A comparative study of CBP, CRP, Micro ESR and blood culture in the diagnosis of neonatal septicemia

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Abstract

Background: Neonatal sepsis is one of the most important causes of morbidity and mortality in children. The incidence was 3% in intramural births, with an overall mortality rate of variation of 18.6%. Aims and Objectives: To evaluate the role of blood culture, micro ESR, CBP, CRP in the diagnosis of sepsis in children. To study sensitivity, specificity, positive predictive values and negative predictive values of the above hematological parameters, either individually or in combination with diagnosis of neonatal sepsis. Method: A study was carried out from January 2015 to December 2015 in hospital, based on, samples of babies with history of infective etiology within the first 28 days of birth. Result: One hundred neonatal cases of clinically suspected septicemia were studied. Culture positive rate was 26%. E coli was the major organism responsible for early onset of sepsis. The highest sensitivity and negative predictive value was found for septic score of more than or equal to 3 by septic scoring, I:T ratio and C-reactive protein with the highest sensitivity, micro ESR and TLC with the highest specificity. Conclusion: CRP emerged as the test with the highest sensitivity. Micro-ESR and TLC had the highest specificity.

Keywords: Neonatal sepsis, Blood culture, C-reactive protein, Micro ESR.

Introduction

Neonatal sepsis in neonates is a clinical syndrome characterized by systemic sign of infection accompanied by bacteremia in the first 28 days of birth [1]. Neonatal sepsis is a complex syndrome caused by an uncontrolled systemic inflammatory response of infectious origin characterized by multiple manifestations which can result in dysfunction and failure of one or more organs and even death [2]. Neonatal sepsis is one of the most important, commonest and major causes of morbidity and mortality. The incidence was 3% among the intramural births, with an overall mortality rate variation of 18.6% [3]. Among the extramural ad mission, sepsis was responsible for 39.7%; on the other hand, indiscriminative overuse of antibiotics, was hazardous for any neonatal unit which may lead to overtreatment and emergence of resistant organism [4, 5]. The clinicians focused on diagnostic test which has high sensitivity and high negative predictive accuracy. These are direct or indirect hematological tests [5]. Septic screens are the batteries of parameter studies in order to improve diagnostic accuracy [5]. Of these, leucocyte count and indices of micro ESR and CRP were the most studied tests which were cost efficient. Therefore, a group of tests was studied to assess the usefulness either singly or in combination with predicting neonatal sepsis. These tests were of total leucocytecount (TLC), Absolute neutrophil count (ANC) immature total ratio (I:T ratio) platelet count, micro ESR and CRP.

Materials and Methods

A study was conducted in the neonatology unit of Princess Esra Hospital attached to Deccan College of Medical Sciences, Hyderabad, Telangana State, India from 1st January 2015 to end of December 2015. In all,
100 neonates were studied. Samples included babies with history of illness of ineffective etiology with the onset within 28 days of birth.

**Inclusive Criteria**: Neonate intramural or extramural delivery, clinically presenting symptoms of sepsis on physical examination such as refusal to feed, lethargy, hypothermia, hyperthermia, vomiting, abdominal distension and diarrhea were studied.

**Exclusive Criteria**: Neonates with lethal congenital anomalies, extreme low birth weight, respiratory distress syndrome and babies treated previously with antibiotics for more than 12 hours were excluded from this study. Detailed clinical examination of each baby was carried out. Gestational age was also calculated. When illness started in the first 72 hours of birth, it was an early onset of disease and after 72 hours, it was late onset. Gestational age of babies was assessed using Ballard Scoring system to classify them as preterm, term, and post term. Blood samples were collected from the neonates suspected for septicemia. The study was divided into three groups for analysis as follows; Proven sepsis, probable sepsis, and no sepsis. Proven sepsis was defined as blood culture or CSF culture or culture from other significant site showing positive for any bacterial growth within 48 to 72 hours. Investigation suggested sepsis. 1 ml of blood sample was collected through peripheral venipuncture in a bottle containing Potassium EDTA. It was subjected to platelet count. Total leucocyte count and differential count analysis were shown on automated cell counters. Band forms with less mature cells were classified as immature polymorpho nuclear leucocytes. I:T ratio was calculated after counting 200 cells. Total leucocytes count, and absolute neutrophil count was recorded.

