e-ISSN: 0975-5160, p-ISSN: 2820-2651

Available online on www.ijtpr.com

International Journal of Toxicological and Pharmacological Research 2023; 13(10); 250-254

Original Research Article

A Hospital Based Prospective Assessment of Platelet Parameters as a Diagnostic Marker in Early Diagnosis of Neonatal Sepsis

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Received: 03-08-2023 / Revised 10-09-2023 / Accepted 12-10-2023
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Conflict of interest: Nil

Abstract:

Aim: The aim of the present study was to evaluate the platelet parameters as a diagnostic marker in early diagnosis of neonatal sepsis.

Methods: This was a prospective study conducted in the Department of Pediatrics, for the period of one year. A total of 620 neonates were admitted during the study period. Based on inclusion and exclusion criteria, 50 neonates were diagnosed as blood culture-positive sepsis and their platelet parameters were compared with those of 60 cases of non-septic neonates.

Results: 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 50 blood culture-positive sepsis, 33 (66%) cases were gram-negative sepsis, 14 (28%) were gram-positive sepsis, and 3 (6%) cases were fungal sepsis. Both the groups were compared for the mean ± SD of platelet parameters. 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 (187123.37 ± 118487.41) lakhs/mm3, higher MPV (9.91 ± 1.54) fL, and had a higher ratio of MPV (fL)/TPC (in lakhs) compared with (9.25 ± 1.44) the non-septic group. The sensitivity, specificity, PPV, NPV values of MPV (cut-off >9 fL) were 64.60%, 54.6%, 52.0%, and 65.15% respectively. The sensitivity, specificity, PPV, NPV of MPV/TPC ratio (>7.2) were 48.8%, 96.40%, 91.9%, and 70.44% respectively.

Conclusion: 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.

Keywords: Mean Platelet Volume, Mean Platelet Volume/Total Platelet Count, Thrombocytopenia.

Introduction

The first month of life is the most vulnerable period of child survival, with 2.4 million newborn dying in 2020. Sub-Saharan Africa has the highest neonatal mortality rate in the world (27 deaths per 1000 live birth) with 43% of global newborn deaths, followed by Central and Southern Asia (23 deaths per 1000 live birth), with 36% of global newborn deaths. [1] With the birth of 25 million children each year India accounts for nearly one fifth of the worlds annual child birth. Every minute one of those babies dies. Prematurity (35 per cent), neonatal infections (33 per cent), birth asphyxia (20 per cent)and congenital malformations (9 per cent) are among the major cause of newborn deaths. [2] Neonatal septicemia is considered one of the leading cause of neonatal mortality globally, more in developing countries like India. [3] Septicemia in neonates is a blood infection that can lead to sepsis which is a clinical syndrome of bacteremia characterized by systemic signs and symptoms of infection in less than 28 days of life. [4]

Neonatal sepsis has been a leading cause of high morbidity and mortality in newborns and is recognized as a global health challenge. [5,6] The incidence of neonatal sepsis in India was 30/1000, as per the neonatal perinatal database (NNPD). [7] The total neonatal mortality rate was 28/1000 live birth of which one third proportion of deaths occur due to sepsis. [8] It is very hard to find out any particular rapid and cost effective hematological test or specific clinical signs to confirm sepsis in neonates. Although it is very requisite to diagnose early a case of neonatal sepsis to initiate early specific treatment as delay in treatment will significantly increase the case fatality and serious morbidity and early initiation of empirical therapy

without diagnosis will lead to serious antibiotic resistance in some cases. Blood culture is the gold standard for diagnosis of neonatal sepsis but it is costly and requires 48-72 hours to get a positive result. Moreover, it is positive only in 35-75% of septic cases and culture become negative in some spurious organisms. [9,10] There is no ideal tests or combination of tests that will definitely point towards diagnosis of sepsis. [11,12]

Platelet production climbs at the beginning of septicaemia due to platelet destruction, and larger and younger platelets are secreted to the peripheral blood. However, bone marrow is repressed subsequently, and thrombocytopenia is seen. [13] Elevated MPV indicates endothelial damages as well as platelet activation. Thrombocyte consumption and MPV values escalate in acute infections. [14] Platelet–large cell ratio (P-LCR) (normal less than 30%) indicates the proportion of platelets greater than 12fl in the circulation in regard to total platelet count which also increased in septicemia due to same cause. [15] MPV/TPC is another important platelet parameter (normal ratio up to 7.2%) which is increased in platelet activation. These platelet parameters can be easily measured by routine blood count analyzer which is rapid and cost effective. There are different studies about platelet parameters in neonatal sepsis which showed beneficial results in most of the studies. [16,17]

The aim of the present study was to evaluate the platelet parameters as a diagnostic marker in early diagnosis of neonatal sepsis.

