Original Research Article

**Thrombocytopenia and thrombocytosis as a predictor of neonatal sepsis: a hospital-based cross-sectional study**

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**ABSTRACT**

Background: Hematological changes in sepsis can aid in the early diagnosis of sepsis even before the culture reports are obtained. Of the various hematological parameters, the platelet count can be an early marker for sepsis. This study was carried out to evaluate the role of thrombocytopenia and thrombocytosis as a marker of sepsis in neonates.

Methods: A cross-sectional study was carried out at neonatal intensive care unit (NICU) at a tertiary care center from 1st January 2014 to 30th September 2014. During this period, 623 neonates had features suggestive of sepsis, of which 190 turned to be having culture-positive sepsis. One hundred and ninety neonates aged between 0 to 28 days of life admitted in NICU with culture-positive sepsis were included in the study. The newborns were categorized based on platelet count and type of organism cultured. Chi square test was used to test statistical significance. P<0.05 was considered statistically significant.

Results: Among 190 participants, 50% had gram-positive organisms in culture, 37.9% had gram-negative sepsis, and 12.1% had fungal sepsis. The prevalence of thrombocytopenia among the participants was 39.47%. There was a statistically significant difference across the type of organism in sepsis (p<0.05). The difference in outcome across the platelet count status was insignificant, with a p=0.391.

Conclusions: The most common organism causing neonatal sepsis was gram-positive organisms, followed by gram-negative organisms and fungus. Thrombocytopenia was present in all forms of neonatal sepsis irrespective of the causation. Hence, thrombocytopenia can be considered as an early marker of neonatal sepsis.

Keywords: Neonatal sepsis, Thrombocytosis, Thrombocytopenia, Hematological changes, Sepsis predictor

**INTRODUCTION**

Globally nearly 1.6 million deaths occur every year due to neonatal sepsis.1 Most deaths occur in developing countries, where the neonatal deaths due to sepsis are almost 60%.2 The incidence of neonatal sepsis in developing countries ranges from 2.2 to 8.6 per 1000 live births.3 Neonatal sepsis acts as a major cause for morbidity and mortality in the neonatal period, especially in low birth weight babies whose prevalence is high in developing countries.4

As per the international sepsis definition conference, sepsis is defined as a clinical syndrome characterized by both infection and systemic inflammatory response syndrome (SIRS).5 In neonates, SIRS is characterized by tachypnea, temperature instability, capillary refill time of 3 sec, white blood cell count (<5000/μl or 34,000/μl), raised C reactive protein (CRP) value, and procalcitonin values. Severe sepsis is characterized by sepsis accompanied by single organ dysfunction, hypoperfusion, or hypotension. Septic shock occurs due to hypotension and hypoperfusion of the organs.6

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a neonatal period often finds its source from the organism from blood, CSF, or urine.

Thrombocytopenia is a major hematological problem encountered in all sick and NICU admitted neonates. Thrombocytopenia in neonates indicates an active underlying disease process. The major cause for thrombocytopenia is increased platelet consumption caused due to various factors like infections, thrombosis, immune-mediated or reduced platelet production. In sepsis, thrombocytopenia occurs in early and late stages. Thrombocytopenia occurs both in gram-positive and gram-negative organism infections. Even before the blood culture is positive for the growth of the organism, thrombocytopenia occurs; hence, it can be considered as an early predictor of neonatal sepsis. Normal platelet count ranges from 150,000 to 450,000 platelets per microliter of blood. Thrombocytopenia is defined as platelet count <150,000 per microliter of blood irrespective of the gestational age. It is the commonest hematological abnormality encountered in NICU.

In view of the high incidence of mortality due to sepsis in NICU, this study was carried out to evaluate the role of thrombocytopenia and thrombocytosis as a marker of sepsis in neonates and to find the prevalence of thrombocytopenia and thrombocytosis among neonates with sepsis. The secondary objectives were to find out the relationship between type of organism (gram-positive and negative, fungus) causing sepsis and thrombocytopenia and to know relationship with neonatal outcome.

METHODS

Study design

This cross-sectional study was done at NICU of Indira Gandhi Institute of child health, Bangalore from 1st January 2014 to 30th September 2014. A total of 190 neonates with culture-proven sepsis were enrolled for the study.

