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ORIGINAL ARTICLE

Neonatal Platelet Parameters as Early Markers for Diagnosis of Neonatal Sepsis

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Abstract

Background: Sepsis in the newborn population is one of the major cause of mortality and morbidity globally. Clinical symptoms and signs give clue for diagnosis of neonatal infection along with various diagnostics methods. This study aims to evaluate neonatal platelet parameters as early and inexpensive tool for diagnosing neonatal sepsis. **Methods**: This was a retrospective study, where 60 neonates with symptoms and signs of sepsis were enrolled and equally distributed into group 1 as neonates whose blood culture was sterile and septic screen was negative and group 2 as the ones with blood culture positive bacteremia. In enrolled neonates with suspected sepsis, before starting antibiotics, septic screen, platelets parameters were noted and MPV/TPC ratio was calculated. Clinical characteristics like need for vasopressor, need of ventilation and duration of NICU stay along with final outcome during hospitalisation were also noted. **Results:** The culture proven sepsis group had total platelet count significantly on lower side of normal (1.94 ± 0.70) and high MPV/TPC ratio (4.91 ± 2.14) along with increased need for vasopressor, ventilation and prolonged stay in NICU (*P*-value of 0.001). The cut off value ≥5.032 of MPV/TPC ratio had specificity of 96.67%, PPV of 92.31%, sensitivity of 40%, NPV of 61.7% and diagnostic accuracy of 68.33%. **Conclusion**: This study concludes that the investigative utility of MPV/TPC surpasses that of TPC or MPV alone in offering enhanced accuracy in early diagnosis of neonatal sepsis.

Keywords: Neonatal sepsis, neonatal platelet parameters, thrombocytopenia, MPV, MPV/TPC ratio

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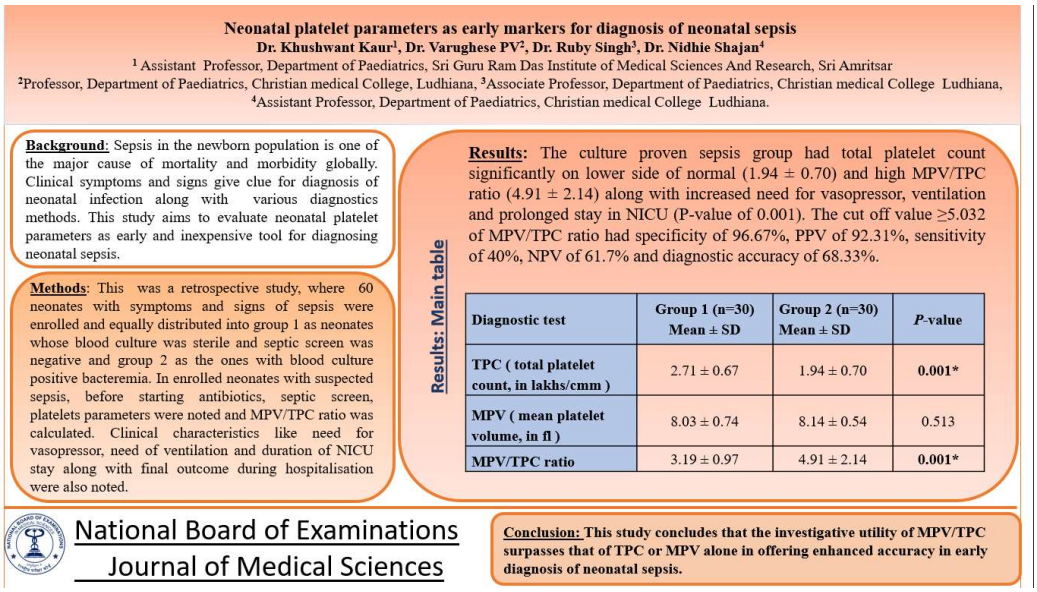
Graphical Abstract



Introduction

Sepsis neonatorum is one of the major causes of morbidity and mortality globally, more in India and in other developing countries. As per the National Family Health Survey 2019-2021 (NFHS-5), the current neonatal mortality rate (NMR) is 24.9 per 1000 live births [1]. The Delhi Neonatal Infection Study (DeNIS) collaboration reported the incidence of 6.2% in culture-positive sepsis and 14.3% in total (culture-positive or culture-negative) sepsis among all NICU admissions [2].

