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**Alterations in Platelet-to-Lymphocyte Ratio, and Some White Blood Cell Indices in Neonatal Thrombocytopenia**

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**Abstract**

Platelets, Neutrophils, Lymphocytes, Neutrophil-to-Lymphocyte ratio (NLR) and Platelet-to-Lymphocyte ratio (PLR) have been studied as haematological and biologic markers of infections and inflammation; however, changes in PLT, NLR and some white blood cell indices in neonatal thrombocytopenia in Nigeria have not been sufficiently evaluated. This study therefore aims to compare these haematological and biologic markers among thrombocytopenic and non-thrombocytopenic new-borns at delivery. A total of 374 new-borns comprising of 35 new-borns with thrombocytopenia and 339 non-thrombocytopenic new-borns were recruited for this study and cord blood was collected from them immediately after delivery. Complete blood count was determined on the cord blood using Sysmex KX-21N auto-haematology analyser while platelet-lymphocyte ratio (PLR) and neutrophil-lymphocyte ratio (NLR) were calculated as ratios of their absolute values respectively. Total white blood cell count ( $15.11 \pm 5.38$  vs  $13.10 \pm 5.14 \times 10^9/L$ ) ( $p < 0.0289$ ) and mean platelet volume ( $10.63 \pm 1.11$  vs  $9.95 \pm 1.14$  fL) ( $p < 0.0008$ ) were significantly higher while mean platelet count ( $113.97 \pm 32.84$  vs  $264.14 \pm 71.51$ ) and platelet-to-lymphocyte ratio ( $28.52 \pm 18.64$  vs  $70.86 \pm 38.87$ ) were significantly lower ( $p < 0.0001$ ) among thrombocytopenic new-borns than non-thrombocytopenic counterparts. There was no significant difference in NLR ( $p > 0.05$ ) between thrombocytopenic and non-thrombocytopenic neonates at birth. The significantly higher leucocyte counts and mean platelet volume

among the thrombocytopenic new-borns may suggest sub-clinical and early on-going infectious process, and platelet destruction may be the possible mechanism for the thrombocytopenia.

**Keywords:** Neonatal thrombocytopenia, new-born, white blood cell indices.

**Introduction**

There is a growing concern about the increasing prevalence of thrombocytopenia at birth. In a recent study, a prevalence of 9.4% thrombocytopenia at birth was observed among apparently healthy new-borns in Delta State, Nigeria (Collins *et al.*, 2018). Thrombocytopenia in a neonate of any viable gestational age is defined as a platelet count less than  $150 \times 10^9/L$  (Wiedmeier *et al.*, 2009) and may result from a number of disease processes ranging from increased platelet destruction, decreased production and splenic sequestration or abnormal pooling based upon the causative process (Lee *et al.*, 2009; Greer *et al.*, 2006). New-borns with thrombocytopenia at birth are termed early onset thrombocytopenia occurring in the first 72 hours of birth.

New-borns with thrombocytopenia often present with varying haematological abnormalities including changes in the number of leucocytes, transient neutropenia, increased number of circulating nucleated red blood cells with or without polycythaemia, and increased erythropoietin levels (Roberts *et al.*, 2008). These haematological abnormalities if not timely and adequately evaluated, could pose

severe threat to the well-being of new-borns. Inflammation has been characterised by changes in the number of neutrophils and lymphocytes (Senturk *et al.*, 2016) while Neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) is used as biologic markers of inflammation (Emokpae *et al.*, 2016). However, inflammation may not be prominent at birth until the end of the second post-natal week following exposure to inflammatory stimuli such as sepsis and bacteraemia (McElrath, 2013).

Total white blood cell count including neutrophils have been observed to increase during bacterial infections, however at birth, neutrophil count may appear normal but with a left shift (Esan, 2016) and thus may not distinguish the presence of bacterial infection. The neutrophil storage pool in the new-born is reported to be limited with an inability to increase stem cell proliferation (Christensen and Rothstein, 1980).