I : T ratios tested as Monroe’s criteria was for term babies and mouzinho criteria for VLBW babies. 0.5 ml to 1 ml of blood sample was taken for micro ESR and CRP estimation. Venous blood was collected in preheparinized microhemocrit glass tube of 7.5 mm length. More than or equal to 15 mm at the end of first hour was taken as significant. C Reactive protein was done by semi quantitative method using Rhelax CRP kit. A positive agglutination with undiluted serum sample corresponding to CRP concentration of 6 micro g/ml was used to calculate each parameter and combination of parameters. Statistical analysis was done using graph pad prism 5 (Graphpad software, Inc, USA). Data is presented as mean ± SD.

Variable difference with P<0.05 is considered statistically significant. The sample size was determined by using the open EPI statistics of 95% confidence to detect the result with 90 % of sample power.

**Results**

One hundred neonates admitted in the neonatal unit, were divided into Group A and Group B. In group A, the neonates were subjected to CBP which included TLC, ANC, I:T ratio, platelet count, micro ESR, CRP, blood culture, and other investigations to support diagnosis. In group B, all investigations were carried out other than blood culture to support diagnosis. The babies were subjected to sepsis in the first 28 days of birth. The study showed that the group comprised 61 males and 39 females in the ratio of 1.56:1

| Test     | Positive Culture | Negative Culture | Positive Culture | Negative Culture | k    |
|----------|------------------|------------------|------------------|------------------|------|
| TLC      | 08               | 08               | 05               | 29               | 0.40 |
| ANC      | 10               | 15               | 15               | 10               | 0.28 |
| I:T Ratio| 13               | 12               | 00               | 25               | 0.52 |
| Platelets| 02               | 09               | 11               | 28               | 0.00 |
| Micro ESR| 07               | 08               | 06               | 29               | 0.30 |
| CRP      | 11               | 18               | 02               | 19               | 0.26 |
| 2 or more| 12               | 18               | 01               | 19               | 0.30 |
| 3 or more| 12               | 09               | 01               | 28               | 0.57 |

Table-1: shows that total leucocyte count suggestive of sepsis i.e positive was found in 16 cases. Of these, 8 cases were bacteriologically positive and of the 34 negative cases, 5 grew organism on culture. I:T ratio of more than 0.2 was found
in 25 cases of which 13 were positive bacteriologically whereas, in 25 negative cases, none was culture positive. ANC was positive in 25 cases of which 10 were proven. Platelet count was less than 1,50,000 per cu.mm in 11 cases of which 2 cases were culture proven. Micro ESR was significantly increased in 15 cases and it was negative in 35 cases of which 6 grew organism.

Table-2: Accuracy of Hematological Parameters.

| Test          | Total Positive | Positive with Proven Sepsis | Sn  | Sp  | PPV  | NPV  | LR  |
|---------------|----------------|-----------------------------|-----|-----|------|------|-----|
| TLC           | 16             | 08                          | 62  | 78  | 50   | 85   |     |
| ANC           | 25             | 10                          | 77  | 59  | 40   | 88   |     |
| I:T Ratio     | 25             | 13                          | 100 | 68  | 52   | 100  |     |
| Platelets     | 11             | 02                          | 15  | 76  | 18   | 72   |     |
| Micro ESR     | 15             | 07                          | 54  | 78  | 47   | 78   |     |
| CRP           | 29             | 11                          | 85  | 51  | 38   | 90   |     |
| 2 or more test positive | 30             | 12                          | 92  | 51  | 40   | 95   |     |
| 3 or more test positive | 21             | 12                          | 92  | 76  | 57   | 96.6 |     |

Table-2 Shows that the proven cases of Sepsis are taken as positive and other cases are taken as negative. Sensitivity of Septic screen is 92%, Specificity is 51%, PPV is 40% and most importantly 95% of the negative predictive accuracy, the prevalence of the proven sepsis is considered as 26%.