Symptoms and signs like lethargy, poor feeding, vomiting, skin pustules, diarrhoea, dehydration, umbilical discharge, fever, apnoea, hypothermia, tachypnoea, grunting, apnea, bradycardia, tachycardia, cyanosis, retractions, hypothermia, hypoglycemia, mottling, shock, abdominal distension, feed intolerance, seizures and sclerema may harbour reservations about early onset sepsis (EOS). As these mentioned findings are not specific for EOS, non infective causes may also present in a similar manner [3].

Neonatal sepsis is a serious, invasive and life threatening infection requiring expeditious therapy. In this condition, pathogens (viral, bacterial or fungal) enter the blood stream and lead to production of various toxins that further evoke a systemic inflammatory response (SIR) [4]. This leads to endothelial cell damage causing adhesion and aggregation of platelets. These toxins or pathogens can binds to platelets directly and then cause aggregation and dispatch of platelets from the circulation [5]. As a result there is destructive thrombocytopenia as platelets are rapidly expedited from the circulation. This prompts the production and release of newer platelets of variable sizes into the circulation.

The platelets when activated, release various inflammatory mediators and cytokines, that regulate inflammation and immune response in the body [6,7]. The cytokines like tissue factors when released in circulation act on coagulation system leading to over-activation and serious complications such as hypoxia followed by ischemia and disseminated intravascular coagulation (DIC) which can finally result in multiple organ dysfunction syndrome {MODS}[8,9].

The mean platelet volume (MPV) is considered a marker of platelet size, function reactivity and average volume of individual platelets [10]. An elevated MPV denotes the activity of the platelets, which can augment the early thrombus formation, followed by aggregation, adhesion and increased risk of severe complications, multiple organ dysfunction syndrome and death [11].

For diagnosing neonatal sepsis, detailed history for signs and symptoms, clinical examination, conventional diagnostic methods (isolation of pathogens from body fluids by cultures) and other diagnostic tests (including CRP, micro-ESR, WBC count, Absolute neutrophil count and IT ratio) are the mainstream methods [11].

Although it is time consuming but still blood culture is the gold standard method for diagnosis of infection in the body. Other limitation is positive blood cultures seen in limited number of cases only [12].

Since there is no ideal test or battery of tests that will help in definite diagnosis of sepsis hence it is imperative to identify a diagnostic test which is feasible, reliable and early, at the same time cost effective with acceptable sensitivity and specificity.

Several studies have been done on the functionality of platelet parameters in neonatal sepsis and have shown promising results [4,13-16]. These platelet parameters {total platelet count (TPC), mean platelet volume (MPV)} can be easily measured by rapid and cost effective routine blood count analyzer. MPV/TPC ratio is another promising platelet parameter that increases in platelet activation.

This study aims to find out the utility of neonatal total platelet count (TPC), mean platelet volume (MPV) and MPV/TPC ratio as an early and economical tool for diagnosing neonatal sepsis.

Material and Methods

This was a single Centre Retrospective descriptive study conducted in the Neonatology unit of a tertiary care hospital in North Western India. After institutional ethics committee clearance (IECBMHR/202401-006), 60 neonates of gestational age ≥34 completed weeks, admitted from January 1st 2022 to December 31st 2023 with suspected sepsis were included in study. Neonates with Major congenital anomalies, initiation of antibiotics prior to sending septic screen, incomplete records and

those born to mothers with immune thrombocytopenia (ITP) were excluded from the study. All the study participants enrolled were categorised into two groups. Group 1 included neonates with sterile/negative blood culture growth, negative sepsis screen and group 2 included neonates who had blood culture positive for sepsis. The patient records were retrieved from the medical records department. Data such as gestational age, birth weight, mode of delivery, gender, neonatal clinical characteristics during hospitalization like need of vasopressor, need of ventilation and duration of NICU stay along with final outcome were recorded. Neonatal septic screen {complete blood count (CBC), C-reactive protein (CRP) and blood culture as per institutional protocol} sent before starting antibiotics was also recorded.

For CBC, machine used was Beckman Coulter DxH800 which is based on the Coulter principle and Impedence method. Different organisms were isolated by Bactec blood culture.

All neonatal platelet parameters in CBC were noted and MPV/TPC ratio was calculated. The interpretation of neonatal MPV/TPC ratio was made easier by calculating it as "MPV(fl)/TPC (in lakhs/cmm)".

Statistical analysis

Results of both the groups were compared by standard statistical method. The data was analyzed using IBM SPSS software (ver. 26.0). The normality of the data was examined with the Kolmogorov–Smirnov test. Chi square test was conducted to compare categorical variables. The independent t-test was used to compare various platelet parameters of group 1 and 2. The maximum product of the sensitivity and specificity from the ROC analysis was used to determine the cut-off values. ANOVA has been used to compare the outcomes of various platelet parameters (TPC, MPV and MPV/TPC ratio) in case of expired neonates. Quantitative data was reported as mean ± standard deviation. Categorical data was presented as frequency (percentage). *P* value of less than 0.05 was considered statistically significant.

