Platelet parameters as a diagnostic marker in early diagnosis of neonatal sepsis- Seeking newer answers for older problems

Santosh Kumar Panda1, Manas Kumar Nayak1, Jenith Thangaraj1, Palash Das1, Rishabh Pugalia2
1Department of Pediatrics, Kalinga Institute of Medical Sciences, KIIT University, Bhubaneshwar, Odisha, 2Department of Pediatrics, Kathiar Medical College, Kathihar, Bihar, India

ABSTRACT

Background: Early identification and intervention of neonatal sepsis can improve the clinical outcome. Blood cultures remain the gold standard for diagnosis but are not easily available and require time. There is a need to identify and validate newer easily available cost-effective investigations, which would help in the diagnosis of neonatal sepsis. Aim: To test the hypothesis that whether platelet parameters, i.e., total platelet count (TPC), mean platelet volume (MPV), and the ratio of MPV/TPC can serve as diagnostic markers in neonatal sepsis. Methods: It was a prospective study conducted in a tertiary care neonatal intensive care unit (NICU). The platelet parameters, i.e., TPC, MPV, and MPV/TPC of blood culture-positive septic neonates were compared with those of non-septic neonates admitted to the NICU. The diagnostic accuracy of the platelet indices was assessed by receiver operating characteristics (ROC) curves and sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). Result: During the study period, 43 blood culture-positive sepsis neonates were compared with 54 cases of non-septic neonates. There was a significant difference in the mean of TPC, MPV, and MPV/TPC ratio between septic groups and non-septic groups. The sensitivity, specificity, PPV, NPV values of MPV (cut-off >9 fl) were 63.40%, 53.8%, 52.0%, and 65.11% respectively. The sensitivity, specificity, PPV, NPV of MPV/TPC ratio (>7.2) were 48.8%, 96.22%, 90.9%, and 70.42% respectively. The area under the curve (AUC) values for TPC, MPV, and MPV/TPC in the ROC analysis were 0.797, 0.641, and 0.809, respectively. Conclusion: Platelet indices MPV and MPV/TPC ratio can be useful in the early diagnosis of neonatal sepsis.

Keywords: Mean platelet volume, mean platelet volume/total platelet count, thrombocytopenia

Introduction

Neonatal sepsis is the second-most important cause of neonatal deaths and also a major cause of hospital admissions.[1] Worldwide, approximately one million neonates die annually due to neonatal sepsis and a majority of them belong to low- and middle-income countries.[2] A multitude of non-specific clinical signs and symptoms such as refusal to feed, lethargy, temperature instability, feeding intolerance, hypotension, respiratory distress, convulsions, mottling, and biochemical and hematological abnormalities such as raised C-reactive protein (CRP), abnormal complete blood count (neutropenia, raised immature neutrophils, thrombocytopenia, etc.) increase the suspicion of sepsis.[3] However, similar symptoms can be seen in varying...
other conditions in neonates known as sepsis mimickers. Hence, different parameters of a complete blood count (CBC) may help the family physicians to identify neonatal sepsis in the community, initiate early treatment, and ensure timely referral.

Laboratory-wise isolation of microorganisms from sterile sites, especially blood or cerebrospinal fluid (CSF), urine is considered the gold standard in diagnosing neonatal sepsis. However, the yield of blood culture varies from 39% to 48% in variously reported literature and takes about 48 h to 7 days for an organism to grow.[4–6]

Testing of newer biomarkers from readily available tests and establishing their diagnostic accuracy to improve the precision in an early diagnosis of neonatal sepsis needs to be done. A CBC is one such affordable and readily available investigation. Platelets have been recognized as a key player in inflammation and thrombosis. Mean platelet volume (MPV) is a surrogate marker of platelet activity and is easily available in automated cell counters. In many physiological and pathological states, platelet activation is indirectly measured from MPV. MPV has been reported to have diagnostic and prognostic values in perinatal infections and inflammation.[7,8] In a recent study by Hayato et al.[9] a high MPV was shown to correlate with neonatal mortality in preterm neonates less than 32 weeks of age.

