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Diagnostic Value of C- Reactive Protein and Hematological Parameters in Neonatal Sepsis

Dr. B.Venkatarao [1], Dr.G.Israel [2], Dr. P. Kamala [3]
1. Associate professor, 2.Assistant professor, 3.professor
Department of Microbiology, Guntur Medical College, Guntur, Andhra Pradesh

Abstract: *Early diagnosis and treatment of the newborn infant with suspected sepsis are essential to prevent severe and life threatening complications. In this era of multidrug resistance, it is mandatory to avoid unnecessary use of antibiotics to treat non-infected infants. Thus rapid diagnostic test(s) that differentiate infected from non-infected infants, particularly in the early newborn period, have the potential to make a significant impact on neonatal care. The purpose of the sepsis screen is to rule out sepsis rather than to rule in sepsis. Traditionally, the sepsis screen consists of 4 items: C-reactive protein (CRP), absolute neutrophil count (ANC), immature to total neutrophil ratio (ITR) and micro-erythrocyte sedimentation rate (µ-ESR).*
Hence, the present study was undertaken to study above rapid diagnostic test(s) that differentiate infected from non-infected infants, particularly in the early newborn period, have the potential to make a significant impact on neonatal care This study on neonatal septicemia comprised of 100 neonates who were clinically suspected as septicemia, from the pediatric NICU ward, Government General Hospital, Guntur was conducted in the department of Microbiology, Guntur Medical College, Guntur spread over for a period of one year. Out of the 100 clinically suspected cases 40 were culture positive, C-reactive protein was positive in 36 proven cases (90%)of neonates septicemia out of 40 blood culture positive cases. C.R.P. test was negative in all 10 control cases. Band cell count / Neutrophil count ratio (> 0.2) - Out of the 40 neonatal sepsis cases 28 were > 0.2 (70%) and 12 cases were <0.2 (30%) Micro ESR - It was > 10 mm in 1st hour in 32 cases (80%) in proven neonatal septicemia. It is < 10mm in 8 cases (20%). Rapid microbe-specific diagnostic tests would assist in the early detection of neonatal sepsis and in safely withholding antibiotics for patients in whom sepsis is unlikely.
Keywords: *Neonatal sepsis, serum CRP, Band cell count, Micro ESR, Total count, Absolute neutrophil count*

I. Introduction

Neonatal sepsis or sepsis neonatorum refers to systemic infection of the newborn characterized by a constellation of a nonspecific symptomatology in association with bacteremia. It was estimated that almost 20 per cent of all neonates develop infection during the neonatal period with the mortality rate reaching as high as 50% for infants who were not treated timely. Neonatal septicemia remains one of the most important causes of mortality despite considerable progress in hygiene, introduction of new antimicrobial agents, and advanced measures for early diagnosis and treatment.

The World Health Organization (WHO) estimated that 1 million deaths per year are due to neonatal sepsis many infections in the neonates can only be established on the basis of etiological agent recovered from blood.

Although blood culture is the gold standard for the diagnosis of sepsis, culture reports would be available only after 48-72 hours. The purpose of the sepsis screen is to rule out sepsis rather than to rule in sepsis. Traditionally, the sepsis screen consists of 4 items: C-reactive protein (CRP), absolute neutrophil count (ANC), immature to total neutrophil ratio (ITR) and micro-erythrocyte sedimentation rate (µ-ESR).

The above Rapid diagnostic test(s) (sepsis screen test) that differentiate infected from non-infected infants, particularly in the early newborn period, have the potential to make a significant impact on neonatal care.

Aims and objectives: To diagnose septicemia earlier by sepsis screen tests
To evaluate Diagnostic value of C reactive protein and hematological parameters in Neonatal sepsis

II. Materials And Methods

This study on neonatal septicemia comprised of 100 neonates who were clinically suspected as septicemia, from the pediatric NICU ward, Government General Hospital, Guntur was conducted in the department of Microbiology, Guntur Medical College, Guntur spread over for a period of one year

Inclusion criteria: Neonates who were clinically suspected of septicemia

- Age < 28 days > 22 weeks of gestation and full term babies.
- Presence of three (3) or more clinical symptoms like refusal of feeds, lethargy, irritability, hypothermia, respiratory distress, jaundice, vomiting, apnoea, abdominal distension, hyperthermia, pustular skin lesions, seizures, sclerema, cyanosis, conjunctival discharge, bulging of anterior frontanellae, diarrhea.