Results

This study was a retrospective cohort that included 60 neonates with signs and symptoms of sepsis. All the neonates enrolled were categorised into two groups. Group 1 included neonates with sterile blood culture, negative sepsis screen and group 2 included neonates with blood culture-positive sepsis.

The study groups were homogenous with respect to demographic profile in terms of gender, gestation, birth weight, mode of delivery, duration of rupture of membranes and presence of meconium stained liquor. No statistically significant difference was noted in distribution of these variables (*P*-value ≥.05). The details are mentioned in Table 1 below.

Table 1. Details of the demographic profile of study population

Demographic Details		Total n=60	Group 1 (Sterile blood Culture, Negative Sepsis Screen) n=30 N (%)	Group 2 (Blood Culture Positive Sepsis) n=30 N (%)	*P*-value
Gender	Males	31	16 (53.33)	15 (50)	0.796
	Females	29	14 (46.67)	15 (50)	
Gestation (in completed weeks)	34-37	26	16 (53.3)	10 (33.33)	0.177
	>37	34	14 (46.67)	20 (66.67)	
Birth Weight (in kg)	<1.5	3	0	3 (10)	0.168
	1.5-2.5	30	17 (56.67)	13 (43.33)	
	>2.5	27	13 (43.33)	14 (46.67)	
Mode Of Delivery	NVD	10	5 (16.67)	5 (16.67)	0.600
	LSCS	49	25 (83.33)	24 (80)	
	ABD	1	0	1 (3.33)	
Duration Of Rupture Of Membranes	I/O	41	19 (63.33)	22 (73.33)	0.227
	<24hrs	13	6 (20)	7 (23.33)	
	>24hrs	6	5 (16.67)	1 (3.33)	
Meconium Stained Liqour	Yes	25	12 (40)	13 (43.33)	0.793
	No	35	18 (60)	17 (56.67)	

A significant difference was observed between the two groups in terms of increased need for vasopressor and ventilation, prolonged stay in NICU and amplified mortality in blood culture positive sepsis group. Among the

neonates who had blood culture positive sepsis, 63.33% required vasopressor, 73.33% required ventilation, all were admitted in NICU for more than 72 hours and 20% had expired (*P*-value = 0.001). The details are mentioned in Table 2.

Table 2. Clinical characteristics of neonates during hospitalization

Clinical Characteristics During Hospitalisation		Total n=60	Group 1 n=30 N (%)	Group 2 n=30 N (%)	*P* – value
Requiring Vasopressor	No	41	30 (100)	11 (36.67)	0.001
	Yes	19	0	19 (63.33)	
Requiring Ventilation	No	38	30 (100)	8 (26.67)	0.001
	Yes	22	0	22 (73.33)	
Duration Of NICU Stay	<48 Hr	4	4 (13.33)	0	0.001
	48-72 Hr	11	11 (36.67)	0	
	>72 Hr	45	15 (50)	30 (100)	
Outcome	Discharge	42	28 (93.33)	14 (46.67)	0.001
	Dama	12	2 (6.67)	10 (33.33)	
	Expired	6	0	6 (20)	

Among 30 blood culture positive sepsis, 66.67% were gram negative sepsis {E.coli (30%), Acinetobacter (30%), Enterobacter (30%), Burkholderia (5%), Klebsiella (5%)}, 23.33% had gram positive sepsis {MRCONS (42.86%), CONS (42.86%), MRSA (14.28%)} and 10% had fungal sepsis {Candida (66.67%), Candida NA (33.3%)}. Among 6 blood culture positive sepsis neonates who expired 50% had gram negative sepsis {E.coli, Acinetobacter, Klebsiella (33.33% each)}, 33.33% had gram positive sepsis {CONS, MRSA (50% each)} and 16.67 % had fungal sepsis (Candida).

As shown in the Table 3, there was no statistically significant difference between two groups with respect to haemoglobin levels, total leucocyte count, percentage of Neutrophil, Lymphocyte, Monocyte and Eosinophil (*P*-value ≥0.05). There is significant difference in the mean of TPC and MPV/TPC ratio between the

two groups. The blood culture proven sepsis group had significantly lower platelet counts (1.94 ± 0.70) and higher MPV/TPC ratio (4.91 ± 2.14) than group 1.

Table 3. Comparison of haematological parameters of CBC between group 1 and group 2.