Table-3: Performance of various combination parameters.

| Combination of Parameters | Total Positive | Positive with Proven | Sn  | Sp  | PPV  | NPV  | LR  |
|---------------------------|----------------|----------------------|-----|-----|------|------|-----|
| Two Test                  |                |                      |     |     |      |      |     |
| ANC + I:T Ratio           | 17             | 10                   | 77  | 81  | 59   | 91   |     |
| TLC + PC                  | 3              | 2                    | 15  | 97  | 67   | 77   |     |
| I:T Ratio + CRP           | 23             | 11                   | 85  | 68  | 48   | 93   |     |
| Three Test                |                |                      |     |     |      |      |     |
| TLC+PC+ I:T Ratio         | 3              | 2                    | 15  | 97  | 66   | 77   |     |
| ANC + I:T Ratio + CRP     | 15             | 11                   | 85  | 89  | 73   | 94   |     |
| I:T Ratio + Micro ESR+ CRP| 13             | 8                    | 62  | 86  | 62   | 86   |     |
| Four Test                 |                |                      |     |     |      |      |     |
| TLC+PC+CRP+ I:T Ratio     | 3              | 2                    | 15  | 97  | 66   | 77   |     |
| Five Test                 |                |                      |     |     |      |      |     |
| TLC+ANC+ I:T Ratio + CRP+ Micro ESR | 7 | 4 | 31 | 92 | 57 | 79 |
| TLC+ANC+I:T Ratio+CRP+ Platelets | 2 | 1 | 08 | 97 | 50 | 75 |
| All Six Test              |                |                      |     |     |      |      |     |
| TLC+ANC+I:T Ratio         | 1              | 0                    | 00  | 97  | 00   | 73   |     |
| +CRP+MicroESR+Platelet Count |            |                      |     |     |      |      |     |

Table 3: Shows that of the two test combination, I:T ratio+CRP emerged as most sensitive tests. It identified 11 out of 23 cases of sepsis of 85% out of the two and also a reasonably good specificity of 69%. TLC +PC emerged as the most specific test combination giving specificity of 97%. ANC + I:T ration + CRP were the best in three test combinations.
with 85% sensitivity and 89% specificity. TLC+PC+I:T ratio produced a specificity of 97%. TLC + I:T ratio + CRP+PC, these four test combinations had a good specificity of 97%. Five and six test combinations identified almost all infants with sepsis.

**Discussion**

The present study was carried out to study rapid diagnosis of septicemia in newborn babies up to first month (28 days of life) by using some indirect hematological tests with reference to age and dependent neutrophil indices. The prevalence of neonatal sepsis was 26% which was comparable with the studies of Philip et al [6] and Gerdes et al [5, 6]. Early diagnosis of neonatal sepsis was found to have prevalence of 7.9% and 5.9% respectively which is much lower than what was observed in Singh et al [7] who observed it to be 27.8% in their study. Joshi et al [8] also reported prevalence of 25.8% which was also similar to these studies. In present study, sensitivity, specificity, positive predictive value, negative prediction value of laboratory parameters were evaluated in relation to blood culture taken as gold standard.

**Table- 4: Total Leucocyte Count (TLC).**

| Authors (Year) | SN% | SP% | PPV % | NPV % |
|----------------|-----|-----|-------|-------|
| Philip et al [6] | 50 | 94 | 40 |
| Rodwel et al [16] | 44 | 92 | 36 | 94 |
| Basu et al [18] | 12 | 93 | 50 | 32 |
| Present Study | 62 | 78 | 50 | 85 |

In Present study TLC has a sensitivity of 61% comparable to Philip et al (6) {50%} and more than that reported by Rodwel et al [16] (44%). Specificity of 78% observed in the present study was more than that reported by Namdeo et al [17] (69%) and less than that of Mishra et al [13] which is (88%). The positive predictive value of 50% observed in the present study is equivalent to the value reported by Basu et al [18]. High negative predictive value of 85% was observed in the present study which was comparable with observations made by Rodwel et al [16] and Philip et al [6].