In the present study, the early diagnostic accuracy of platelet parameters, i.e., total platelet count (TPC), MPV, and the ratio of MPV/TPC for neonatal sepsis were studied.

### Materials and Methods

#### Methods

This was a prospective study conducted between January 2019 and January 2020 in a tertiary care neonatology unit. The study protocol was presented to Institutional Ethics Committee and all the data were collected after taking the informed consent of parents. The procedures were followed in accordance with the ethical standards as per the Helsinki Declaration of 1975. All neonates who presented with clinical signs and symptoms, i.e., lethargy, respiratory distress, temperature instability, feed intolerance, hypotension, and seizure suggestive for neonatal sepsis were enrolled in one arm. Neonates admitted in the absence of the above clinical conditions and CBC had been done for part of their evaluation were enrolled in the control arm. Clinical conditions affecting neonatal platelet counts, i.e., neonates with syndromic babies, chromosomal aneuploidy, hydrops fetalis, hypoxic–ischemic encephalopathy, intrauterine growth restriction (IUGR) neonates, neonatal polycythemia, hemolytic anemia, need of exchange transfusion, a past history of platelet transfusion, maternal conditions, i.e., idiopathic thrombocytopenic purpura (ITP), collagen vascular diseases, gestational hypertension, toxoplasma, rubella, cytomegalovirus, herpes (TORCH) infection were excluded from both arms. As per the treatment protocol, for neonates who presented with clinical symptoms and signs suggestive of neonatal sepsis, intravenous antibiotics were initiated after sending CBC, CRP, and blood cultures. Neonates were diagnosed as blood culture-positive sepsis or clinical sepsis based on the presence or absence of isolation of microbes, respectively. Platelet parameters of blood culture-positive septic neonates were compared with the platelet parameters of control neonates.

#### Analysis of sample

Blood was collected by venipuncture in the same sitting in which blood culture was collected. Platelet parameters, such as TPC, MPV of CBC obtained from the Coulter counter (automated analyzer, Beckman Coulter, LH 780, California) were recorded. Blood culture-positive sepsis was diagnosed by Bac T/Alert and VITEK-2 blood culture methods and the organism causing neonatal sepsis was recorded.

#### Statistical analysis

Continuous variables were statistically described in terms of mean and standard deviation (SD) and categorical variables as frequencies (number of cases) and percentages (%). A comparison of platelet indices between sepsis and control groups was done using a $t$-test. Diagnostic accuracy of the platelet indices for neonatal sepsis was assessed by receiver operating characteristics (ROC) curves and sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated. $P$ values < 0.05 were considered statistically significant. All data were analyzed using statistical software STATA 15.1.

#### Results

### General information

A total of 656 neonates were admitted during the study period. Based on inclusion and exclusion criteria, 43 neonates were diagnosed as blood culture-positive sepsis and their platelet parameters were compared with those of 54 cases of non-septic neonates [Figure 1]. The demographic characteristics and clinical conditions of both groups are described in Table 1. Both the groups were comparable for weight and gestational age. There was no significant difference in the gender distribution of the patient or the mode of delivery in the septic and non-septic groups. Among 43 blood culture-positive sepsis, 28 (65.10%) cases were gram-negative sepsis, 12 (27.90%) were gram-positive sepsis, and 3 (7%) cases were fungal sepsis. The clinical findings and isolated microorganisms of septic neonates and indications of CBC of non-septic neonates are shown in Table 2.

