

# Correlation of platelet count and platelet indices with neonatal sepsis-diagnostic and prognostic indicator

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## Abstract

**Introduction:** To evaluate variations in platelet count and platelet indices- mean platelet volume (MPV) and platelet distribution width (PDW) in neonatal sepsis. **Methodology:** This study was conducted over a period of one year in Neonatal Intensive Care Unit of Mata Chanan Devi Hospital, New Delhi. Neonates with confirmed as well as probable sepsis were included in the study. Platelet count, MPV and PDW were monitored three times: at diagnosis (1<sup>st</sup> day), 3<sup>rd</sup> day and 7<sup>th</sup> day of sepsis. **Results:** Among 100 cases, culture proven sepsis was present in 17 neonates. Fungal sepsis occurred in 2 cases (11.76%), 7 neonates (41.18%) had gram positive sepsis and 8 neonates (47.06%) had gram negative sepsis. Thrombocytopenia was present in 38% and thrombocytosis in 6% cases. Mild thrombocytopenia was noted in 55.26%, moderate thrombocytopenia in 31.58% and severe thrombocytopenia in 13.16% babies. Culture positive neonates had high prevalence of thrombocytopenia. High mortality was found in moderate to severe thrombocytopenic neonates, and these babies also had high MPV and high PDW. **Conclusions:** Thrombocytopenia was more common than thrombocytosis in neonatal sepsis. Prevalence of thrombocytopenia was significantly high in culture proven sepsis (64.7%). There was statistically significant difference in mean platelet count on day 1, day 3 and day 7 of sepsis among culture positive and culture negative neonatal sepsis. Neonates with culture proven sepsis had high MPV on day 7 and high PDW on day 1 of sepsis. There was high MPV and high PDW in neonates who developed thrombocytopenia and also in expired babies. Platelet count and platelet indices can be used as early diagnostic and prognostic biomarkers for neonatal sepsis.

**Key words:** Mean platelet volume (MPV), Neonatal sepsis, Platelet count, Platelet distribution width (PDW), Thrombocytopenia.

## Introduction

Neonatal sepsis causes significant mortality and long term morbidity in neonates, especially for preterm infants of very low birth weight [1-5]. In 2015, 2.7 million deaths, or roughly 45% of all under-five deaths, occurred during first 28 days of life [6]. More than one-third of the estimated 4 million neonatal deaths around the world each year are caused by severe infections and a quarter (around one million deaths) are due to neonatal sepsis/ pneumonia alone [7].

Neonatal sepsis is one of the commonest causes of neonatal mortality contributing to 15% of all neonatal deaths [8]. Some population-based studies have reported clinical sepsis rates ranging from 49 to 170/1000 live births in rural India [9]. Incidence is not changed much over the past decade, and the fatality due to sepsis is between 30% and 65% [10].

Sepsis is a non-specific inflammatory defence mechanism and is considered a generalized process where every organ and system can be involved. The haemostatic system is frequently disturbed during sepsis.

Manuscript received: 6<sup>th</sup> August 2017  
Reviewed: 16<sup>th</sup> August 2017  
Author Corrected: 25<sup>th</sup> August 2017  
Accepted for Publication: 30<sup>th</sup> August 2017

Haematological changes induced in neonatal sepsis have been used to make an early diagnosis and to detect complications. Changes in platelet count and platelet indices such as mean platelet volume (MPV) and platelet distribution width (PDW) induced by neonatal sepsis have been the focus of various studies.

Thrombocytopenia is used as an early but non-specific marker of sepsis in neonates [11]. Platelet size can be analysed using MPV and PDW, and it correlates with platelet activity. MPV is measurement of average size of platelets; large platelets are more active and have high thrombotic potential. High MPV indicates an increased quantity of young platelets in the circulation [12]. In neonatal period, MPV ranges from 10-12 fl [12, 13].

There are high MPV levels in destructive thrombocytopenia and low MPV levels in hypoproliferative thrombocytopenia [14, 15]. Platelet distribution width (PDW) is an indicator of variation in platelet size. Normal values of PDW are between 10 % and 17.9 % [16].