Sample size calculation

The sample size was calculated assuming the proportion of thrombocytopenia in neonatal sepsis as 58.5% as per the study by Agrawal et al. Other parameters considered for sample size calculation were 8% absolute precision and a 95% confidence level. The following formula was used for sample size as per study by Daniel et al.

\[ n = \frac{Z^2 \cdot p \cdot (1 - p)}{d^2} \]

Where \( n \) = sample size, \( Z = Z \) statistic for a level of confidence level=1.960, \( p = \) expected prevalence/proportion of outcome= 0.585, \( d = \) precision >0.08.

The required sample size as per the calculation mentioned above was 146. To account for a non-participation rate of about 30%, another 44 subjects were added to the sample size. Hence final required sample size was 190. During the study period, a total of 623 neonates were admitted into the NICU for sepsis, of which 190 were culture-proven sepsis. They were recruited as the study participants.

Methods used

Complete blood counts, CRP, and blood culture were done as a part of the sepsis evaluation from all participants after collecting blood at admission. All the blood samples were drawn under strict aseptic precautions from peripheral veins for blood culture before starting antibiotic therapy. Blood was taken in a bottle containing blood culture media. They were categorized based on the platelet count once the blood culture is positive.

The current study was done to find the relationship between platelet count and the type of organism causing sepsis. The study also tried to find the relationship between platelet count and outcome in these neonates with culture-proven sepsis. Total WBC count<5000, being a proven marker of sepsis, was compared with platelet count.

Operational definitions

Neonatal period was considered from birth till 28 days after delivery. Neonatal sepsis acts as a major cause for morbidity and mortality in the neonatal period, especially in low birth weight babies whose prevalence is high in developing countries.

As per international sepsis definition conference, sepsis is defined as a clinical syndrome characterized by both infection and systemic inflammatory response syndrome (SIRS). In neonates, SIRS is characterized by tachypnea, Temperature instability, capillary refill time of 3 secs or more, white blood cell count (<5000/µl or 34,000/µl), raised C reactive protein value, and procalcitonin values. Severe sepsis is characterized by sepsis accompanied by single organ dysfunction, hypoperfusion, or hypotension. Neonates with culture-proven sepsis are those who had a clinical picture of septicemia, and the organism causing sepsis was isolated from blood culture.

Thrombocytopenia was considered when the platelet counts were less than 1.5 lakhs per cubic millimeters. Platelet count was considered normal if it was more than or equal to 1.5 lakhs and less than 5 lakhs per cubic millimeters. Thrombocytosis was defined as platelet count more than or equal to 5 lakhs per cubic millimeters. Mild thrombocytopenia when 100,000-150,000/mm³. Moderate as 50,000-99,999/mm³ and severe as <50,000/mm³.

Early-onset sepsis was defined as clinical features of sepsis appearing within 72 hours of birth, while late-onset sepsis was defined as clinical features of sepsis appearing after 72 hours of life.
Inclusion criteria

All neonates aged 0 to 28 days presenting with the hypothermia or fever, refusal of feeds, lethargy, seizures, excessive cry, vomiting; abdominal distension; respiratory distress; bulging anterior fontanelle; delayed capillary filling time, sclerema, bleeding, shock, and found to be culture-positive were included in the present study.

Exclusion criteria

Neonates with congenital anomalies and antibiotics administered prior were excluded from the study. All neonates admitted with culture-negative sepsis were excluded from the study. Mother with a history of ITP, Systemic lupus erythematosus, and those on medications causing thrombocytopenia during pregnancy were excluded.

Maternal history, history of birth events, clinical features, and complete blood count details were recorded in a pre-structured data collection proforma. Based on the blood culture reports, the neonates were classified as those who had gram-positive sepsis, gram-negative sepsis, and fungal sepsis.

Ethical aspects

The institutional ethics committee of Indira Gandhi Institute of child health, Bangalore approved this study. Confidentiality was maintained, and the identity of the neonates was kept unknown. Informed written consent was obtained from the parents/guardian of the neonates. Data confidentiality was maintained.

Statistical methods

The neonatal outcome, platelet count, and sepsis were considered as the primary outcome variables. The type of organism was considered as the primary explanatory variable. Demographic parameters like gender, inborn/referred, type of organism, hemoglobin, birth weight, gestational age, total WBC count, and diagnosis were considered as other study relevant variables.

