Original Research Article

Modified hematological sepsis score: an easy and cost effective measure to combat neonatal septicemia

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ABSTRACT

Background: Early diagnosis of neonatal sepsis continues to be a problem because of subtle and non-specific clinical features. Blood culture is the gold standard, but it takes several days and is expensive. The hematological scoring system (HSS) consisting of different blood parameters could be an effective and simple method to help diagnose and treat neonatal sepsis. Aim of the present study was undertaken to highlight the importance of HSS in the early diagnosis and evaluation of neonatal septicemia.

Methods: This was a prospective study done in a peripheral hospital in Kerala. The inclusion criteria involved all inborn babies above 34 weeks gestation. Exclusion criteria involved babies with congenital anomalies, congenital heart diseases, pathological jaundice, birth weight less than 2kg and babies requiring NICU admission, 550 babies were included in the study. Cord CRP and 48 hours CRP was taken. At 48 hours, blood samples were also taken for Total count (TC), Absolute Neutrophil Count (ANC), Platelet count, and peripheral smear. Blood culture was taken for haematological scoring.

Results: Individually, though parameters like TC, ANC, 48 hrs CRP and platelet count had excellent sensitivity (100%) and NPV (100%), their specificity was low 65%-82%. Degenerative changes showed sensitivity 94.1%, specificity 91% and NPV 99.8%. HSS score >5 and >6 had better specificity and NPV.

Conclusions: HSS scoring can be used to safely exclude neonatal sepsis, thus avoiding unnecessary antibiotic exposure in newborns and undue worry for parents.

Keywords: Blood parameters, C-Reactive protein, Newborn, Sepsis, Septicemia

INTRODUCTION

Over the years, there have been many advances in neonatology but neonatal sepsis continues to be a matter of deep concern for all neonatologists worldwide. The current neonatal mortality rate in India is 25/1000 live births. In developing countries, clinically diagnosed sepsis is present in 49-170 per 1000 live births and culture-proven sepsis in 16 per 1000 live births. Early onset septicemia can be contracted from the mother via transplacental route, ascending infection, passage through an infected birth canal, or exposure to infected blood at delivery. There are no particular blood tests or clinical signs to confirm a neonate has septicemia. The rational approach practiced by most neonatologists is to initiate antibiotic therapy in all infants with clinically suspected bacterial infection and to discontinue treatment if blood culture remains sterile and clinical signs disappear. This leads to unnecessary antibiotic use in newborn period leading to antibiotic resistance, making the need for effective sepsis screen markers crucial. Although a positive blood culture still remains the gold standard for
diagnosing sepsis, the procedure is expensive, takes 48-72 hours, and can have low yield due to insufficient sample volumes, intermittent or low-density bacteraemia, or suppression of bacterial growth by intrapartum antibiotics.\textsuperscript{4,5} Moreover, microbiological culture facilities in many peripheral hospitals in India are still far from optimal. Most neonatologists, therefore, are forced to rely, even today, on a sepsis screen (which includes various haematological markers) for a quick and reliable diagnosis.

There have been many studies based on Rodwell’s haematological sepsis score.\textsuperscript{6} Individually, each of the parameter studied showed poor performance, but a combination of them has excellent sensitivity.\textsuperscript{7-9} In recent years, many other parameters like Interleukins, adhesion molecules and immunoglobulins have been extensively studied as markers of neonatal sepsis but these tests are not readily available in all labs and are expensive.\textsuperscript{10} In a developing country like India, those septic parameters were needed that are quick, easy and less expensive in addition to being highly sensitive and specific. In this study, authors have modified the haematological sepsis score by including cord and 48 hours CRP and by excluding abnormal fT ratio and micro ESR. Though there have been studies across India regarding the use of HSS with different combinations of haematological parameters, only few have been done in Kerala regarding this.

**METHODS**

This is a prospective study done in a peripheral hospital in Kerala from January 2018 - June 2019. Based on a study conducted by Vani Krishnamurthy et al, proportion of patients with sepsis was 12\%.\textsuperscript{11} Sample size $n = \frac{Z_a^2 \times p \times q}{d^2}$ $Z_a$ at 95\% CI = 1.96

$p = \text{proportion of patients with sepsis} = 12\%$

$q = 100-p = 88\%$, $d = \text{precision}=3\%$

Minimum sample required, $n = 451$. 550 cases were taken in the study.