Changes in Platelet parameters like MPV and PDW are helpful in diagnosis of neonatal sepsis but these indices have not been extensively studied in neonatal sepsis. Hence the present study was undertaken to evaluate thrombocytopenia and variations in platelet indices in neonatal sepsis.

## Materials and Methods

It was a descriptive study conducted over a period from January 2016 to December 2016 in neonatal intensive care unit of Mata Chanan Devi Hospital, New Delhi. This study was approved by ethics and scientific committee of Mata Chanan Devi Hospital. During the study period 100 consecutive neonates (both inborn and out born) with clinical signs and symptoms of sepsis along with either positive culture (confirmed neonatal sepsis) or other laboratory findings suggestive of bacterial infection without positive culture (probable sepsis) were included after taking written informed consent from parents.

**Inclusion Criteria:** Any neonate who has clinical signs and symptoms of sepsis along with either positive culture or other laboratory findings suggestive of bacterial infection without positive culture.

## Exclusion Criteria

- Neonates with congenital anomalies, Hypoxic ischemic encephalopathy, Hyaline membrane disease, Congenital heart disease.
- Congenital and acquired causes of thrombocytopenia other than sepsis.
- Babies without parental consent.

Confirmed neonatal sepsis was defined as the presence of clinical signs and symptoms of sepsis with isolation of pathogen from blood (bactac), CSF or urine, and probable sepsis as the neonate presenting with clinical features and laboratory parameters consistent with infection without a positive culture. Possibility of sepsis will be considered with one or more following clinical feature: Temperature instability, convulsions, bulging fontanelle, lethargic or unconscious, poor activity, apnea / tachypnea, respiratory distress, tachycardia / bradycardia, hypotension, feeding intolerance, abdominal distension and necrotizing enterocolitis.

Blood sample (for blood culture and laboratory parameters) was drawn from the neonates who were having the clinical signs of sepsis. In laboratory parameters septic screen [17, 18], it includes following tests,

1. TLC: - total leucocyte count
2. Absolute Neutrophil Count
3. Peripheral smear for band cells: - slides stained with gram stain and examined under oil immersion light microscope, a ratio of immature neutrophils (bands) to mature neutrophils > 0.2 taken as positive.
4. Micro ESR:- micro ESR > 15mm at 1st hour will be considered as positive.
5. CRP- >6mg/dl will be considered positive; slide agglutination (latex) method will used (semi quantitative).

Sepsis screen will be considered positive if 2 or more parameters are positive.

Platelet count, MPV and PDW were performed by automatic haematological coulter counter as part of complete blood count. Platelet count, MPV and PDW were monitored three times: at diagnosis (1<sup>st</sup> day), 3<sup>rd</sup> day and 7<sup>th</sup> day of sepsis.

Thrombocytopenia was defined as platelet count  $<1.5$  lacs/ $\mu$ l and it was classified as mild if platelet count was between 1 lac/ $\mu$ l and  $<1.5$  lacs/ $\mu$ l, moderate if platelet count was between 0.5 lac/ $\mu$ l and  $<1$  lac/ $\mu$ l and severe if platelet count was  $<0.5$  lac/ $\mu$ l [19].

Blood culture, Urine routine and microscopy, urine culture, chest X-ray and any other relevant investigations wherever indicated (CSF,

Neuroimaging etc) were done in neonates suspected of sepsis. Early onset sepsis was defined as age at onset of sepsis  $<72$  hours and late onset sepsis was defined as age at onset of sepsis  $\geq 72$  hours of life.

Statistical analysis: After compilation of data, statistical analysis was done using SPSS version 17.0 software and P- value  $<0.05$  was considered significant.

## Results

In our study out of 100 cases, 76 babies were preterm and 24 babies were term, mean  $\pm$  SD gestational age was  $34.45 \pm 2.94$  weeks. Mean  $\pm$  SD birth weight was  $2.07 \pm 0.60$  kg. Early onset sepsis was present in 27 cases, while late onset neonatal sepsis was present in 73 cases; mean  $\pm$  SD age at onset neonatal sepsis was  $4.05 \pm 2.64$  days.

Culture proven sepsis was present in 17 neonate and remaining 83 cases were culture negative sepsis (probable sepsis). Among the culture positive 17 cases, 2 cases (11.76%) had fungal sepsis, 7 neonates (41.18%) had gram positive sepsis, and 8 neonates (47.06%) had gram negative sepsis.