### Table 1: Demographic profile of septic and non-septic neonates

| Demographic variables | Sepsis ($n=43$) | Non-septic ($n=54$) | $P$ |
|-----------------------|---------------|---------------------|-----|
| Gestational age (weeks) | 33.29±3.78 | 34.18±4.21 | 0.282 |
| Birth weight (g) | 1950±970 | 1870±869 | 0.668 |
| Mode of delivery (LSCS) | 20 | 22 | 0.568 |
| Sex (male) | 23 | 24 | 0.375 |
Panda, et al.: Platelet parameters in neonatal sepsis

Hematological parameters

Both the groups were compared for the mean ± SD of platelet parameters. Thrombocytopenia (defined as a platelet count of less than 1,50,000 lakhs/mm$^3$) was found in 17 (39.5%) septic neonates and 2 (3.7%) non-septic neonates. There was a significant difference in the mean ± SD of TPC, MPV, and MPV/TPC ratio between septic groups and non-septic groups. The septic group had significantly lower platelet counts (187146.34 ± 118467.41) lakhs/mm$^3$, higher MPV (9.97 ± 1.56) fL, and had a higher ratio of MPV (fL)/TPC (in lakhs) compared with (9.34 ± 2.7) the non-septic group [Table 3].

Diagnostic accuracy of various parameters

The sensitivity, specificity, PPV, NPV values of the above diagnostic parameters using cut-off values are described in Table 4. In this study, the area under the curve (AUC) for TPC, MPV, and MPV/TPC in ROC analysis was 0.797, 0.641, and 0.809, respectively [Figure 2].

Discussion

Early- and late-onset neonatal sepsis have high mortality; the survivors may have many long-term morbidities.[10] Clinical signs and symptoms of neonatal sepsis are nonspecific and also present in many conditions called sepsis mimickers. Overuse of antibiotics is associated with the emergence of antibiotic-resistant pathogens, neonatal gut dysbiosis, and increased risk for necrotizing enterocolitis.[10] Considering the moderate yield of blood culture positivity, the alternative diagnostic test for neonatal sepsis should have higher sensitivity and specificity. Different parameters from a CBC such as MPV, neutrophil-lymphocyte ratio, and red cell distribution width, are being studied as markers of neonatal sepsis.[12] Platelet count is well studied in neonatal sepsis and MPV is upcoming as a sensitive marker in recent studies although it is not utilized in day-to-day bedside neonatal practice. However, there is a significant difference in MPV/TPC ratio between septic neonates (9.34 ± 2.7) and non-septic neonates (3.76 ± 1.2) in this study. The sensitivity, specificity, PPV, and NPV of MPV/TPC ratio at cut-off ≥7.2 was 48.8%, 96.2%, 90.9%, and 70.42%, respectively. The present study explored the test quality of MPV/TPC ratio based on AUC (0.809) in ROC analysis, which is very good. To the best of our knowledge and existing literature search, this is the first study to consider MPV/TPC as a diagnostic test in neonatal sepsis. In this study, both TPC and MPV/TPC had very high specificity (around 96%) and PPV (90%) with a sensitivity of around 40 to 50%, which denotes a lower false positivity rate. In comparison to the above platelet parameters, MPV has higher sensitivity (63%) but lower specificity (53%), suggesting MPV could be raised by other inflammatory conditions. Both maternal and neonatal clinical conditions biasing the platelet counts are excluded from both septic and non-septic arms, this strict adherence of inclusion and exclusion criteria to analyze platelet parameters is the strength of our study.