**Inclusion criteria**

All inborn newborn babies $\geq 34$ weeks gestational age.

**Exclusion criteria**

- Birth weight $<2$kg
- Babies with congenital anomalies/ malformations
- Babies with congenital heart diseases
- Pathological jaundice
- Babies getting admitted to NICU.

After taking into consideration of both inclusion and exclusion criteria, 550 babies were included in this study.

The study was approved by the ethics committee. Parental consent was obtained for the newborns enrolled in the study. Babies were divided into three groups:

- **Group A** (proven sepsis): All babies with culture positive and clinical features of sepsis - 17.
- **Group B** (probable sepsis): All babies with culture negative but with clinical suspicion of sepsis and abnormal CRP or blood parameters – 217.
- **Group C** (normal babies): All babies with blood culture negative and no evidence of sepsis - 316.

Mothers with the following risk factors were taken into consideration for probable sepsis;

- Febrile illness in the mother within 2 weeks of delivery.
- Meconium stained liquor.
- Untreated or partially treated urinary tract infection.
- Premature rupture of membranes.

Clinical parameters for neonatal sepsis included in our study were tachypnea, hypoglycemia, hypo/ hyperthermia, refusal to feeds, vomiting, poor perfusion, lethargy, apnea and irritable cry.

CRP was measured using latex agglutination test kit. Visible agglutination of latex particles constituted a positive result which indicated a level of CRP $\geq 0.6$ mg/dL. Approximately 3 ml of blood was collected from the umbilical cord after clamping and cutting of the cord. At 48 hours, about 3-4ml blood was drawn using a sterile syringe, out of which 1ml of the blood sample was allowed to clot in a sterile bottle to collect serum for the estimation of C-reactive protein and the remaining 2ml of blood was collected in a sterile bottle containing the anticoagulant EDTA (2-2.5 mg/ml) for estimation of Total WBC count (TC), Absolute neutrophil count (ANC) and platelet count. TC were done on a Neubauer counting chamber. Differential counts were performed on Leishman stained blood smears by counting at least 200 cells. All these were done by the pathologist who was blinded to the study. In this study, antibiotics were started based mostly on the 48 hours blood parameters in addition to clinical parameters.

Blood culture was done for all Group A (17) and Group B (217) babies using 3ml of venous blood collected from a peripheral vein by sterile procedure and before the commencement of antibiotics. The blood was aseptically introduced into aerobic and anaerobic culture media. Inoculated blood culture media were considered negative if there was no growth after continuous incubation for up to 7 days. After 3 days of antibiotics, again on Day 5 of life, CRP was taken for all Group A and Group B babies. If CRP levels were decreasing or in normal range and the blood culture was sterile, the antibiotics were stopped and babies were discharged. A follow up CRP was taken on day 10 or on follow up.
**Statistical analysis**

Data were entered in MS Excel and analysis was done using SPSS 16.0 version. Data were presented as percentages since they were categorical in nature. Association between categorical variables were assessed using Chi-square test. Sensitivity, specificity, Positive Predictive Values (PPV) and Negative Predictive Values (NPV) were calculated to assess the validity of screening tool and p-values were also calculated, p-value < 0.005 was considered statistically significant.

**RESULTS**

There were total 550 babies included in the study. The parity of the mother, type of delivery, sex of the baby had no effect in proven sepsis category, 16 babies (94.1%) were term gestation and only 1 preterm baby (5.9%). 10 babies (58.8%) were male and 7 babies (41.2%) were female in proven sepsis category. According to growth according to gestational age, 14 babies (82.4%) were AGA, 2 babies (11.8%) were SGA and only 1 baby (5.9%) was IUGR in proven sepsis group, 217 newborns were found to have probable sepsis. In probable sepsis category, 107 babies (49.3%) were male and 110 babies (50.7%) were female. According to growth according to gestational age, 188 babies (86.6%) were AGA, 14 babies (6.5%) were SGA and 15 babies (6.9%) were IUGR.