The difference of these studies, when individual predictors of sepsis are considered varied in positive test criteria. I:T ratio was found to have higher sensitivity of 10% which is equivalent to Gerdes et al [5] which is (100%) and comparable to Rodwel et al [6] which is (96%) and Basu et al [18] which is (92%). Philip et al [6]. Mishra et al [13] studied band cell to neutrophil ratio and found to have lower sensitivity as compared with I:T ratio i.e 90% and 92% respectively. Specificity of 68 % observed in the present study was comparable with 71% as observed by Rodwell et al [16] which is (44%). Specificity of 78% observed in the present study was more than that reported by Namdeo et al [17] as which is less than that reported by Mishra et al [13] which is (88%). Positive predictive value of 50% observed in the present study is equivalent to Basu et al [18]. High negative predictive value of 85% observed in the present study was comparable with observation made by Rodwelet al [16] philip et al [6]. I: T ratio was found to have higher sensitivity of 100% which is equivalent to Gerdes et al [5] which is (100%) and comparable to Rodwel et al [16] which is (96%) and Basu et al which is [18] (92%). Philip et al [6], Mishra et al [13] studied band cells to neutrophil ratio and found to have lower sensitivity as compared with I : T ratio i.e 90% and 92% respectively. Specificity of 68% observed in the present study was comparable with 71% as observed by Rodwelet al [16], which is higher than reported Gerdes et al [5]. Most importantly I:T ratio has got the highest negative predictive value amongst all neutrophils indices i.e of 100%.

**Table-5: Micro ESR**

| Author (year) | Sn(%) | SPC% | PPYC% | NOVC |
|---------------|------|------|-------|------|
| Philip et al [6] | 30 | 97 | 43 | - |
| Gerdes et al [5] | 31 | 94 | 24 | 95 |
| Singh et al [7] | 55 | 81 | - | - |
| Present study | 54 | 78 | 47 | 78 |

(Table: - 5 Shows that Micro- ESR was found to have specificity more than sensitivity, of 78% over 54% reported in the present study which was comparable with that observed by Singh et al [7] which was 81%, and Gerdes et al [5] which was 94% of specificity, Philip et al [6] was reported 97% of specificity and 30% of sensitivity. Singh et al [7] observed sensitivity of 55% which is comparable with the present study value of 54% of sensitivity.)
CRP was found to have the highest sensitivity in the tests other than neutrophils indices i.e. I:T ratio in the present study. The observation is similar to Singh et al [7] and comparable with Gerdes et al [5] and Basu et al [18]. It has the specificity of 51% which is less than that of Basu et al [18]. High negative predictive value of 90% was found in the study of CRP which is comparable with Basu et al [18] and lower than reported by Gerdes et al [5]. CRP test was a good indicator with high sensitivity and affected by other factors like both weight, gestational age and onset of disease. In the present study two or more test positives had sensitivity of 92%, specificity of 51%, positive and negative predictive value of 40% and 95% respectively which are comparable with that of Philip et al [6] who showed the sensitivity of 93%. Specificity-88% and positive predictive value as 39%. These Values were also comparable with 3 of Singh et al [7] 86% of sensitivity and 90% specificity. Amongst two test combinations I: T ratio + CRP emerged as the most sensitive (85%) and with specificity (68%) and TLC +PC emerged as the most specific test with specificity of 97% in two test combinations. Sharma et al [11], reported CRP + B:N ratio of having sensitivity 100% and specificity 51.7%. Singh et al [7] reported ANC + CRP as the most specific one, whereas Philip et al [6],reported ANC +BN: TN as the most useful combination with highest positive predictive value. Sharma et al [11], reported CRP+ micro ESR + Toxic generation, as the most useful in three test combinations with sensitivity of 100% and specificity of 82% which is comparable with the present study. The present study had higher number of low birth weight babies as 80%, out of which 22% were below 1500 gm weight. These observations were similar to those reported by Sharma et al [11] (70%), Ahmed et al [10] (60%) and Joshi et al [8]. However, they reported 116 (50.4%) as low birth weight cases in their study. The present study had 60% cases of early onset sepsis while cases with late onset septicemia were 40%. Sharma et al [13] observed 33 cases (66%) of more than 7 days of age. Incidences of refusal to feed was comparable with Guha et al [7] who observed it to be 66.25% higher than reported by Ahmed et al [10] (27%) and Weber et al [12] as 41.5%. Lethargy and respiratory distress cases were seen as 80% and 44% respectively in the present study. Guha et al [7] and Sharma et al [11] reported similar incidences of lethargy as 61.25% and 60% cases respectively. Hyperthermia was found in 10 cases in the present study, which were preterm babies indicating that hyperthermia was more common in preterm babies with septicemia. Chandana et al [14] reported 14% incidences of hyperthermia and 12% incidences of hypothermia.