In this study, 65.10% of cases were gram-negative sepsis, 27.90% were gram-positive sepsis, and 7% cases were fungal sepsis. A similar bacteriological profile of neonatal sepsis was also reported by various studies in the Indian subcontinent.[13-15] The mean TPC of septic neonates was significantly lower compared to that of non-septic neonates. Thrombocytopenia with a TPC < 1.5 lakh/mm$^3$ had sensitivity, specificity, PPV, and NPV of 41.5%, 96.15%, 89.47%, and 67.56%, respectively. The probable pathophysiology is neonatal sepsis-induced endothelial damage and the formation of microthrombi, which lead to the consumption of platelets. The imbalance between the consumption and production from the bone marrow leads to low platelet counts in neonatal sepsis. In a study by Ree et al.[16] the incidence of thrombocytopenia was 49% in their cohort of 460 septic neonates. Brown et al.[17] reported severe thrombocytopenia (TPC counts below 50,000/mm$^3$) associated with neonatal sepsis and necrotizing enterocolitis. Thrombocytopenia has also been described as a reliable early diagnostic marker in necrotizing enterocolitis.[18]
Guida et al. reported that the mean MPV increased by 0.30 fL (95% confidence interval [CI]: 0.12—0.47) from baseline during diagnosis of blood culture-positive sepsis in very low birth weight neonates. In a meta-analysis incorporating 11 studies on 932 septic neonates, the MPV was significantly higher among septic neonates compared to healthy neonates. Mohsen et al. reported that with MPV >10.2 fL, the sensitivity, specificity, PPV, and NPV for diagnosis of neonatal sepsis were 71%, 63%, 74%, and 59%, respectively. In this study, on using a cut-off of MPV >9 fL, the sensitivity, specificity, PPV, and NPV for diagnosis of neonatal sepsis were 63.4%, 53.8%, 52.0%, and 65.11%, respectively.

Catal et al. found a positive correlation between MPV and CRP in neonatal sepsis. At MPV cut-off value of 10.35 fL for diagnosis of neonatal sepsis, the sensitivity was 97.8% and the specificity was 78.7% (AUC = 0.949; \( P < 0.001 \)). Increased turnover of platelets leads to the release of younger platelets. The megakaryocyte thrombopoietin levels are in a dynamic state during neonatal sepsis, which explained the pathological changes in platelet indices. The younger platelets are larger in size and more granular and hence there is an increase in the MPV. Becchi et al. found a negative correlation (\( r = -0.34; P < 0.001 \)) between TPC and MPV in septic patients. MPV is an economical and statistically significant marker of neonatal sepsis with mean MPV of 9.56 fL and 8.58 fL among blood culture-positive septic and non-septic neonates, respectively.

A recent meta-analysis was conducted by Gerasimos-Panagiotis et al. to determine the diagnostic accuracy of MPV in neonatal sepsis. They found a sensitivity of MPV in neonatal sepsis found to be around 0.675 (95% CI: 0.536—0.790) and specificity of 0.733 (95% CI: 0.589—0.840), respectively, at an optimal cut-off point of 9.28 fL.
The ratio of MPV to TPC has been tested in several inflammatory and septic conditions such as pneumonia and bacterial sepsis. MPV/TPC ratio is a better predictor than MPV or TPC alone for mortality among septic adult patients. In a study on 120 adult septic patients, a higher MPV/TPC ratio with cut-off >3.71 on admission was a significant risk factor for 28-day mortality (AUC = 0.81; \( P = 0.001 \)). In another study by Djordjevic et al. on 392 critically ill patients admitted in the surgical ICU found that MPV/TPC ratio offered no advantage over platelet count or MPV alone. In the ROC analysis of platelet indices in this study, the AUC of MPV/TPC (0.809) was better than that of MPV (0.641) and TPC (0.797). We found that the ratio of MPV/TPC had better diagnostic performance than individual MPV and TPC for distinguishing septic and non-septic neonates. MPV/TPC ratio is an upcoming biomarker in neonatal sepsis and shows promising results as per our analysis. There is sparse literature on MPV/TPC ratio as a diagnostic test in neonatal sepsis and the authors recommend that this ratio needs further studies as a diagnostic marker.