Descriptive analysis was carried out by mean and standard deviation for quantitative variables, frequency, and proportion for categorical variables. The association between explanatory variables and categorical outcomes was assessed by cross tabulation and comparison of percentages.

The chi-square test was used to test statistical significance. \( p<0.05 \) was considered statistically significant. coGuide version V.1.0 was used for statistical analysis. \(^{13}\)

RESULTS

A total of 190 subjects were included in the final analysis. The mean platelet count was 205246.03±145038/mm\(^2\) and ranged between was 6000 to 674000/mm\(^2\) in the study population. Among the people with sepsis, 82 (43.2%) participants had early-onset neonatal sepsis, and 108 (56.8%) participants had late-onset neonatal sepsis. Out of 190 babies, 37 (19.5%) babies were <2.5 kg and 153 (80.5%) babies were >2.5 kg. Among neonates, a majority of 50% of participants had gram-positive, followed by 37.9% of participants who had gram-negative and 12.1% participants had the fungal sepsis. Out of 190 subjects, 14 (7.4%) subjects had expired, and remaining 176 (92.6%) subjects were discharged. The prevalence of the thrombocytopenia among the participants was 39.47% as shown in the Table 1.

Among the people with neonatal sepsis, 67 (35.26%) participants had sepsis without focus. Sepsis with pneumonia, sepsis with hyperbilirubinemia, sepsis with meningitis, sepsis with septic arthritis, sepsis with urinary tract infection, and sepsis with cellulitis were the other diagnosis made (Table 2).

Of 72 neonates presenting with sepsis caused by gram-negative organisms, 36 (50%) had thrombocytopenia. The number of neonates with severe thrombocytopenia was more among sepsis caused by gram-negative organisms (22.2%).

There was a statistically significant difference across the type of organism in sepsis (\( p<0.05 \)) (Table 3).

The difference in outcome across the platelet count status was insignificant, with a \( p \) value of 0.391. Among the cases in a mild thrombocytopenia group, 1 (4.35%) case had <5000 total WBC count, and 22 (95.65%) cases had \( \geq 5000 \) total WBC count. Among the cases in the moderate thrombocytopenia group, 2 (7.14%) cases had <5000 total WBC count, and 26 (92.86%) cases had \( \geq 5000 \) total WBC count. Among the cases in the severe thrombocytopenia group, 4 (16.67%) cases had <5000 total WBC count, and 20 (83.33%) cases had \( \geq 5000 \) total WBC count. Among the cases in the thrombocytosis group, all of the 10 (100%) cases had \( \geq 5000 \) total WBC count. Among the cases in the normal platelet count group, 7 (6.67%) cases had <5000 total WBC count, and 98 (93.33%) cases had \( \geq 5000 \) total WBC count as shown in the Table 4.

Among the cases with a combination of total WBC count (<5000) and thrombocytopenia, 5 (71.43%) cases had expired, and 2 (28.57%) cases had been discharged. Among the cases with a combination of total WBC count >5000 and thrombocytopenia, 2 (28.57%) cases had expired, and 5 (71.43%) cases had been discharged (Table 5).
Table 1: Summary of baseline characteristics (N=190).

