative

S. No	Pt. Name	Age	Sex	Maturity	TLC	ANC	Immature Neutrophils	I:T	I:M	D.C.	PLT	SCORE	CRP	BC
1	B/o Manjula	1	F	TERM	31,400	5433	1317	0.21	0.3	NO	0.6	4	R	N
2	B/O Mahananda P	10	F	TERM	4,800	1690	1190	0.19	0.3	SEEN	1.5	5	R	P
3	B/O Santha bai	20	M	TERM	23,700	10191	700	0.16	0.2	SEEN	1.9	3	NR	N
4	B/O Chanaamma M	1	M	TERM	4500	3920	600	0.15	0.18	NO	0.2	2	NR	N
5	B/O Basamma	1	M	PRETERM	4,000	1530	1070	0.18	0.22	SEEN	0.4	5	R	P
6	B/O Roopa patil	1	M	PRETERM	16,500	7090	2890	0.24	0.33	NO	0.5	5	R	P
7	B/o Kashibai	20	M	TERM	4900	1650	730	0.14	0.23	SEEN	1	2	NR	N
8	B/O Shilpi	1	M	TERM	13,100	6970	1190	0.21	0.3	SEEN	1.2	4	R	N
9	B/O Shabana D	1	M	PRETERM	4,500	1,250	950	0.23	0.3	SEEN	1	6	R	P
10	B/O Isharatamma	1	F	TERM	15,400	9850	1980	0.2	0.27	SEEN	2.7	3	R	N
11	B/O Laxmi	22	M	TERM	8,000	3760	940	0.16	0.22	SEEN	0.25	2	NR	N
12	B/o shivamma U	2	M	PRETERM	24500	16950	4250	0.25	0.33	SEEN	1.8	6	R	P
13	B/O Megha T	1	M	TERM	10900	7980	1260	0.23	0.31	SEEN	3.03	4	NR	N
14	B/O Sumitra	1	M	PRETERM	4850	1690	1080	0.24	0.32	NO	2.9	5	R	P
15	B/O Vaishali	1	F	TERM	13000	6240	1270	0.19	0.25	SEEN	1.5	2	NR	N
16	B/O Mayamma	1	M	PRETERM	35703	19310	14530	0.23	0.3	SEEN	2.7	6	R	P
17	B/O Mahananda J	1	M	TERM	21900	10930	4570	0.24	0.33	SEEN	3.68	6	R	P
18	B/O Yashoda M	1	M	TERM	25600	11700	3670	0.27	0.37	NO	2.7	5	R	P
19	B/o Shaminabee	1	M	TERM	7900	6080	1890	0.18	0.28	SEEN	2.3	3	NR	N
20	B/o Anjuma	1	M	TERM	4600	2570	1540	0.19	0.26	SEEN	2.7	3	NR	N
21	B/o Bhagyashree	3	F	TERM	4900	1420	680	0.23	0.34	SEEN	1.98	5	R	P
22	B/o Mananada	1	F	PRETERM	25800	10780	4380	0.29	0.38	SEEN	1.8	6	R	P
23	B/O Renuka	1	F	PRETERM	4200	1560	1150	0.25	0.34	SEEN	0.9	7	R	P
24	B/o Shanu bai	1	M	TERM	20500	8980	1030	0.18	0.23	SEEN	2	3	R	N
25	B/O Roopa	3	M	PRETERM	4000	1140	570	0.27	0.38	SEEN	0.3	6	R	P
26	B/O Renuka	1	M	PRETERM	24000	10380	1220	0.2	0.31	SEEN	0.6	6	R	P
27	B/o Basamma	1	F	TERM	14900	8930	3530	0.21	0.3	NO	1.9	4	NR	N

28	B/O Vijayalaxmi B	1	F	TERM	24000	11750	4130	0.26	0.33	SEEN	1.5	6	R	P
29	B/o Renuka	7	M	TERM	10300	4326	1070	0.19	0.27	SEEN	3.1	1	NR	N