Abdominal distension and vomiting were seen in 13 and 6 cases respectively whereas diarrhea was found in 9% babies. These findings were similar to those reported by Mishra et al [13]. In the present study, the total leucocyte count sensitivity was 61% comparable with Philip et al 50% and more than that reported by Rodwell et al [16]. Specificity of 78% observed in the present study was more than that reported by Namdeo et al [17] as 69% and less than that of Mishra et al [13] which is 88% positive predictive value of 50% observed in the present study was equivalent to Basu et al [18]. In the present study, platelet count (<1,50,000/mm$^3$) emerged as the test with good specificity but poor sensitivity. It was found to have specificity of 76% which was comparable with that reported by Mishra et al [13] 79% and Basu et al [18], as 71%. Micro ESR was found to have specificity more than sensitivity, of 78% over 54% reported in the present study which was comparable with that observed by Singh et al [22] which 81% and Gerdes et al [21] which is 94%. In the present study, 100 cases were enrolled on clinical suspicion of sepsis.

Cultural positivity rate was 26% which was similar to that of Singh et al [22] 28% Joshi et al [8] which was 25.8%. In the present study, staphylococcal sp. were the commonest isolates 4/13 along with Ecoli 4/13 cases followed by klebsiella in 3/13 cases. Blood culture was positive in 12 cases with a blood culture positivity rate of 24%. Ecoli was the commonest organism implicated for early onset sepsis in the present study i.e. 43%. staph sp 50% was the commonest organism responsible for late onset of sepsis, klebsiella spp 33% and Ecoli 17% were also responsible with 43% cases of early onset of sepsis. In the present study, 23.3% Boo et al [23] was reported as the highest mortality associated with Klebsiella Sp of 63.8% followed by Ecoli in 57.7% and staph 27.3%, but in the present study, there was no mortality related to Klebsiella Sp. Ahmed et al [10] studied 30 cases of which 12 cases expired overall mortality and case fatality.

## Table-6: C reactive Protein.

| Author (year) | Sn(%) | SPC% | PPYC% | NOVC |
|---------------|-------|------|-------|------|
| Phillipe et al [6] | 47 | 86 | 22 | - |
| Singh et al [7] | 80 | 91 | - | - |
| Gerdes et al [5] | 93 | 85 | 28 | 99 |
| Basu et al [18] | 92 | 68 | 92 | 86 |
| Present study | 85 | 51 | 38 | 90 |

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rate was 28% in the present study. Karthikeyan [24] was observed as case fatality rate of 13.5% which was lower than the present study Joshi et al [8] observed mortality of 32.2% which was higher than the present study. Mortality amongst culture positive cases in present study was 31% which was lower as compared with that of Mishra et al [25] who observed mortality of 61.7%. Among gram negative septicemia 71% died whereas mortality was only 49% in gram positive septicemia.

**Conclusion**

Neonatal septicemia is a life threatening emergency. Blood culture positive rate was 26%. E.coli was the major organism responsible for early onset of sepsis and staph sp. was the common culprit for late onset of sepsis. Highest sensitivity and negative predictive values were found for a septic score of more than or equal to 3 by septic scoring. For prevention of neonatal septicemia, it requires to improve survival rate, better approach with early initiation of appropriate antibiotics.

It also requires infection control policies at the national level for effective management of such infections. To define the need for antibiotic therapy, several tests recommended as helpful in diagnosis neonatal sepsis were evaluated.

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