| Parameter                                           | Summary (%) |
|-----------------------------------------------------|-------------|
| Gender (Male: female ratio)                         | 120.70      |
| Inborn: Referred (ratio)                            | 159:31      |
| **Organism**                                       |             |
| Coagulase-negative *Staphylococci*                  | 80 (42.11)  |
| Non-fermenting gram-negative bacilli                | 23 (12.11)  |
| Methicillin-resistant *Staphylococcus aureus*       | 6 (3.16)    |
| *Staphylococcus aureus*                             | 2 (1.05)    |
| *E.coli*                                            | 6 (3.16)    |
| Gram positive cocci (undifferentiated)              | 1 (0.53)    |
| Other organisms                                    | 72 (37.9)   |
| Haemoglobin (mean ± SD) g/dl                        | 13.66±3.83  (4.10 to 22.60) |
| Total count (mean ± SD) count/mm³                   | 16980.32±11623.22 (1510 to 59000) |
| Platelet (mean ± SD) count/mm³                      | 205246.03±145038 (6000 to 674000) |
| **Sepsis**                                          |             |
| Early onset neonatal sepsis                         | 82 (43.2)   |
| Late onset neonatal sepsis                          | 108 (56.8)  |
| **Birth weight (kg)**                               |             |
| <2.5                                                | 37 (19.5)   |
| >2.5                                                | 153 (80.5)  |
| **Type of organism**                                |             |
| Gram positive                                      | 95 (50)     |
| Gram negative                                      | 72 (37.9)   |
| Fungus                                              | 23 (12.1)   |
| Gestational age (Term: Pre term)                    | 168:22      |
| **Platelet count status**                           |             |
| Thrombocytopenia                                    | 75 (39.47)  |
| Mild thrombocytopenia                               | 23 (12.1)   |
| Moderate Thrombocytopenia                           | 28 (14.7)   |
| Severe Thrombocytopenia                             | 24 (12.6)   |
| Thrombocytosis                                      | 10 (5.3)    |
| Normal platelet count                               | 105 (55.3)  |
| **Total WBC count (count/mm³)**                     |             |
| <5000                                               | 14 (7.4)    |
| ≥5000                                               | 176 (92.6)  |
| **Neonatal outcome**                                |             |
| Expired                                             | 14 (7.4)    |
| Discharged                                          | 176 (92.6)  |

Table 2: Descriptive analysis of diagnosis in the study population (n=190).

| Diagnosis                                         | Percentages (%) |
|---------------------------------------------------|-----------------|
| Sepsis without focus                              | 67 (35.26)      |
| Sepsis with Pneumonia                             | 58 (30.53)      |
| Sepsis with hyperbilirubinemia                    | 19 (10)         |
| Sepsis with meningitis                            | 17 (8.9)        |
| Sepsis with septic arthritis                      | 14 (7.4)        |
| Sepsis with urinary tract infection               | 13 (6.8)        |
| Sepsis with cellulitis                            | 2 (1.05)        |
DISCUSSION

Neonatal sepsis is a life-threatening condition deems rapid and early diagnosis to give timely treatment. Thrombocytopenia is one of the most common hematological manifestations seen in neonatal sepsis. Thus platelet count can be considered as an early marker of neonatal sepsis.14

In this current study, the prevalence of thrombocytopenia among neonates with culture-proven sepsis was 39.47%. Severe thrombocytopenia was seen among neonates with sepsis caused due to gram-negative organisms. Thrombocytosis was present in 6.31% of the neonates with culture-positive sepsis. Total WBC count<5000 is a proven marker of sepsis. Hence this study was conducted to correlate platelet count and neonatal outcome with the Total WBC count. The majority, 92.6%, of the neonates had a total WBC count more than or equal to 5000.

In our study, 37.9% of neonatal sepsis was caused due to gram-negative organisms, whereas 50% of the neonates had gram-positive organisms causing sepsis. Coagulase-negative staphylococci were the most common (47.11%) organism causing sepsis in this present study. Similar observations were made by Rahman et al, where 29% of participants had staphylococcal sepsis.15 This can be attributed to the constant presence of *Staphylococcus* as a commensal in the skin, nasopharynx, gastrointestinal tract and can spread via the health care workers.16 This emphasizes the need for maintaining hygienic handling of the neonates. In the study done by Khassawneh et al and Jordan had gram-negative organism as the most common cause of neonatal sepsis in contrast to our study.16

Fungal sepsis was observed in 12.1% of the neonates in this study. Similar findings were seen by Sundaram et al, where 11% of the septic neonates were due to fungal sepsis.16 In a study by Guida et al, 8% of the neonates had fungal sepsis.17 Also, In the study done by Singh et al, the mortality rate was 37.1% among the neonates with sepsis, which was in contrast to the current study, where the mortality rate was 7.4%.18 This might be attributed to the pseudomonal infection, which was common among the neonates in the study by Singh et al.18 In our study, we found that low birth weight babies (80.5%) were higher in proportion

Table 3: Comparison of type of organism with sepsis and platelet count status (n=190).