Diagnostic test	Group 1 (n=30) Mean ± SD	Group 2 (n=30) Mean ± SD	*P*-value
Hemoglobin (g/dl)	17.28 ± 2.32	17.26 ± 2.62	0.975
TLC (total leucocyte count, cells/cmm)	14950.0 ± 4759.15	14273.0 ± 5.88.79	0.597
Neutrophil (%)	60.50 ± 13.1	67.1 ±13.26	0.057
Lymphocyte (%)	31.80 ±11.7	27.47 ±11.33	0.15
Monocyte (%)	2.73 ± 1.62	2.27 ± 1.76	0.289
Eosinophil (%)	3.53 ±4.71	1.43 ±1.59	0.024
TPC (total platelet count, in lakhs/cmm)	2.71 ± 0.67	1.94 ± 0.70	0.001
MPV (mean platelet volume, in fl)	8.03 ± 0.74	8.14 ± 0.54	0.513
MPV/TPC ratio	3.19 ± 0.97	4.91 ± 2.14	0.001

The receiver operator curve characteristics (ROC) analysis for comparing the accuracy of various parameters of the platelets for neonatal sepsis is shown in Figure 1 and Table 4. The area under the curve for total platelet count (TPC), mean platelet volume (MPV) and MPV/TPC ratio was 0.784, 0.573 and 0.764 respectively. There difference in the mean ± SD of TPC (1.47 ± 0.68) and MPV/TPC ratio (6.61 ± 2.66) of expired neonates who had blood culture positive sepsis was significant.

Table 4. ROC analysis of platelet parameters for neonatal sepsis.

Diagnostic test	AUC (95% CI)	Cut-off point	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Diagnostic Accuracy (%)	*P*-value
TPC (Lakhs/cmm)	0.784 (0.672, 0.897	≤1.5	36.67	96.67	91.67	60.42	66.67	0.001
MPV(fL)	0.573 (0.425, 0.721)	≥7.850	63.33	56.67	28.89	62.22	68.33	0.333
MPV/TPC ratio	0.764 (0.647, 0.882)	≥5.032	40	96.67	92.31	61.7	68.33	0.001

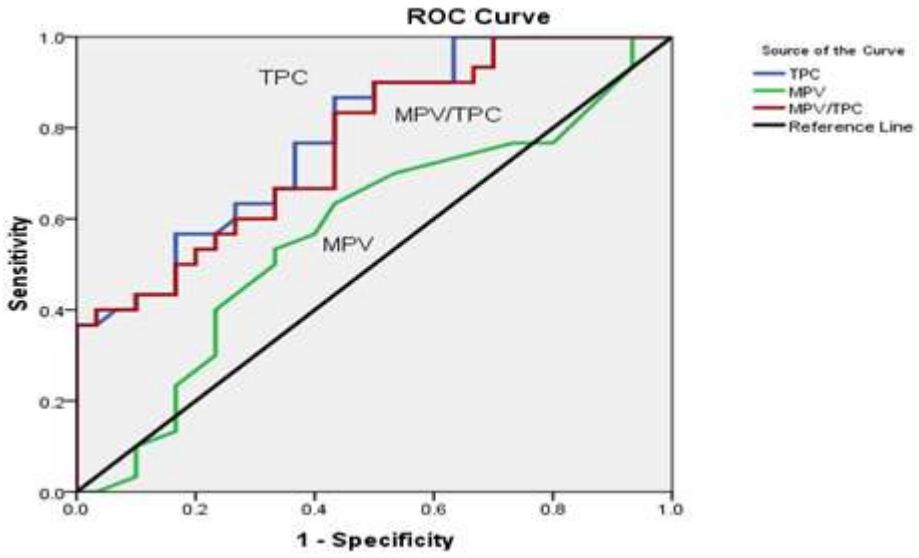


Figure 1. ROC curve analysis of TPC, MPV and MPV/TPC ratio for neonatal sepsis.

Discussion

Sepsis neonatorum being a fatal infection, remains a top reason of morbidity and mortality in newborns. Timely detection and management is pivotal to prevent consequences associated with bacteremia in newborns and improve impacts yet diagnosing it poses a big challenge. In our study 66.67% of newborns had blood culture positive for gram negative organisms, 23.33% had blood culture positive for gram positive organisms and 10% had fungal sepsis. Profile of bacteria, similar to our study, have been reported in various other studies [15,17,18].

Numerous studies have shown variable results for various platelet variables including TPC, MPV [4,10,11,13,15,16], platelet distribution width {PDW}[13,16,19,24] and platelet large cell ratio {P-LCR} [24].