Thrombocytopenia was present in 38% cases, thrombocytosis was found in 6% cases and 56% cases had the normal platelet count. Among all thrombocytopenic neonates 55.26% babies had mild thrombocytopenia, 31.58% had moderate thrombocytopenia and 13.16% babies had severe thrombocytopenia.

Thrombocytopenia was common on day 1 of neonatal sepsis (present in 38% babies), and platelet count gradually improved (90% babies had normal platelet count on day 7). On day 1 of sepsis Mean  $\pm$  SD platelet count was  $1.89 \pm 0.95$  lacs/ $\mu$ l, while on day 3 and day 7 it was  $1.82 \pm 0.74$  lacs/ $\mu$ l and  $2.14 \pm 0.60$  lacs/ $\mu$ l respectively, thus mean platelet count also improved as sepsis get controlled after instituting the antimicrobial therapy.

In this study mean platelet count in discharged babies was  $1.66 \pm 0.63$  lacs/ $\mu$ l and in babies who expired it was  $0.56 \pm 0.12$  lacs/ $\mu$ l, difference was statistically significant ( $p < 0.001$ ). But there was no correlation of platelet count with gestational age and age at onset of sepsis (table 1).

**Table-1: Relationship of platelet count (average of day 1, day 3 and day 7 of sepsis) with various factors.**

Variables		Platelet count			P value
		No thrombocytopenia (number of cases)	Thrombocytopenia (number of cases)	Mean $\pm$ SD (lacs/ $\mu$ l)	
Gestational age	Preterm (n=76)	50	26	$1.64 \pm 0.62$	$>0.05$
	Term (n=24)	12	12	$1.56 \pm 0.76$	
Age at onset of sepsis	EOS (n= 27)	20	7	$1.79 \pm 0.63$	$>0.05$
	LOS (n=73)	42	31	$1.56 \pm 0.65$	
Outcome	Discharged (n=96)	62	34	$1.66 \pm 0.63$	<b><math>&lt;0.001</math></b>
	Expired (n=4)	0	4	$0.56 \pm 0.12$	

We found statistically significant difference in platelet count on different days among culture positive and culture negative neonatal sepsis (table 2).

**Table-2: Platelet count on different days among culture positive and culture negative neonatal sepsis.**

Platelet count on different days	Culture		P value
	Negative sepsis (n=83)	Positive sepsis (n=17)	
Day 1	2.00 ± 0.96	1.23 ± 0.51	<0.05
Day 3	1.91 ± 0.72	1.24 ± 0.64	<0.05
Day 7	2.20 ± 0.57	1.75 ± 0.65	<0.05

The prevalence of thrombocytopenia in gram positive sepsis was 86%, while in gram negative sepsis and fungal sepsis it was 50% in both.

Among different type of organisms, all neonates with Staphylococcus infection had thrombocytopenia followed by Klebsiellapneumoniae sepsis (67%) and fungal sepsis (50%).

The mortality rate was 4% and all expired babies had thrombocytopenia. In neonates with mild thrombocytopenia there was no mortality, while the mortality among neonates with moderate thrombocytopenia and severe thrombocytopenia was 20% and 25% respectively which was statistically significant ( $p < 0.001$ ). Platelet transfusion for thrombocytopenia was required in 15.78% of cases.

The mortality of thrombocytopenic neonates who received platelet transfusion was 33.3% compared to 2.1% mortality in babies who didn't receive transfusion ( $p < 0.05$ ).

We found statistically significant correlation of mean platelet volume with age at onset of sepsis (high MPV in late onset sepsis), platelet count (high MPV in thrombocytopenic neonates) and outcome (high MPV in expired babies) (table 3).