**Table 1: Modified hematological sepsis screen.**

| Parameter                          | Abnormal value | Score |
|------------------------------------|----------------|-------|
| Cord CRP                           | ≥0.6 mg/dl     | 1     |
| 48 hours CRP                       | ≥0.6 mg/dl     | 1     |
| Abnormal leucocyte count at 48 HRS | ≤5000/mm³      | 1     |
| Absolute Neutrophil Count (ANC) at 48hrs | <1500/mm³      | 1     |
| Thrombocytopenia at 48HRS          | ≤1.5 lac/mm³   | 1     |
| Degenerative changes               |                |       |
| Toxic granules                     |                |       |
| Cytoplasmic vacuolations           |                | 1     |

**Table 2: Demographic table of the study population.**

| Characteristics                     | Number (%) | Proven sepsis (17) | Probable sepsis (217) |
|-------------------------------------|------------|--------------------|-----------------------|
| Primiparity of mother               |            |                    |                       |
| Single                              | 209 (38%)  | 8 (47.1%)          | 72 (33.2%)            |
| Multiparity                         | 341 (62%)  | 9 (52.9%)          | 145 (66.8%)           |
| Type of delivery                    |            |                    |                       |
| Normal vaginal delivery             | 228 (41.5%)| 5 (29.4%)          | 88 (40.6%)            |
| Instrumental delivery               | 64 (11.6%) | 5 (29.4%)          | 30 (13.8%)            |
| Cesarean delivery                   | 258 (46.9%)| 7 (41.2%)          | 99 (45.6%)            |
| According to gestation              |            |                    |                       |
| Term                                | 451 (82%)  | 16 (94.1%)         | 181 (83.4%)           |
| Preterm                             | 99 (18%)   | 1 (5.9%)           | 36 (16.6%)            |
| According to birth weight           |            |                    |                       |
| 2000-2499g                          | 84 (15.3%) | 1 (5.9%)           | 29 (13.4%)            |
| 2500-2999g                          | 241 (43.8%)| 10 (58.8%)         | 96 (44.2%)            |
| 3000-3499g                          | 187 (34%)  | 4 (23.5%)          | 75 (34.6%)            |
| 3500-3999g                          | 38 (6.9%)  | 2 (11.8%)          | 17 (7.8%)             |
| Single/ multiple births             |            |                    |                       |
| Single                              | 534 (97.1%)| 17 (100%)          | 210 (96.8%)           |
| Twin gestation                      | 16 (2.9%)  | 0 (0.0%)           | 7 (3.2%)              |
| According to sex of baby            |            |                    |                       |
| Male                                | 281 (51.1%)| 10 (58.8%)         | 107 (49.3%)           |
| Female                              | 269 (48.9%)| 7 (41.2%)          | 110 (50.7%)           |
| According to growth for gestational age |          |                    |                       |
| Aga (appropriate for gestational age)| 460 (83.6%)| 14 (82.4%)         | 188 (86.6%)           |
| SGA (small for gestational age)     | 54 (9.8%)  | 2 (11.8%)          | 14 (6.5%)             |
| IUGR (intrauterine growth retardation)| 36 (6.6%) | 1 (5.9%)           | 15 (6.9%)             |
| Clinical features of sepsis in baby |            |                    |                       |
| Apnea                               | 10 (1.8%)  | 0 (0.0%)           | 10 (4.6%)             |
| Tachypnea                           | 63 (11.5%) | 8 (47.1%)          | 55 (25.3%)            |
| Vomiting                            | 31 (5.6%)  | 0 (0.0%)           | 31 (14.3%)            |
| Lethargy                            | 12 (2.2%)  | 1 (5.9%)           | 11 (5.1%)             |
According to clinical risk features in baby, none of the babies in proven sepsis had apnea or vomiting. 8 babies (47.1%) had tachypnoea and 5 babies (29.4%) had refusal to feeds. In probable sepsis category, 55 babies (25.3%) had tachypnoea, 31 babies (14.3%) had vomiting, 16 babies (7.4%) had refusal to feeds, 12 babies (5.5%) had hypothermia and 76 babies (35%) had no risk factors. Maternal risk factors were urinary tract infections - 3 (17.6%) in probable sepsis and 44 (20.3%) in proven sepsis; fever seen in 6 mothers (35.3%) in proven sepsis and 22 mothers (10.1%) in probable sepsis. All 10 cases of premature rupture of membranes were seen in mothers of babies with probable sepsis.

Table 3 shows the HSS score 0-2 were seen in 115 babies of Group B (26.7%). HSS score 3-4 were seen in 48 babies (98%) of Group B. HSS score ≥ 5 were seen in 16 babies (22.9%) in Group A and 54 babies (77.1%) in Group B. All the values are significant p<0.001. All babies (119) with HSS score above 3 were started on antibiotics.