Exclusion criteria:

- Extreme prematurity <22 weeks of gestation
- Gross congenital anomalies
- Undergone surgery

Specimen collection

Samples collection: One ml of collected blood sample was inoculated into blood culture bottle containing 5-10ml brain heart infusion broth for isolation of the bacteria. 1.8 ml for micro ESR, absolute neutrophil count and band cells, CRP and remaining few drops used for TC and DC.

Separation of serum from blood

The 1 ml of blood preserved for serological test was kept undisturbed at room temperature for 30-45 minutes to allow clot formation. The supernatant fluid was centrifuged at low speed (1500 rpm for 5-10 mins) and used for qualitative and quantitative tests of CRP. Serum was collected aseptically using sterile Pasteur pipette. The present study was conducted to evaluate the usefulness of positive rapid screening test like c-reactive protein (quantitative), total leucocyte count, ratio of band cells and neutrophil count, absolute neutrophil count and micro ESR The remaining part was centrifuged at 1500-2000 rpm for 10-15 minutes. After centrifugation, the deposit was used for gram stain as per the standard procedures.

The isolates from blood were identified by colony morphology, gram staining, motility and biochemical reactions as per the standard procedures

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1. CRP qualitative analysis

One drop of patient serum, positive control, negative control were placed in separate test circle of the glass slide. One drop of CRP latex antigen suspension was added, mixed and spread the fluid over the entire area of the particular cell with separate mixing sticks. The slide was tilted back and forth for two minutes. The agglutination was noted. All the positive samples were screened for quantitative test.

2. C-reactive protein estimation (CRP quantitative test)

The cut off values for positive rapid screening test is >0.4 mg/dl.

C-reactive protein level was determined quantitatively by using CRP latex kit (Biosystems).

Principle of the method

Serum C - reactive protein (CRP) causes agglutination of the latex particles coated with antihuman c-reactive protein. The agglutination of the latex particles is proportional to the CRP concentration and can be measured by turbidimetry.

Contents

A. Reagent
B. Reagent
S. Standard

Composition

A. Reagent: Glycine buffer 0.1 mol/L, sodium azide 0.95 g/L, pH 8.6.
B. Reagent: Suspension of latex particles coated with anti-human CRP antibodies, sodium azide 0.95 g/L.
S. CRP Standard: Human serum. (C-reactive protein concentration is stated on the vial label).

Reagent preparation

Working reagent

Smaller working reagent volumes can be prepared by mixing 1 ml of reagent B + 4ml of Reagent A. The reagent A and B thoroughly shaken before pipetting.

Procedure (nephlometric)

1. The working reagent and the instrument was brought to 37^0c.
2. The working reagent 1.0 mL and sample 7 µL taken into cuvette.

3. Both were mixed and immediately the cuvette was inserted into the instrument
4. The absorbance was recorded at 540 nm after 10 seconds (A1) and after 2 minutes (A2).

Calculations
The CRP concentration in the sample is calculated using the following general formula.
(A2-A1) sample / (A2-A1) Standard x C standard = C sample (mg/L).

Reference value
Neonates 0.4 ≥ mg /l

2. Total leucocyte count (TLC): < 5,000 cells /cu.mm

To calculate total leucocyte count, WBC pipette was used and all the WBC cells were counted in Neubauer counting chamber. The WBC were identified under low magnification. The leucocyte appeared as round, shiny (refractile) darkish dots, with a halo around them. The cells were counted in the 4 WBC squares of 16 squares each (total of 64 squares).