**Table-3: Relationship between various factors with mean platelet volume (average of day 1, day 3 and day 7 of sepsis).**

MPV (fl) comparison		Mean±SD (fl)	P value
Gestational age	Preterm (n=76)	11.30 ± 0.82	>0.05
	Term(n=24)	11.55 ± 0.93	
Age at onset of sepsis	Early onset sepsis (n=27)	11.08 ± 0.87	<0.05
	Late onset sepsis (n=73)	11.46 ± 0.83	
Platelet count	Thrombocytopenia(n=38)	12.01 ± 0.73	<0.001
	No thrombocytopenia(n=62)	10.96 ± 0.66	
Outcome	Discharged (n=96)	11.31 ± 0.83	<0.05
	Expired (n=4)	12.62 ± 0.30	

No significant correlation of MPV with gestational age (table 3), and certain type of organism (gram positive, gram negative and fungal sepsis).

There was significant difference in mean MPV levels among culture positive and culture negative sepsis only on day 7 of sepsis ( $p$  value <0.05).

We found high PDW level in babies with thrombocytopenia, which was statistically significant from neonates without thrombocytopenia ( $p < 0.001$ ).

Significantly high PDW level was present in expired babies (mean PDW=14.60±0.28%) in comparison to discharged babies (mean PDW=12.96±1.13%),  $p$  value <0.05 (table 4).

**Table-4: Relationship between various factors and platelet distribution width (average of day 1, day 3 and Day 7 of sepsis).**

PDW (%) comparison		Mean±SD (%)	P value
Gestational age	Preterm (n=76)	12.97±1.18	>0.05
	Term (n=24)	13.21±1.06	
Age at onset of sepsis	Early onset sepsis (n=27)	12.99±1.44	>0.05
	Late onset sepsis (n=73)	13.04±1.04	
Platelet count	Thrombocytopenia(n=38)	13.67±0.98	<0.001
	No thrombocytopenia(n=62)	12.63±1.08	
Outcome	Discharged (n=96)	12.96±1.13	<0.05
	Expired (n=4)	14.60±0.28	

There was no significant association of PDW with gestational age, age at onset of sepsis and different type of organism.

We observed significant difference in mean platelet distribution width levels among culture positive and culture negative sepsis on day 1 of sepsis ( $p < 0.05$ ).

## Discussion

Neonatal sepsis is a life threatening condition which needs urgent diagnosis and proper management. The early signs and symptoms of sepsis in the newborn are nonspecific and subtle and might be easily confused with other non-infectious causes. A definitive diagnosis of neonatal sepsis can be made only with a positive blood culture. However, it may yield false positive results due to contamination or negative results even with severe infection. The most important approach is early diagnosis and treatment of neonates with sepsis. Thus there is need for alternative early valid markers of neonatal sepsis.

Thrombocytopenia affects up to 20-50% of all neonates admitted in neonatal intensive care unit [20]. In our study prevalence of thrombocytopenia was 38% which was similar to other studies [21, 22]. Charoo BA [23] observed that 27% of babies with sepsis developed mild, 20% moderate and 12.5% developed severe thrombocytopenia.

The prevalence of thrombocytopenia among neonates with early onset sepsis and late onset sepsis was 25.92% and 42.46% respectively. Rabindran et al [24] observed the prevalence of thrombocytopenia about 56.94% among late onset sepsis while 48.38% among early onset neonatal sepsis while Jeremiah ZA et al [19] noted the prevalence of thrombocytopenia among early onset sepsis 84.84% and

Charoo et al [23] noted the prevalence of thrombocytopenia among late onset sepsis as 59.5%.

Among 76 preterm babies, 26 babies (34.21%) had thrombocytopenia while 12 babies (50%) among term neonates developed thrombocytopenia ( $p > 0.05$ ). Similarly Abdalla Alshorman et al [25] also reported no statistical significant difference in the platelet response to infection whether being full term or premature neonates.

We found significant high prevalence of thrombocytopenia (64.7%) in culture proven neonatal sepsis. Results were statistically significant when compared with culture negative neonatal sepsis ( $p = 0.003$ ). In studies by Guida et al [26] and Mannan MA et al [27] they observed the prevalence was 50% among babies with culture positive sepsis.

In our study platelet nadir for fungal sepsis was 48,000/ $\mu$ l compared to 40,000/ $\mu$ l for gram positive sepsis and 52,000/ $\mu$ l for gram negative sepsis. In study by Sartaj A Bhat et al [28] noted that lowest platelet count for fungal sepsis, gram positive sepsis and gram negative sepsis was 1.4 lacs/ $\mu$ l, 20,000/ $\mu$ l and 34,000/ $\mu$ l respectively.