In Table 4, cord CRP has low sensitivity (64.7%) and specificity (87.4%). Total count, ANC, 48 hrs CRP and platelet count have 100% sensitivity and NPV, but their specificity is low 65%-82%. Degenerative changes showed sensitivity 94.1%, specificity 91% and NPV 99.8%.

| Parameters                  | Sensitivity | Specificity | NPV  | PPV   | p value |
|-----------------------------|-------------|-------------|------|-------|---------|
| Total count                 | 100%        | 81.2%       | 100% | 14.5% | <0.001  |
| ANC                         | 100%        | 80.3%       | 100% | 13.9% | <0.001  |
| Platelet count              | 100%        | 80.7%       | 100% | 14.2% | <0.001  |
| Degenerative changes        | 94.1%       | 91.0%       | 99.8%| 25.0% | <0.001  |
| Cord CRP                    | 64.7%       | 87.4%       | 98.7%| 14.1% | <0.001  |
| 48 hrs CRP                  | 100%        | 65.1%       | 100% | 8.4%  | <0.001  |

In Table 5, hematological parameters like total count, ANC, platelet count and 48 hours CRP were seen in all 17 cases (100%) of proven sepsis category (Group A). 16 out of 17 babies (94.1%) had abnormal degenerative changes. In Group B, only 46-48% babies had abnormal total count, ANC and platelet count. 186 out of 217 babies (85.7%) had abnormal 48 hours CRP. Degenerative changes were seen in only 48 (22.1%) out of 217 babies. Abnormal cord CRP was seen only in 11 babies (64.7%) in Group A and in 67 babies (30.9%) in Group B. Thus, it can be concluded that cord CRP by itself is not a good parameter for sepsis in both Group A and B.

Table 6 shows, HSS score up to >4 has 100% sensitivity and NPV but specificity is low (73.5% -84.6%). HSS score ≥5 and >6 has better specificity and NPV. Thus, it can be safely said that all babies with HSS score >4 are highly prone to develop sepsis and hence complete sepsis work up should be done for these babies.
In proven sepsis category, blood culture reports showed 7 babies had E. Coli, 6 babies had staphylococcus, 2 babies had klebsiella growth and 2 babies showed pseudomonas growth. Intravenous antibiotics were started according to the antibiotics protocol followed in our hospital and continued for 48-72 hours for 119 babies. The repeat CRP on day 5 was taken for these babies and antibiotics were stopped if the CRP and blood culture reports are negative. Repeat CRP was taken for all these babies at first follow up.

**DISCUSSION**

Neonatal sepsis was one of the common causes of neonatal mortality contributing to 16% of all intramural deaths, other causes being preterm birth, severe infection, and asphyxia. The wide variations between studies in methods and results, the lack of precision in the definition of sepsis and the lack of standardized reference values makes it impossible to predict which neonate is at risk and which is not. Thus, neonatal sepsis continues to be a neonatologist’s dilemma to correctly diagnose whether baby has sepsis and to initiate treatment as early as possible.

Cord blood is the earliest hematologic sample that can be obtained from a newborn in a risk free, painless and non-invasive manner. Since CRP does not cross placenta, the elevated levels are due to production of CRP in the neonate. The definitive diagnosis of sepsis is made by a positive blood culture, which requires a minimum of 48-72 hours, yields a positive result in 30-40% of cases. However, the probable sepsis group, which has negative blood culture with clinical signs of sepsis, comprises a difficult diagnostic group and could not be ignored because the fatal infection had been reported in another study in the presence of negative blood culture.  

Cord CRP as an effective marker to predict EONS has been studied extensively with widely varied results. When blood culture was considered as the standard cut off point, CRP was found to be an effective marker for neonatal sepsis with sensitivity ranging from 87-92%. The discrepancy in the sensitivity of CRP in different studies is due to variations in diagnostic criteria, the time of onset of the infection (early or late) and different methods of CRP estimation (latex agglutination method or radio-immunodiffusion technique). Our study shows that cord CRP is more specific but 48 hours CRP is more sensitive in predicting neonatal sepsis. Hence 48 hours CRP is better predictor than cord CRP in sepsis screening (Table 4 and 5).