### Table 2: Clinical conditions of septic and non-septic neonates with isolated microorganisms of septic neonates

| Clinical presentation of septic neonates | Total no (43) | Non-septic neonates | Total no (54) |
|-----------------------------------------|--------------|---------------------|--------------|
| Respiratory distress                    | 31           | Hyperbilirubinemia  | 13           |
| Mechanical ventilation                  | 17           | Transient tachypnea of new born | 11 |
| Shock                                   | 25           | LGA/hypoglycemia    | 3            |
| Feed intolerance                        | 13           | Asymptomatic preterm neonates, (Gestational age <34 weeks) | 13 |
| Meningitis                              | 6            | Asymptomatic preterm neonates, (Gestational age >34 weeks) | 11 |
| Fever                                   | 3            | Meconium stain liquor | 3 |

| Isolated microorganisms from blood culture |
|--------------------------------------------|
| Lombia pneumoniae (n=14)                   |
| Escherichia coli (n=7)                     |
| Acinetobacter baumannii (n=4)              |
| Burkholderia cepacia (n=3)                 |
| Gram-positive sepsis (n=12)                |
| Staphylococcus aureus (n=3)                |
| Staphylococcus haemolyticus (n=6)          |
| Staphylococcus epidermidis (n=3)           |
| Fungal sepsis (n=3)                        |
| Candida albicans (n=2)                     |
| Candida krusei (n=1)                       |

### Table 4: Comparison of hematological parameters of CBC and CRP between sepsis and non-septic neonates

| Hematological parameters | Sepsis (n=43) | Non-septic (n=54) | \( P \) |
|--------------------------|---------------|-------------------|-------|
| TPC (cell/mm\(^3\))      | 187146.34±118467.41 | 285192.30±109704.24 | <0.001 |
| MPV (in fl)               | 9.97±1.56     | 9.22±1.44         | 0.019 |
| MPV/TPC                   | 9.34±2.7      | 3.76±1.2          | <0.001 |

### Table 3: Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) of CRP, TPC, MPV, and MPV/TPC ratio

| Diagnostic test | Cut-off point | Sensitivity | Specificity | PPV | NPV |
|-----------------|---------------|-------------|-------------|-----|-----|
| TPC             | <150,000 cells/mm\(^3\) | 41.5%      | 96.15%      | 89.47% | 67.56% |
| MPV             | ≥9 fl         | 63.4%      | 53.8%       | 52.0% | 65.11% |
| MPV/TPC         | ≥7.2         | 48.8%      | 96.2%       | 90.9% | 70.42% |

*For easier interpretation of MPV/TPC ratio, the value was calculated as MPV (fl)/TPC (in lakhs)*

The ratio of MPV to TPC has been tested in several inflammatory and septic conditions such as pneumonia and bacterial sepsis. MPV/TPC ratio is a better predictor than MPV or TPC alone for mortality among septic adult patients. In a study on 120 adult septic patients, a higher MPV/TPC ratio with cut-off >3.71 on admission was a significant risk factor for 28-day mortality (AUC = 0.81; \( P = 0.001 \)). In another study by Djordjevic et al. on 392 critically ill patients admitted in the surgical ICU found that MPV/TPC ratio offered no advantage over platelet count or MPV alone. In the ROC analysis of platelet indices in this study, the AUC of MPV/TPC (0.809) was better than that of MPV (0.641) and TPC (0.797). We found that the ratio of MPV/TPC had better diagnostic performance than individual MPV and TPC for distinguishing septic and non-septic neonates. MPV/TPC ratio is an upcoming biomarker in neonatal sepsis and shows promising results as per our analysis. There is sparse literature on MPV/TPC ratio as a diagnostic test in neonatal sepsis and the authors recommend that this ratio needs further studies as a diagnostic marker.

### Summary

Low TPC, high MPV, and a high ratio of MPV/TPC are sensitive markers of neonatal sepsis, which, when used in conjunction with typical clinical history, can be used as early diagnostic markers of neonatal sepsis. The diagnostic performance of MPV/TPC is better than that of TPC or MPV alone in neonatal sepsis.