| Parameter                  | Type of organism | Gram-positive (n=95) (%) | Gram-negative (n=72) (%) | Fungus (n=23) (%) | Chi square | P value |
|----------------------------|------------------|-------------------------|--------------------------|------------------|------------|---------|
| Sepsis                     | Early onset      | 33 (34.73)              | 44 (61.1)                | 5 (21.73)        | 16.507     | <0.001  |
|                            | Late onset       | 62 (65.26)              | 28 (38.89)               | 18 (78.26)       |            |         |
| Platelet count status      |                  |                         |                         |                  |            |         |
| Thrombocytopenia           | Mild             | 15 (15.78)              | 5 (6.94)                 | 3 (13.04)        |            |         |
|                            | Moderate         | 7 (7.36)                | 15 (20.83)               | 6 (26.08)        |            |         |
|                            | Severe           | 5 (5.26)                | 16 (22.22)               | 3 (13.04)        |            |         |
|                            | Thrombocytosis   | 6 (6.31)                | 4 (5.72)                 | 0 (0)            |            |         |
|                            | Normal platelet  | 62 (65.26)              | 32 (44.44)               | 11 (47.82)       |            |         |

* No statistical test was applied due to 0 subjects in the cell

Table 4: Comparison of outcome and total WBC with platelet count status (n=190).

| Parameter                  | Platelet count status | Platelet count status | Platelet count status | Platelet count status | Platelet count status | Platelet count status | Platelet count status | Platelet count status | Platelet count status |
|----------------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|
|                            | Mild (n=23) (%)       | Moderate (n=28) (%)   | Severe (n=24) (%)      | Thrombocytopenia (n=10) (%) | Normal platelet count (n=105) (%) | Chi-square | P value |
| Neonatal outcome           | Expired               | 1 (4.35)              | 1 (3.57)              | 4 (16.67)            | 1 (10)                | 7 (6.67)              | 4.116     | 0.391     |
|                            | Discharge             | 22 (95.65)            | 27 (96.43)            | 20 (83.33)           | 9 (90)                | 98 (93.33)            |           |           |

Table 5: Comparison of outcome between a combination of total count <5000 and thrombocytopenia (n=14).

| Outcome                    | Combination of total count (<5000) and thrombocytopenia | Chi-square | Fisher exact p value |
|----------------------------|----------------------------------------------------------|------------|----------------------|
|                            | Positive (n=7) (%)                                       | 2.571      | 0.286                |
|                            | Negative (n=7) (%)                                       |            |                      |
compared to newborns with normal birth weight. The outcome was also poor in newborns with low birth weight than those with normal birth weight.

92.6% of the neonates had a total WBC count more than or equal to 5000. The difference in the proportion of neonatal outcome and total WBC count was statistically significant. As per the current study findings, thrombocytopenia was more commonly observed in neonatal sepsis than thrombocytosis. Thrombocytosis was present in 6.31% of the neonates with culture-positive sepsis. Because of a small proportion of cases with thrombocytosis, of which six neonates had gram-positive sepsis and 4 had gram-negative sepsis and no mortality, it was nonconclusive about its role in predicting sepsis as well as the outcome.

All sepsis-causing organisms can cause thrombocytopenia in neonates. The platelets are considered active participants in the host defence system. The various factors that are attributed to thrombocytopenia are increased platelet consumption caused due to endothelial damage, platelet destruction by bacterial and fungal toxins. The cell wall component-lipid A of gram-negative bacteria destroys the platelets. The current study has added evidence towards the role of thrombocytopenia as a predictor of neonatal sepsis.

The limitation of the current study being a single-center study with a limited sample size reduces the generalisability of findings. A future study focusing on the role of thrombocytopenia on a large sample is recommended.

CONCLUSION

The common organism producing sepsis was gram-positive organisms followed by gram-negative and fungus. Thrombocytopenia was more in neonates with sepsis caused due to gram-negative organisms. Severe thrombocytopenia was seen more among neonates with sepsis caused due to gram-negative organisms. However, further studies involving larger samples with multiple centers are needed to prove these gram-negative organisms’ associations with thrombocytopenia. Thrombocytopenia was more prevalent in sepsis among low-birth-weight neonates. Combination of thrombocytopenia and total count <5000 appears promising in predicting mortality, albeit further studies involving larger samples are needed. Thrombocytopenia acts as an early predictor of neonatal sepsis and also the outcome of the neonates. Clinical correlation and platelet counts can be used together to suspect early sepsis and provide timely, appropriate treatment.

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