Calculation = no. of cells x depth factor x dilution factor / area counted
= N x 0.1 x 1/20
(N= number of cells
Depth factor = 0.1
Dilution factor = 1/20)
= N x 50

3. Absolute neutrophil count
Calculation
ANC = (% neutrophils + %bands) x (WBC) / (100)

Differential count (DC)
Fixing the blood films:
8-10 drops of Leishman's stain were poured on specimen slide from a drop bottle and covered the entire surface and allowed the stain to remain undisturbed for 1-2 minutes (fixation time).

Staining the blood film
- After fixation time was over, an equal number of drops of distilled water were added to the stain from a drop bottle.
- The stain and water were mixed by gently blowing.
- A glossy greenish layer (serum) soon appeared on the surface of the diluted stain. The diluted stain was allowed to remain on the slide for 6-8 minutes (staining time).

Observation and results
- Two drops of cedar wood oil was placed in the Centre of the smear.
- The cells were focused under oil immersion lens.
- The slide was examined all over at the head and tail ends along the edges.

The **neutrophils** appeared in 10-14 µm in size, multilobed (2-6 lobes) blue-violet coloured nucleus, closely packed violet pink granules of cytoplasm. The percentage distribution of each type of WBC was calculated.
Normal: Neonates and infants 40-50 %.

4. Band cell count to total neutrophil count ratio (I/T ratio): 0.2

I/T ratio = no. of band cells / no. of neutrophils

The immature polymorph nuclear leucocytes, (stab cells) characterized by further condensation of nuclear chromatin and transformation of nuclear shapes into sausage and band forms with more or less uniform diameters throughout their length, measuring 10- 16 µm and the nucleus centrally or eccentrically placed with light purplish blue.Cells without their complete formation of distinct lobes (usually connected by a filamentous strand) are classified as band forms.

Juvenile/band forms 10-16 µm size nucleus is centrally or eccentrically placed and band shape of uniform thickness, light purplish blue. They normally constitute <6% neutrophils; an increase may point to an inflammatory process.Band stage (stab forms) : characterized by band like shape of the nucleus with constant diameter throughout and condensed nuclear chromatin. The nucleus is deeply indented and horse shoe shaped.

5. Micro ESR

Determination of erythrocyte sedimentation rate (ESR) in capillary blood can be of value in evaluating sick newborns. Micro ESR requires a small amount of blood which can be obtained easily by heel prick and this test is easy to perform.

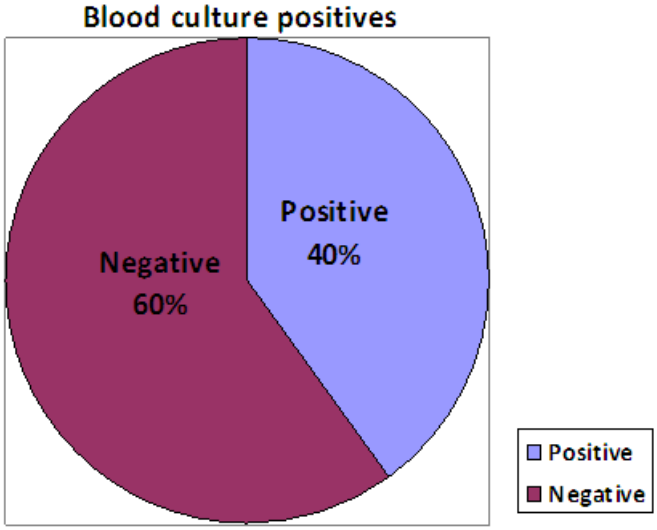
Hematocrits also were determined, and ESR values were corrected for the hematocrits by means of standard charts. All tests were performed within two hours of obtaining the blood.

III. Results

Table 1

Out of 100 clinically suspected cases 40 were culture positive

Total cases	Culture positive	Culture negative
100	40	60
%	40%	60%



Rapid diagnostic tests to detect neonatal septicemia

1.c-reactive protein (>0.4mg) by latex agglutination test was down in all cases, and it was positive in 36 proven cases (90%)of neonates septicemia out of 40 blood culture positive cases. C.R.P. test was negative in all 10 control cases.