30	B/o sangeeta	1	M	PRETERM	26200	12370	5290	0.23	0.3	NO	2.1	5	R	P
31	B/o Asmamma	1	M	PRETERM	4900	1670	990	0.25	0.33	SEEN	2.1	5	R	P
32	B/o vidhyarani	1	F	TERM	21300	10900	4710	0.29	0.4	SEEN	2.9	6	R	P
33	B/O Sangeeta M	1	M	TERM	23800	6700	1970	0.18	0.24	SEEN	2	4	R	N
34	B/O Vijayalaxmi	2	M	PRETERM	4600	1196	512	0.27	0.37	SEEN	1	6	R	P
35	B/O Shrutamma	1	M	TERM	4100	1770	1190	0.29	0.4	SEEN	2.5	6	R	P
36	B/O Gayatri	5	M	PRETERM	20700	10270	1370	0.27	0.37	SEEN	1.02	6	R	P
37	B/O Bibi Ayesha	1	M	TERM	14100	8420	3190	0.27	0.38	SEEN	2.2	5	R	P
38	B/O Bhagyashri	1	M	TERM	18700	7724	1190	0.19	0.24	SEEN	1.9	2	NR	N
39	B/O Shaila Shirabur	1	M	TERM	20100	3280	665	0.18	0.25	NO	1.8	1	NR	N
40	B/O Deepa	1	F	PRETERM	22000	10310	4690	0.24	0.32	SEEN	0.9	7	R	P
41	B/O Jyothi	1	F	TERM	15100	9060	2710	0.29	0.4	SEEN	0.2	6	R	P
42	B/O Anita	1	F	TERM	4600	1370	230	0.16	0.2	NO	0.6	3	NR	N
43	B/O Bharati	1	M	TERM	4200	2580	880	0.19	0.23	SEEN	1.9	2	NR	N
44	B/O Laxmi	1	M	TERM	23100	10830	1170	0.18	0.23	SEEN	2.8	3	R	N
45	B/O Vidya	1	F	TERM	4300	1590	1030	0.17	0.24	SEEN	2.01	6	R	P
46	B/O Manjula	1	F	TERM	20400	8400	1170	0.19	0.26	SEEN	0.3	4	R	N
47	B/o pavitra	2	M	TERM	16300	2620	770	0.18	0.24	SEEN	3.05	1	NR	N
48	B/O savitha	1	M	TERM	21900	9760	2170	0.18	0.24	SEEN	3.7	4	NR	N
49	B/o Nakusabai	1	M	TERM	21400	9700	2450	0.17	0.28	SEEN	2.77	4	NR	N
50	B/o Renuka	1	M	TERM	6800	4320	1270	0.29	0.38	SEEN	2.8	3	NR	N
51	B/ o Geeta	1	F	TERM	18800	8900	4960	0.19	0.23	NO	2.96	2	NR	N
52	B/O Nagamma	1	M	PRETERM	10600	9570	2350	0.24	0.32	SEEN	0.25	6	R	P
53	B/o Kavitha	1	M	PRETERM	16700	9350	2260	0.28	0.41	SEEN	0.35	6	R	P
54	B/o Rekha	1	M	PRETERM	12300	8070	2250	0.24	0.33	SEEN	0.9	6	R	P
55	B/O Bharati T	1	F	TERM	19900	8345	1430	0.28	0.39	SEEN	2.2	4	R	N
56	B/O Priyanka	5	F	PRETERM	4500	1670	1170	0.27	0.41	SEEN	1.5	6	R	P

57	B/O Renuka	1	M	TERM	26700	10187	4450	0.19	0.27	NO	2.72	3	NR	N
58	B/O Sumayamma	5	M	PRETERM	27700	10130	4990	0.28	0.34	SEEN	0.25	7	R	P
59	B/O Manjula	1	F	TERM	15225	6226	698	0.12	0.19	NO	1.9	1	NR	N