Some studies have shown decrease in platelet count is associated with higher mortality. Charoo BA et al [23] reported significantly higher mortality in

patients with thrombocytopenia (26.8%) in comparison to non-thrombocytopenic group (11.1%) and also reported that mortality was highest in patients with severe thrombocytopenia. Olmez et al [29] showed that a  $\geq 30\%$  decline in platelet count is associated with increased mortality. Another study by Rastoqi et al. [30] concluded that decrease in platelet count among preterm neonates is associated with increased mortality.

We observed high mortality rate among neonates who required platelet transfusion for thrombocytopenia. Similarly Sartaj A Bhat et al [28] observed that among thrombocytopenic babies who received platelet transfusion the mortality was 42.85% compared to (0%) mortality in babies who had not received transfusion. Christense et al. [31] had mentioned that mortality rate of those receiving platelet transfusion was twice than those babies receiving none.

There were no significant differences between preterm and fullterm neonates related to MPV levels, similarly Abdalla Alshorman et al. [25] reported high MPV almost equal in both premature and full term babies. MPV in neonates with early onset sepsis and late onset neonatal sepsis was  $11.08 \pm 0.87\text{fl}$  and  $11.46 \pm 0.83\text{fl}$  respectively ( $p < 0.05$ ). Catal F et al [32] observed no significant differences between early and late onset of sepsis related to MPV levels.

MPV of thrombocytopenic babies was  $12.01 \pm 0.73\text{fl}$  and in babies without thrombocytopenia was  $10.96 \pm 0.66\text{fl}$  ( $p < 0.001$ ). Sartaj A Bhat et al [28] noted the average MPV of patients with thrombocytopenia and without thrombocytopenia  $11.74 \pm 1.08\text{fl}$  and  $9.59 \pm 1.04\text{fl}$  respectively.

We found statistically significant high MPV in expired babies (non-survivors) in comparison to discharged babies (survivors) ( $p < 0.05$ ). Catal F et al [32] also reported significant difference between survivors and non-survivors in MPV levels. There was high PDW level in babies with thrombocytopenia, which was statistically significant as compared to neonates without thrombocytopenia ( $p < 0.001$ ). In our study mean PDW of the cases discharged was  $12.96 \pm 1.13\%$  and expired was  $14.60 \pm 0.28\%$  ( $p < 0.05$ ). Guclu E et al [33] also reported significantly high PDW in non-survivors.

## Conclusion

Thrombocytopenia is a common complication in neonatal sepsis and means platelet count improves over 7 days as sepsis gets controlled. Neonates with culture proven sepsis have high prevalence and severity of thrombocytopenia, high MPV and high PDW, so these can be used as early diagnostic biomarkers of neonatal sepsis. Mortality in sepsis cases increases with severity of thrombocytopenia. Mean platelet count of expired babies remained significantly lower on all 3 days as compared with discharged babies, so thrombocytopenia is a good prognostic marker of neonatal sepsis. There is positive correlation of high MPV and PDW with thrombocytopenia in neonatal sepsis. Significant difference was found between survivors and non-survivors of neonatal sepsis in terms of platelet count, MPV and PDW.

**Funding:** Nil, **Conflict of interest:** None initiated, **Perission from IRB:** Yes

**Disclosure-** The authors declare no conflict of interest.

**Author Contribution-** AKT contributed to conception and design of this study, DKC performed and collected the data, AKT, DKC, SN and JC drafted and analysed the manuscript, AKT critically reviewed and supervised the whole study. All authors read and approved the final manuscript.

## Abbreviations

CRP- c reactive protein

CSF-cerebrospinal fluid

ESR- erythrocyte sedimentation rate

TLC-total leucocyte count

MPV- meanplatelet volume

PDW- platelet distribution width

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#### How to cite this article?

Choudhary D.K, Tiwari A.K, Narang S, Chhabra J. Correlation of platelet count and platelet indices with neonatal sepsis-diagnostic and prognostic indicator. *J PediatrRes*. 2017;4(08):511-518.doi:10. 17511/ijpr.2017.i08.03.

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