2.Band cell count / Neutrophil count ratio (> 0.2) - Out of the 40 neonatal sepsis cases 28 were > 0.2 (70%) and 12 cases were <0.2 (30%)

3. Micro ESR - It was > 10 mm in 1st hour in 32 cases (80%) in proven neonatal septicemia. It is < 10mm in 8 cases (20%).

4. Total count - It was <5000 in 8 cases (20%) in proven neonatal septicemia.

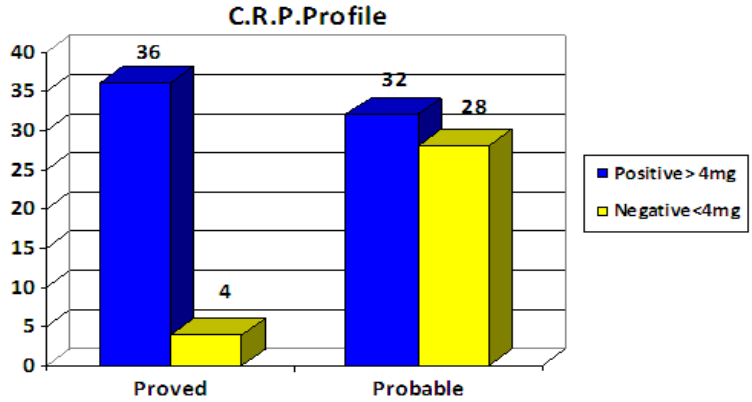
5. Absolute neutrophil count (<1500 and >7500) - It was 20 cases (50%) in proven neonatal septicemia.

Sepsis screen test to detect neonatal septicemia.

1. C.R.P.Profile (quantitative test)

Table 2

CRP quantitative	Cases (100)		Control group (n=10)
	Proved (40)	Probable (60)	
Positive >0.4	36 (TP)	32 (FP)	0
Negative <0.4	4 (FN)	28 (PN)	10



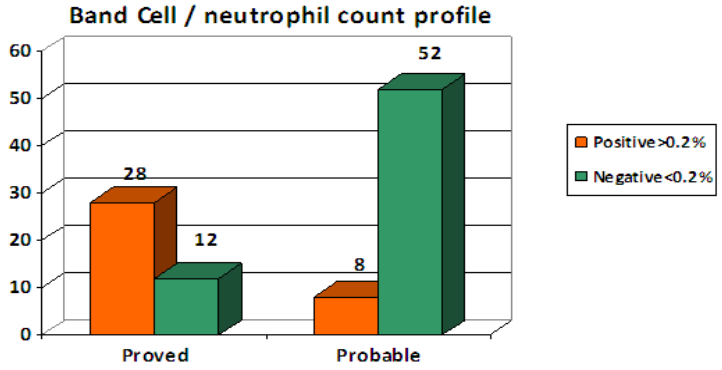
Comparison of culture positivity with CRP.

Test	Positive
Culture	40
CRP	36

Band cell count / neutrophil count profile

Table 3

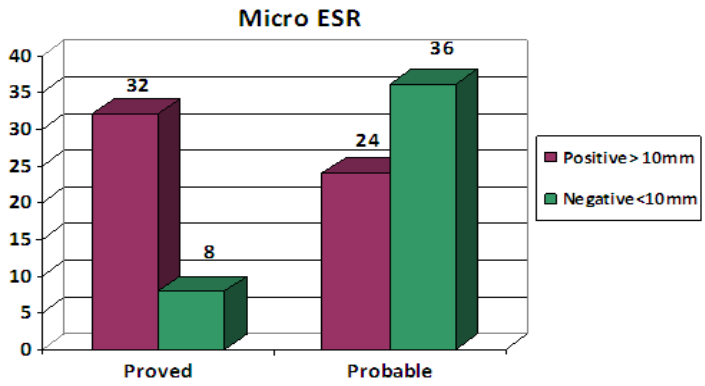
Band cell count / neutrophil count	Cases (100)		Control group (n=10)
	Proved (40)	Probable (60)	
Positive >0.2	28(TP)	8 (FP)	0
Negative <0.2	12 (FN)	52 (PN)	10