60	B/O Aasha	1	M	TERM	18600	8759	3318	0.17	0.29	SEEN	3.64	3	R	N
61	B/O Santha bai	1	M	TERM	19340	6368	3593	0.17	0.26	SEEN	3.49	3	NR	N
62	B/O Rajeshwari B	6	M	PRETERM	24530	11395	7565	0.27	0.38	SEEN	0.35	7	R	P
63	B/O Rabiya	1	M	TERM	3000	1770	560	0.18	0.24	SEEN	2.41	3	R	N
64	B/O Bhisimilla	1	M	TERM	15800	7648	2995	0.2	0.26	SEEN	2.3	4	NR	N
65	B/O Savitha B	1	M	TERM	5200	1645	530	0.17	0.26	SEEN	0.3	3	R	N
66	B/O Sunanda	1	F	TERM	17800	7165	2920	0.16	0.25	SEEN	2.3	3	R	N
67	B/O Seema	11	M	TERM	14900	7060	2330	0.16	0.27	SEEN	0.6	4	R	N
68	B/O Premamma	2	M	TERM	18900	10530	2050	0.19	0.24	NO	3.02	2	NR	N
69	B/O Kusuamma	12	M	PRETERM	4200	1080	545	0.23	0.3	NO	1.01	5	R	P
70	B/O Vijaya	2	M	TERM	16200	7264	2130	0.18	0.24	SEEN	0.4	4	R	N
71	B/o Manjula P	1	M	TERM	25100	12964	6190	0.19	0.29	NO	3.45	3	R	N
72	B/O Jaibun	2	M	TERM	18700	11200	1240	0.23	0.3	NO	1	4	R	N
73	B/O Jayashree	1	M	PRETERM	4600	1400	590	0.2	0.3	SEEN	3.1	5	R	P
74	B/O Aasha B	1	F	TERM	16800	8320	1850	0.19	0.24	SEEN	1.9	3	R	N
75	B/O Sangeetha	3	F	TERM	14000	4200	830	0.19	0.25	NO	0.8	1	NR	N
76	B/O Sherin	12	F	TERM	22700	8970	2155	0.18	0.27	NO	1.8	4	NR	N
77	B/O Prema B	1	F	TERM	16600	9773	3645	0.18	0.28	NO	0.75	3	NR	N
78	B/O Lalebee	3	F	TERM	4300	2730	1720	0.16	0.24	sEEN	1.8	3	NR	N
79	B/O Haseena	1	F	TERM	20200	7240	3475	0.18	0.26	NO	0.6	4	R	N
80	B/O Indrabai	1	M	TERM	23790	9165	4155	0.19	0.29	NO	0.9	4	R	N
81	B/O Shrutamma	4	M	TERM	16700	8655	2345	0.19	0.28	NO	0.9	4	R	N
82	B/O Honamma	1	F	PRETERM	3700	950	350	0.19	0.23	SEEN	2	5	R	P
83	B/O Kamala	1	M	TERM	21800	7590	3236	0.16	0.27	NO	1.9	3	NR	N
84	B/O Malamma	1	M	PRETERM	4700	1430	650	0.26	0.37	SEEN	0.75	6	R	P
85	B/O Sherinamma	10	M	TERM	4500	3230	860	0.16	0.24	NO	0.15	3	NR	N

86	B/o Rekha P	1	F	TERM	8700	2670	690	0.17	0.24	NO	0.45	4	R	N
87	B/O Bhouramma	1	M	TERM	13700	7790	1675	0.19	0.27	NO	2.3	3	NR	N
88	B/O Sangubai	1	F	TERM	10150	6700	1530	0.18	0.28	NO	1.9	3	NR	N
89	B/O Sujatha	1	F	TERM	13700	5490	1120	0.16	0.26	NO	2.03	1	NR	N
90	B/O Aasma	1	M	TERM	17300	9830	2620	0.16	0.25	NO	0.85	4	R	N