### Conclusion

Neonatal sepsis is an important health problem. Neonatal sepsis needs early diagnosis and early initiation of treatment to have a better outcome. Complete blood count is an easily available investigation to primary care physicians who are the first contact physicians for most of the cases with neonatal sepsis. A CBC along with clinical symptoms and signs, varying parameters of CBC can prove to be an invaluable tool in diagnosing neonatal sepsis, especially to the primary care physician. It needs to be accurately diagnosed and earlier intervention is required to prevent complications. Apart from TPC, MPV, and MPV/TPC ratio are new biomarkers that can increase the diagnostic accuracy of neonatal sepsis. CBC parameters such as low TPC, high MPV, and high MPV to TPC ratio at designated cut-off values serve as important diagnostic markers when used alone or together. To conclude, all neonates presenting with signs and symptoms of neonatal sepsis should have at least a CBC done, along with other markers of sepsis. Platelet parameters, i.e., TPC, MPV, and MPV/TPC ratio should also be utilized for early diagnosis of neonatal sepsis.
Limitations
There were a few limitations of this study. It was conducted in a single neonatal care unit with relatively small sample size. The serial follow-up of platelet parameters in sepsis neonates was not studied. The prediction of mortality in neonatal sepsis based on initial platelet parameters or serial follow-up platelet parameters needs further evaluation. Hence, a prospective multicentric study involving a larger sample size considering neonatal and perinatal factors should be incorporated to improve the strength of future studies.

Declaration of patient consent
The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

References
1. GBD 2015 Mortality and Causes of Death Collaborators. Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980-2015: A systematic analysis for the Global Burden of Disease Study 2015. Lancet 2016;388:1459-544.
2. Fleischmann-Struzek C, Goldfarb DM, Schlattmann P, Schlapbach LJ, Reinhart K, Kissoon N. The global burden of paediatric and neonatal sepsis: A systematic review. Lancet Respir Med 2018;6:223-30.
3. Shane AL, Sánchez PJ, Stoll BJ. Neonatal sepsis. Lancet 2017;390:1770-80.
4. Shaaban HA, Saﬁwat N. Mean platelet volume in preterm: A predictor of early onset neonatal sepsis. J Matern Fetal Neonatal Med 2020;33:206-11.
5. Aydemir C, Aydemir H, Kokturk F, Kulah C, Mungan AG. The cut-off levels of procalcitonin and C-reactive protein and the kinetics of mean platelet volume in preterm neonates with sepsis. BMC Pediatrics 2018;18:253.
6. Omran A, Maaroof A, Saleh MH, Abdelwahab A. Salivary C-reactive protein, mean platelet volume and neutrophil lymphocyte ratio as diagnostic markers for neonatal sepsis. J Pediatr (Rio J) 2018;94:827.
7. Cekmez F, Tanju IA, Canpolat YE, Aydinoz S, Aydemir G, Karademir F, et al. Mean platelet volume in very preterm infants: A predictor of morbidities? Eur Rev Med Pharmacol Sci 2013;17:134-7.
8. Wang J, Wang Z, Zhang M, Lou Z, Deng J, Li Q. Diagnostic value of mean platelet volume for neonatal sepsis: A systematic review and meta-analysis. Medicine (Baltimore) 2020;99:e21649.
and neonatal sepsis: A systematic review and meta-analysis of diagnostic accuracy. J Matern Fetal Neonatal Med 2021;4:1-13.

27. Oh GH, Chung SP, Park YS, Hong JH, Lee HS, Chung HS, et al. Mean Platelet volume to platelet count ratio as a promising predictor of early mortality in severe sepsis. Shock 2017;47:323-30.

28. Djordjevic D, Rondovic G, Surbatovic M, Stanojevic I, Udovicic I, Andjelic T, et al. Neutrophil-to-lymphocyte ratio, monocyte-to-lymphocyte ratio, platelet-to-lymphocyte ratio, and mean platelet volume-to-platelet count ratio as biomarkers in critically ill and injured patients: Which ratio to choose to predict outcome and nature of bacteremia? Mediators Inflamm 2018;2018:3758068.