Micro ESR profile

Table 4

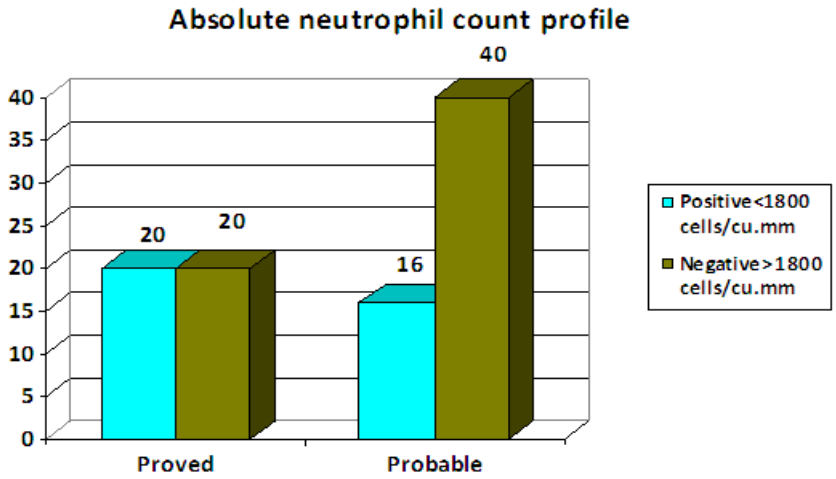
Micro ESR	Cases (100)		Control group (n=10)
	Proved (40)	Probable (60)	
Positive >10mm	32 (TP)	24 (FP)	0
Negative <10mm	8 (FN)	36 (PN)	10



4. Absolute neutrophil count profile

Table 5

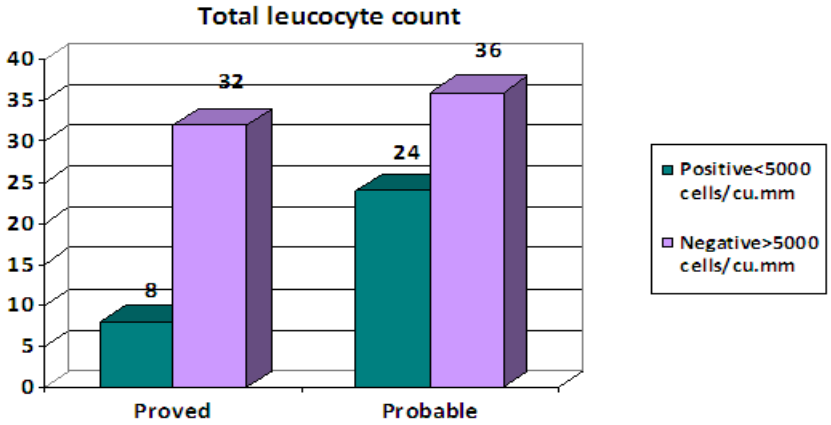
Absolute neutrophil count	Cases (100)		Control group (n=10)
	Proved (40)	Probable (60)	
Positive <1800 cells/cu.mm	20 (TP)	16 (FP)	0
Negative > 1800 cells/cu.mm	20 (FN)	40 (PN)	10



5. Total leucocyte count

Table 6

Total leucocyte count	Cases (100)		Control group (n=10)
	Proved (40)	Probable (60)	
Positive <5000 cells/cu.mm	8(TP)	24(FP)	0
Negative > 5000 cells/cu.mm	32 (FN)	36(PN)	10