91	B/O Manamma	1	M	TERM	16700	6230	3320	0.19	0.28	SEEN	3.75	3	R	N
92	B/O Shahin	9	F	TERM	20300	8170	3730	0.18	0.27	SEEN	4.01	4	R	N
93	B/O Gundakka	1	M	TERM	24700	7320	2420	0.16	0.24	NO	3.5	3	NR	N
94	B/O Ashwini	1	M	TERM	9320	4790	1920	0.15	0.24	SEEN	1.1	4	R	N
95	B/O Sangeetha	1	F	TERM	15100	6230	1350	0.18	0.26	SEEN	4.15	3	NR	N
96	B/O Sundrabai	4	M	TERM	17300	8230	2180	0.14	0.24	SEEN	2.2	4	R	N
97	B/O Anuradamma	1	M	TERM	4670	1930	850	0.18	0.25	NO	2.12	1	NR	N
98	B/O Gayatri P	1	M	PRETERM	28300	9750	2530	0.16	0.24	NO	1.25	4	R	N
99	B/O Jyothi Sajjan	2	F	TERM	10300	7170	2320	0.17	0.27	SEEN	2.3	3	R	N
100	B/O Mahadevi	1	M	TERM	12500	7450	2480	0.18	0.27	SEEN	1	4	R	N
101	B/O Sabnaam	1	F	TERM	15400	9850	2980	0.19	0.28	SEEN	0.45	4	R	N
102	B/O Roopali	1	M	TERM	20300	8326	2070	0.16	0.25	NO	2.2	3	NR	N
103	B/O Shobha	1	M	PRETERM	28500	14250	5350	0.23	0.3	SEEN	1.01	7	R	P
104	B/O Chandini	2	F	TERM	4000	1820	600	0.15	0.24	NO	0.3	2	NR	N
105	B/O Vijayalaxmi	3	M	PRETERM	18200	9264	2130	0.16	0.24	NO	0.4	4	R	N
106	B/o Kavitha B	1	M	TERM	5200	1745	530	0.17	0.26	SEEN	0.3	4	R	N
107	B/O Kashiamma	10	M	TERM	3900	1650	730	0.14	0.24	NO	1	2	NR	N
108	B/O Shiavamma	1	M	PRETERM	24500	16950	3250	0.25	0.33	SEEN	0.2	6	R	P
109	B/O Sangabai	1	F	TERM	10250	6700	2530	0.18	0.27	SEEN	1.5	3	R	N
110	B/O Laxmi P	10	F	TERM	9100	5060	940	0.16	0.22	NO	0.25	1	NR	N
111	B/O Bhauramma	1	M	TERM	13700	7790	1675	0.21	0.28	SEEN	2.5	3	R	N
112	B/O Reamma	2	M	PRETERM	4850	1390	1680	0.24	0.32	NO	2.9	5	R	P
113	B/O Sherin	5	M	TERM	7500	2930	1460	0.16	0.24	NO	0.15	3	R	N
114	B/O Kamalamma	1	M	TERM	7800	4090	1936	0.16	0.25	SEEN	1.9	3	R	N

115	B/O Vaishali B	2	F	TERM	13000	6240	1070	0.19	0.27	NO	1.4	2	NR	N
116	B/O Preramma	1	M	TERM	20200	9340	2475	0.19	0.26	NO	0.6	4	R	N
117	B/O Indramma	1	M	TERM	18790	7165	2155	0.17	0.28	NO	0.9	3	R	N
118	B/O Mayamma P	1	M	PRETERM	35703	19310	4530	0.23	0.3	SEEN	0.7	6	R	P
119	B/O Somamma	1	M	PRETERM	10600	6773	1645	0.17	0.28	SEEN	1.3	4	R	N
120	B/O Shailu	2	M	TERM	19100	3280	665	0.18	0.25	NO	2.1	1	NR	N