Total leukocyte count of <5000 and Absolute neutrophil count <1800/mm³ were considered significant for sepsis. Leukopenia was a better predictor of sepsicaemia as compared to leukocytosis as it has higher specificity. Total neutrophil counts rise after birth and reach their peak levels at 6 to 8 h of life. There have been studies which show good specificity with high PPV and some that show no significant relation for neonatal sepsis. Study shows both total count and ANC individually had excellent sensitivity and NPV, but specificity ranging from 80-81% (Table 4 and 5).

Degenerative changes in neutrophils like cytoplasmic vacuolization and toxic granulation were reported to be a valuable adjunct in the early detection of neonatal bacterial infections shown in our study with specificity of 91% and sensitivity 94.1% of and NPV 99.8%.

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**Table 5: Performance of sepsis screen parameters in all three groups of babies.**

| Parameters          | Groups                  |     |     |     | p value |
|---------------------|-------------------------|-----|-----|-----|---------|
|                     | Proven sepsis (N=17)    |     |     |     |         |
| Total count         | 17 (100%)               | 100 |     | 0   | <0.001  |
| ANC                 | 17 (100%)               | 105 |     | 0   | <0.001  |
| Platelet count      | 17(100%)                | 103 |     | 0   | <0.001  |
| Degenerative changes| 16 (94.1%)              | 48  |     | 0   | <0.001  |
| Cord CRP            | 11 (64.7%)              | 67  |     | 0   | <0.001  |
| 48 hrs CRP          | 17 (100%)               | 186 |     | 0   | <0.001  |

**Table 6: Performance of HSS score.**

| HSS score | Sensitivity | Specificity | NPV  | PPV  | p value |
|-----------|-------------|-------------|------|------|---------|
| >2        | 100%        | 73.5%       | 100% | 10.8%| <0.001  |
| >3        | 100%        | 80.9%       | 100% | 14.3%| <0.001  |
| >4        | 100%        | 84.6%       | 100% | 17.2%| <0.001  |
| >5        | 94.1%       | 89.9%       | 99.8%| 22.9%| <0.001  |
| >6        | 64.7%       | 96.6%       | 98.8%| 37.9%| <0.001  |
contrast, lower sensitivity was observed in some.\textsuperscript{5,8,9} The presence of toxic granules indicates the production of unusual PMNs during infection and stress induced leucopoiesis.\textsuperscript{24} Studies showed that thrombocytopenia could be used as an early but nonspecific marker for diagnosis of sepsis which is similar to our study which shows 100% sensitivity and 100% NPV and specificity 80.7\%.\textsuperscript{5,25,26} Thrombocytopenia is due to increased platelet destruction and sequestration seen in infections, and decreased platelet production due to damage of megakaryocytes.

The earliest hematological sepsis study was done by Rodwell et al.\textsuperscript{4} Over the years, different combinations of these blood parameters have been used and many studies formulated over their effectiveness for diagnosing neonatal septicemia.\textsuperscript{26,27} Few studies in India too reported that HSS was a simple, rapid and effective screening tool of sepsis with excellent NPV and specificity.\textsuperscript{8,9,24} The combination of HSS and CRP for diagnosing neonatal septicemia are few.\textsuperscript{28} The HSS more than or equal to 4 was more significant in early diagnosis of neonatal sepsis compared to CRP and procalcitonin level with $p=0.001$.\textsuperscript{29} This was also similar to this study which shows that combination of hematological parameters with 48 hours CRP was good to exclude sepsis (Table 3 and 6).

There were some limitations in the study. Firstly, this study was done in a small peripheral hospital. Secondly, authors did not include parameters of the sepsis screen such as micro-erythrocyte sedimentation rate and the ratio of immature to mature neutrophils which is a major drawback. Though many of our enrolled babies had clinical evidence of sepsis, positive blood culture results were found only in 17 neonates.

**CONCLUSION**

There are no ideal tests for the diagnosis of early onset neonatal sepsis. HSS is simple, quick and cost-effective test with high sensitivity and specificity for the early diagnosis of neonatal sepsis and a score $>4$ may guide to make decisions for judicious use of antibiotic treatment. It may also useful in decreasing mortality, duration of hospital stay and the cost of treatment.

**Recommendations**

There is an utmost need for regular analysis of sepsis in all nurseries with their bacteriological profile and their sensitivity pattern, which will help to prevent antibiotic resistance. This modified score is yet to be validated in a bigger study to improve the accuracy of this screening test.

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