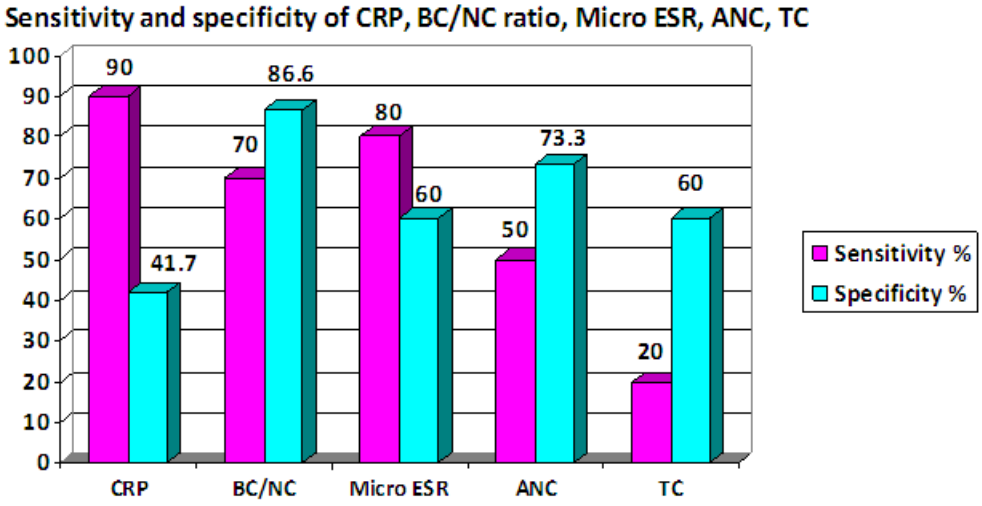
Specificity and sensitivity

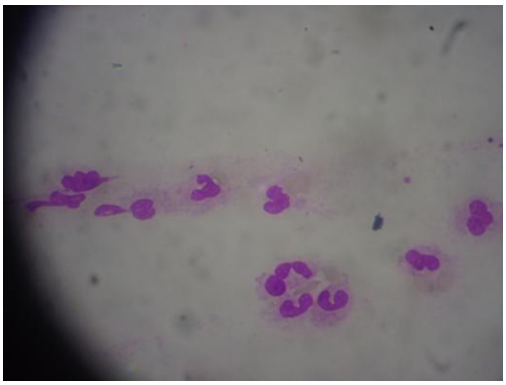
C.R.P sensitivity 90% and specificity 41.17%; **BC/NC** ratio sensitivity 70% and specificity 86.6%; **micro ESR** sensitivity was 80% and specificity 60%. **Total count** sensitivity 20% and specificity 60%. **ANC** sensitivity 50% and specificity 73.3%.

Table 7

Table showing sensitivity and specificity of CRP, BC/NC ratio, Micro ESR, absolute neutrophil count, total leucocyte count.

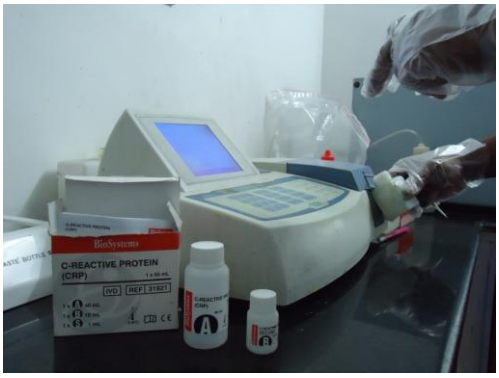
Sepsis screen test	Sensitivity	Specificity	Positive predictive value
CRP >0.4mg	90%	41.7%	52.9%
2BC/NC ratio	70%	86.6%	77.7%
Micro ESR > 10 mm	80%	60%	57.1%
Absolute neutrophil count <1800 cells/cu.mm	50%	73.3%	55.5%
Total leucocyte count <5000 cells/cu.mm	20%	60%	25%