121	B/O Sangeetha P	1	F	TERM	15100	6230	1650	0.19	0.27	SEEN	4.15	3	R	N
122	B/O Mahadevi B	1	M	PRETERM	21900	7930	1270	0.24	0.33	SEEN	0.7	6	R	P
123	B/O Manamma P	1	M	PRETERM	15100	9664	2190	0.19	0.29	NO	3.2	3	R	N
124	B/O Anjumamma	1	F	TERM	9600	1770	230	0.16	0.2	NO	0.5	1	NR	N
125	B/O Indramma B	1	M	PRETERM	8790	4165	1155	0.17	0.28	NO	0.9	3	R	N
126	B/O Reshamma	3	M	TERM	6300	2730	720	0.16	0.25	SEEN	1.9	3	R	N
127	B/O Yashoda P	1	M	PRETERM	4600	1600	970	0.27	0.37	NO	2.7	5	NR	P
128	B/O Bharati	1	M	TERM	4200	1980	880	0.19	0.23	SEEN	2.1	2	NR	N
129	B/O Preamma	2	M	TERM	10600	6773	1645	0.15	0.26	SEEN	1	4	R	N
130	B/O Bhagyashree P	2	F	PRETERM	4900	1520	1180	0.33	0.4	SEEN	1.98	5	NR	P
131	B/O Renuka	1	M	TERM	6800	4320	1270	0.18	0.29	SEEN	1.24	4	R	N
132	B/O Shrutamma B	2	M	PRETERM	12400	9700	2450	0.16	0.24	SEEN	1	4	R	N
133	B/O Aashamma	1	M	PRETERM	29900	14970	3790	0.25	0.33	SEEN	0.9	6	R	P
134	B/O Seetamma	3	F	TERM	14000	4200	830	0.19	0.25	NO	0.8	2	NR	N
135	B/O Nakusabai	1	M	TERM	12400	9700	2450	0.17	0.26	NO	0.9	3	R	N
136	B/O Aayesha	1	M	PRETERM	4100	1120	3190	0.27	0.38	SEEN	0.5	6	NR	P
137	B/O Reshma	1	M	TERM	14900	8930	2530	0.19	0.28	SEEN	1.15	4	R	N
138	B/o Shanu bai P	1	M	PRETERM	20500	14980	4730	0.18	0.23	SEEN	2.5	4	R	N
139	B/O Jayamma	1	F	TERM	4500	1400	2990	0.2	0.3	SEEN	0.12	5	NR	P
140	B/O Sujatha B	1	F	TERM	13700	5490	1120	0.18	0.26	NO	2.45	1	NR	N
141	B/O Anjuamma	1	M	TERM	8600	5570	1140	0.19	0.26	SEEN	1	3	R	N
142	B/O Sushma	10	F	TERM	4400	1080	2345	0.23	0.31	NO	0.55	5	NR	P
143	B/o Shamina P	1	M	TERM	21900	6080	1790	0.15	0.26	NO	1.1	4	R	N

144	B/O Rekha B	1	F	TERM	4300	1070	950	0.24	0.33	SEEN	0.9	6	R	P
145	B/O Manjula	1	F	TERM	15225	9226	1196	0.12	0.19	NO	1.9	1	NR	N
146	B/O Meghamma	1	F	TERM	10900	7980	1860	0.19	0.28	SEEN	1.02	4	R	N
147	B/O Isharatamma P	1	F	TERM	15400	9850	1980	0.18	0.27	NO	1	3	R	N
148	B/O Geetamma B	2	F	TERM	18600	9570	3350	0.24	0.32	SEEN	0.2	6	R	P
149	B/O Shilpa P	2	M	TERM	13100	8970	2790	0.18	0.27	SEEN	1	4	R	N
150	B/O Reshma T	2	M	TERM	23700	10191	1700	0.16	0.26	SEEN	1.2	4	NR	N