Platelet count unfolds as a pivotal parameter for infection in newborns, often manifesting with a lower platelet count in severe cases. Our study showed that 91.67% of neonates who had culture positive bacteremia, had total platelet count below 1.5Lakhs/cmm. Our findings were comparable with the study done by Choudhary [19] where the incidence of thrombocytopenia was noted among 81.12% of the septicemic neonates. A study by Choudhary [16] found 64.7% neonates with culture proven neonatal septicemia had thrombocytopenia. The TPC cut off scale of 1.5lakh/cmm was further validated by our study, showing that among 12 neonates with this threshold, 91.67% neonates had culture positive bacteremia which was statistically significant (*P*-value 0.0001). There are various other studies that have affirmed thrombocytopenia as an important indicator of neonatal infection [14,20,21,22].

Mean platelet volume (MPV) is a measure of average size of platelets in blood which increases during platelet activation. In our study, the cut off value of MPV ≥7.850fl revealed a diagnostic accuracy of 68.33% but was found to be statistically insignificant (*P*-value ≥0.05). However out of 30 neonates with bacteremia, 63.33% had MPV values above the cut off value. Findings similar to our study were observed in a study by Karne [15] where MPV was increased in neonates with proven sepsis but did not show any relation between sepsis and MPV. Choudhary [19], Panda [23] and Bagchi [24] had higher MPV cut off values >10.8 fl, ≥9 fl and >10.5fl respectively {(*P*-value <0.0001)} as compared to our study. The sensitivity (63.33%) in our study was comparable to that reported by Panda [23] (63.4%) inferring that upper value of MPV may also occur in other systemic inflammatory conditions.

The MPV/TPC ratio has been studied in pneumonia and bacterial infection to identify platelet dysfunction, activation and consumption indicative of sepsis and inflammation in the body. In our study, a high MPV/TPC ratio (4.91 ± 2.14) was noted in neonates with bacteremia and those who had expired (6.61 ± 2.66) with significant *P*-value. At cut off value ≥5.032, MPV/TPC ratio had good specificity of 96.67%, positive predictive value (PPV) of 92.31%, sensitivity of 40%, negative predictive value (NPV) of 61.7% and diagnostic accuracy of 68.3%. Panda et al. [23] revealed high specificity (96.2 %), PPV (90.9%) at cut off value of ≥7.2 and Bagchi [24] reported similar observations of higher specificity (95.6%) and high PPV (90.2%) at cut off value ≥7.2 which further reinforces the findings in our study. Oh GH et al in their study done on adult population with blood culture proven bacteremia, stated that MPV/TPC ratio value exalted than cut off of 3.71 at admission was considerably associated with mortality risk (*P*-value = 0.001) [11]. Djordjevic et al. in his study done on critically ill patients, reported that

amongst all platelet parameters like platelet count, MPV and MPV/TPC ratio, ratio offered no advantage over other two parameters [21].

In the ROC analysis of platelet parameters of our study, area under the curve (AUC) for MPV/TPC ratio was **(**0.764) better than MPV. We observed that MPV/TPC ratio had better specificity, PPV, sensitivity, NPV and diagnostic accuracy than MPV. Although AUC for TPC was (0.784) more than MPV/TPC ratio (0.764) but MPV/TPC ratio had identical specificity but higher PPV, NPV, sensitivity and diagnostic accuracy than TPC.

Regarding the MPV/TPC ratio, the cut of value of ≥5.032 was further solidified by the fact that the 13 neonates who had value above the cut off, 92.3% neonates had blood culture-positive septicemia which was statistically significant (*P*-value 0.001). Our study exemplify excellent specificity (96.67%) and high positive predictive value (92.31%) for MPV/TPC ratio, shore it up to be superior upcoming diagnostic marker in neonatal sepsis.

Conclusion

From the current study, it was concluded that amongst platelet parameters, low TPC and a higher MPV/TPC ratio at the designated cut off values, fulfil the role as important marker for diagnosis of infection in neonates and should be utilized as early marker for diagnosis of sepsis neonatorum. These parameters when combined with detailed history, complete clinical examination and CRP can be used as early, ubiquitous and cost effective markers for diagnosis of sepsis neonatorum. The diagnostic performance of MPV/TPC is preeminent to MPV or TPC alone in early diagnosis of sepsis in neonatal population.

Limitation

It was a single centre retrospective study with a small sample size. Hence a prospective, multicentric study with large sample size will add strength to future studies.

Statements and Declarations

Conflicts of interest

The authors declares that they do not have conflict of interest.

Funding

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