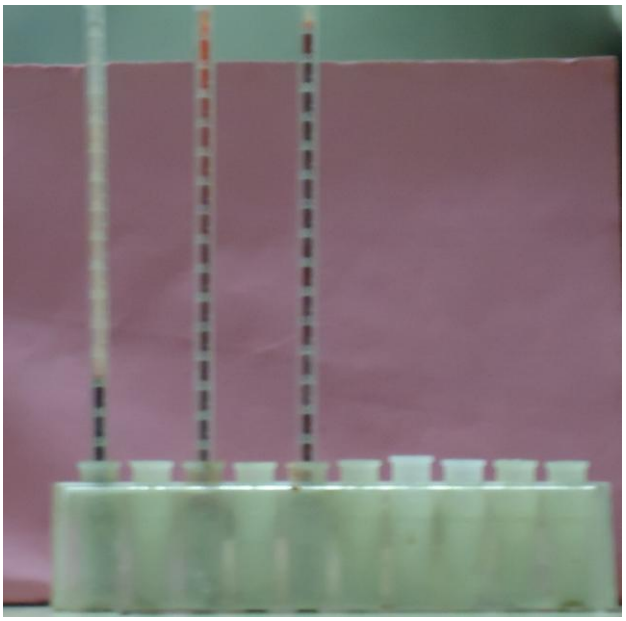


Band neutrophils

CRP – qualitative test



CRP – quantitative test (Neflometric)



Micro ESR

IV. Discussion

For the effective management of neonatal septicemia cases, study of the bacteriological profile with their antibiotic pattern plays a significant role.

In the present study, out of the 100 clinically suspected neonates, 40 (40%) were culture positive which correlated with Zakariya BP et al (41.6%) and Rakhee Agarwal (42.7%) **(Table No. 1)**

C reactive protein test was positive in 36% of culture proven cases (90%). The sensitivity was 90% and specificity was 41.17% and positive predictive value was 52.9%. Rekha Sriram found CRP to be the single most sensitive 90%, and specific test in distinguishing infected form non-infected group. Anita Chandana et al had found C.R.P to be 90.9% sensitive in diagnosing infection when it was present. The sensitively to C.R.P in the present study was closely correlating with the findings of M.Singh et al (1997).

CRP is synthesized within six to eight hours of exposure to an infective process or tissue damage, with a half life of 19 hours, and may increase more than 1000-fold during an acute phase response.16 The ranges of sensitivity and specificity for diagnosis of early onset sepsis ranges are 43–90% and 70–78% respectively.14 The specificity and positive predictive value of CRP ranges from 93% to 100% in late onset sepsis. Thus CRP is a "specific" but "late" marker of neonatal infection.15 CRP as a diagnostic marker in neonates has higher sensitivity and specificity than total neutrophil count and immature to total neutrophil ratio.17 NNPD

The differences in the results of this parameter shown by the different studies was due to variations in the diagnostic criteria, the time of onset of infection (early or late) and different methods of CRP estimation. **(Table No. 2 and7)**

In this study band cells by neutrophil count ratio (≥0.2) was positive in 28 cases (70%). The sensitivity was 70% and specificity was 86.6% and the positive predictive value was 77.7%. Rekha Sriram found 52.2% of sensitivity and 56.5% specificity and positive predictive value were 82.8%. Different studies have shown variable results in this parameter which may be due to variations in the blood sampling time, the severity of infection, the age of the neonates. **(Table No. 3 and 7)**

In the present study the Micro ESR (>10 mm) was positive in 32 cases (80%). The sensitivity was 80% and specificity was 60% and the positive predictive value was 57.1%. Rekha Sriram found micro ESR 73.1% sensitive and 56.2% specificity and the positive predictive value 32.8% in diagnosing infection. These variations were due to the fact that at least four hours are required for hematological response to develop after the onset of infection and blood samples collected and analyzed before this will yield normal results. **(Table No. 4 and 7)** In the present study absolute neutrophil count (< 1800 cells/cu.mm) was positive in 20 cases (50%). The sensitivity was 50%, specificity was 73.3% and the positive productive value was 55.5%. Rekha Sriram, found ANC of 50% sensitivity and 49.6% specificity and the positive predictive value was 3.5% which was very low. **(Table No. 5 and 7)** In the present study the total leucocyte count (<5000 cells/cu.mm) was positive in 8 cases (20%). The sensitivity was 20%, specificity was 60% and the positive productive value was 25%. Rekha Sriram has found 63.6% sensitivity 51% specificity and the positive predictive value was 12.1%. From this study when single test were considered CRP was the most sensitive 90% test to detect sepsis and BN ratio is more specific 86.6%. **(Table No. 6 and 7)**

V. Conclusion

The number of lives lost in the perinatal and neonatal period exceeds that of any other period in life of a similar duration. In order to sustain gains in child survival made in recent decades, attention must be focused on reduction of morbidity and mortality in the newborn period. The evaluation of tests for neonatal sepsis is important because the infection may present a very serious threat to the baby.