Materials and Methods

This was a prospective study conducted in the Department of Pediatrics, SKMCH, Muzaffarpur, Bihar, India for the period of one year. A total of 620 neonates were admitted during the study period. Based on inclusion and exclusion criteria, 50 neonates were diagnosed as blood culture-positive sepsis and their platelet parameters were compared with those of 60 cases of non-septic neonates. 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 was 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/AIert 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

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

Demographic variables	Sepsis (*n*=50)	Non-septic (*n*=60)	*P*
Gestational age (weeks)	32.28±3.77	33.17±4.26	0.289
Birth weight (g)	1945±960	1865±862	0.675
Mode of delivery (LSCS)	23	28	0.578
Sex (male)	28	29	0.314

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.

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

Clinical presentation of septic neonates	Total (50)	Non‑septic neonates	Total (60)
Respiratory distress	33	Hyperbilirubinemia	15
Mechanical ventilation	18	Transient tachypnea of new born	12
Shock	26	LGA/hypoglycemia	4
Feed intolerance	15	Asymptomatic preterm neonates, (Gestational age <34 weeks)	14
Meningitis	7	Asymptomatic preterm neonates, (Gestational age >34 weeks)	12
Fever	4	Meconium stain liquor	3
Isolated microorganisms from blood culture Gram-negative Sepsis (n=33) *Klebsiella pneumoniae* (n=16) *Escherichia coli* (n=8) *Acinetobacter baumannii* (n=5) *Burkholderia cepacia* (n=4) Gram-positive sepsis (n=14) *Staphylococcus aureus* (n=4) *Staphylococcus haemolyticus* (n=7) *Staphylococcus epidermidis* (n=3) Fungal sepsis (n=3) *Candida albicans* (n=2) *Candida krusei* (n=1)			

Among 50 blood culture‑positive sepsis, 33 (66%) cases were gram‑negative sepsis, 14 (28%) were gram‑positive sepsis, and 3 (6%) cases were fungal sepsis.

Table 3: Comparison of hematological parameters of CBC and CRP between sepsis and non‑septic neonates

Hematologicalparameters	Sepsis (*n*=50)	Non‑septic (*n*=60)	*P*
TPC (cell/mm^3)	187123.37±118487.41	285184.30±109708.24	<0.001
MPV (in fL)	9.91±1.54	9.25±1.44	0.012
MPV/TPC	9.36±2.7	3.76±1.2	<0.001

Both the groups were compared for the mean ± SD of platelet parameters. 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 (187123.37 ± 118487.41) lakhs/mm3, higher MPV (9.91 ± 1.54) fL, and had a higher ratio of MPV (fL)/TPC (in lakhs) compared with (9.25 ± 1.44) the non‑septic group.

Table 4: Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) of CRP, TPC, MPV, and MPV/TPC ratio

Diagnostictest	Cut‑off point	Sensitivity	Specificity	PPV	NPV
TPC	<150,000 cells/mm^3	42.8%	95.15%	88.48%	68.52%
MPV	(≥9fL)	64.6%	54.6%	52.0%	65.15%
MPV/TPC	(≥7.2)	48.8%	96.4%	91.9%	70.44%

The sensitivity, specificity, PPV, NPV values of MPV (cut‑off >9 fL) were 64.60%, 54.6%, 52.0%, and 65.15% respectively. The sensitivity, specificity, PPV, NPV of MPV/TPC ratio (>7.2) were 48.8%, 96.40%, 91.9%, and 70.44% respectively.

Discussion

Neonatal sepsis is the second‑most important cause of neonatal deaths and is also a major cause of hospital admissions. [18] Worldwide, approximately one million neonates die annually due to neonatal sepsis and a majority of them belong to low‑ and middle‑income countries. [19] 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. [20] 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. [21]

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 50 blood culture-positive sepsis, 33 (66%) cases were gram-negative sepsis, 14 (28%) were gram-positive sepsis, and 3 (6%) cases were fungal sepsis. 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. [22] 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. [23] 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.

Both the groups were compared for the mean ± SD of platelet parameters. 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 (187123.37 ± 118487.41) lakhs/mm3, higher MPV (9.91 ± 1.54) fL, and had a higher ratio of MPV (fL)/TPC (in lakhs) compared with (9.25 ± 1.44) the non-septic group. The sensitivity, specificity, PPV, NPV values of MPV (cut-off >9 fL) were 64.60%, 54.6%, 52.0%, and 65.15% respectively. The sensitivity, specificity, PPV, NPV of MPV/TPC ratio (>7.2) were 48.8%, 96.40%, 91.9%, and 70.44% 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 [24] the incidence of thrombocytopenia was 49% in their cohort of 460 septic neonates. Brown et al [25] reported severe thrombocytopenia (TPC counts below 50,000/ mm3) associated with neonatal sepsis and necrotizing enterocolitis. Thrombocytopenia has also been described as a reliable early diagnostic marker in necrotizing enterocolitis. [26] The younger platelets are larger in size and more granular and hence there is an increase in the MPV. [27,28] Becchi et al [29] 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. [30] A recent meta-analysis was conducted by Gerasimos- Panagiotis et al. [31]

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 with various parameters is an easily available investigation. 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.

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