The mainstay for therapy for Neonatal Septicemia being appropriate supportive care, antibiotics used based on susceptibility testing of organism isolated. Rapid microbe-specific diagnostic tests would assist in the early detection of neonatal sepsis and in safely withholding antibiotics for patients in whom sepsis is unlikely.

Among the sepsis screen parameters studied CRP was the most sensitive test of 90%, BC/NC ratio was the most specific of 86.6% and 77.7% positive predictive value. Continuing surveillance of Neonatal infections, Local patterns and antibiotic sensitivity of pathogens is vital to determine trends in the infection improve reliability of the data and guide empiric antibiotic therapy & preventive measures.

References

[1]. A.K. Mane, N.V. Nagdeo, VR Thomdura - Study of neonatal septicemia in tertiary care hospital – rural Nagpur, 2010. 110.
[2]. Agarwal DS, Chowdary P, Srivastava G, Saini L – Study of neonatal infection. Indian Pediatr 1985: 122. 3d
[3]. Aggarwal R, Sarkar N, Deorari AK, Paul VK. – Sepsis in the newborn. Indian J Pediatr. 2001; 68 (12): 1143 – 7. 12d
[4]. Ahmed Ghafoor T, Waqar T, Ali S, Aziz S, mahamud S. – Diagnostic value of C-reactive protein and haematological parameters in Neonatal sepsis JCPSP. 2005; 15 (3) : 152 – 6. 11d
[5]. Alojipan LC, Andrews BF - Neonatal sepsis, Clin. Pediatr 2005: 14:181-185. R14
[6]. Andrade SS, Bispo PJ, Gales AC (2008) - Advances in the microbiological diagnosis of sepsis. Shock 30: Suppl 141–46. R67
[7]. Anita chandana M. Nagaraja rao et al – Indian J.Pediatr. 1999 ; 55:947-953. 1 d
[8]. Anita Sharma, CV. Krishna murthy – Evaluation of sepsis screen for diagnosis of neonatal septicemia. I11
[9]. Arnon S, Litmanovitz I. - Diagnostic tests in neonatal sepsis. Curr Opin Infect Dis. Jun 2008;21(3):223-7. R7 Bauer, Kirby, Sherris and Truck, 1966. Am. J. Clin. Path 45 :493. M15
[10]. Bommen M. - Major common problems in neonatal infection, Journal of Applied Medicine 1997; 13:1 09-117.r49 Gerdes JS. –
[11]. Diagnosis and management of bacterial infections in the neonate. Pediatr Clin N Am. 2004; 51 : 939-59. 14d.
[12]. Gotoff S P, Behrmamn R E. - Neonatal septicemia J.Pediatrics 1980 ; 76 : 142-153. R50
[13]. Gupta SK, U. Sharma, ML Gupta – A rapid diagnostic method for diagnosis of neonatal septicemia. Indian Pediatr. 2003 ; 29:153-155. 9d
[14]. Jaswal RS, Kaushal RK, Goel A, Pathania K. - Role of C-Reactive protein in deciding duration of antibiotic therapy in neonatal septicemia. Indian Pediatr. 2003 Sept; 40: 880-3. 10d
[15]. Kindmark C-O. – The concentration of C-reactive protein in sera from healthy individuals 1972.m10
[16]. Namedo U K et al. - Hematological Indices for early diagnosis of neonatal septicemia. Indian Pediatr 1995 ; 22: 287-291. R60.