Platelets have been reported to increase in diameter in response to infections following their rapid release from the spleen (Boyraz *et al.*, 2014) as well as in cases of thrombocytopenia caused by rapid platelet destruction with a resultant bone marrow release of immature larger platelets into circulation (Reddy *et al.*, 2014). It is important to determine changes in PLR, MPV and WBC counts in neonatal thrombocytopenia to enable adequate clinical management of this increasingly recognized condition. Therefore, this study seeks to determine the alterations in the levels of neutrophil-to-lymphocyte ratio, PLR, mean platelet volume (MPV), platelet counts, total leucocyte counts as well as lymphocyte counts and neutrophil counts as possible markers of inflammation and/or infection in new-borns with thrombocytopenia.

## Materials and Methods

### Study population

This is a cross-sectional study conducted at Central Hospital, Warri and Federal Medical Centre, Asaba in Delta State, Nigeria. Three hundred and seventy-four (374) apparently healthy neonates at birth constituted the subjects for this study.

Informed consent was obtained from the mothers of the new-borns prior to delivery and before specimen collection. The study was approved by the ethical committees of both health institutions (FMC/ASB/28, 20<sup>th</sup> April, 2016 and CHW/ECC VOL1/095, 4<sup>th</sup> August, 2016).

### Sample collection and processing

Five millilitres (5mL) of Cord blood was collected from the umbilical cord of all neonates immediately after delivery and dispensed into ethylene di-amine tetra-acetic acid (EDTA) container and mixed. White blood cell counts, absolute neutrophil and lymphocyte counts, platelet counts and mean platelet volume were estimated within 3 hours of collection using haematology auto-analyser model Sysmex KX-21N, (Sysmex Corporation, Kobe, Japan) according to the manufacturer's instruction. Neutrophil lymphocyte ratio (NLR) was determined by dividing the absolute values of the neutrophil by the absolute values of lymphocyte. Similarly, the ratio of the absolute values of platelet count to lymphocyte count gave the platelet-lymphocyte ratio (PLR).

### Statistical analysis

The data were compared between thrombocytopenic and non-thrombocytopenic new-borns by student t-test using the statistical software INSTAT® (Graph Pad Inc., La Lolla, CA, USA). The level of significant was set at  $p < 0.05$ .

### Results

From table 1, the mean total white blood cell counts among thrombocytopenic new-borns was significantly higher ( $p = 0.0289$ ) when compared with mean total white blood cell count of non-thrombocytopenic new-borns. There was no significant difference in absolute neutrophil and lymphocyte counts, as well as in NLR values between thrombocytopenic and non-thrombocytopenic new-borns ( $p > 0.05$ ). Platelet counts and PLR values ( $p < 0.0001$  each) were significantly lower in thrombocytopenic new-borns while MPV values ( $p = 0.0008$ ) were higher in thrombocytopenic when compared with non-thrombocytopenic new-borns.

**Table 1: Comparison of some haematological parameters between thrombocytopenic and non-thrombocytopenic new-borns.**

Parameters	Thrombocytopenic new-borns N=35	Non-thrombocytopenic new-borns N=339	p-value
WBC ( $\times 10^9/L$ )	15.11 $\pm$ 5.38	13.10 $\pm$ 5.14	0.0289*
NEUT ( $\times 10^9/L$ )	8.43 $\pm$ 4.64	7.29 $\pm$ 3.48	0.0754
LYMP ( $\times 10^9/L$ )	5.12 $\pm$ 2.28	4.56 $\pm$ 2.21	0.1556
PLT ( $\times 10^9/L$ )	113.97 $\pm$ 32.84	264.14 $\pm$ 71.51	<0.0001*
NLR	1.95 $\pm$ 1.58	2.65 $\pm$ 12.31	0.7373
PLR	28.52 $\pm$ 18.68	70.86 $\pm$ 38.87	<0.0001*
MPV (fL)	10.63 $\pm$ 1.11	9.95 $\pm$ 1.14	0.0008*

**Key:** WBC=White Blood Cell counts; NEUT=Neutrophil counts; LYMP= Lymphocyte counts; NLR= Neutrophil-Lymphocyte Ratio; PLR= Platelet-Lymphocyte Ratio; MPV= Mean Platelet Volume; PLT= Platelet count.

**Discussion**

The neutrophil to lymphocyte ratio (NLR), because it is calculated using neutrophils and lymphocytes is regarded to be a more stable and useful index than the absolute counts as marker of several diseases. The NLR has been reported as a predictor of severity and clinical outcome in patients with community acquired pneumonia (Loonen *et al.*, 2014; de Jager *et al.*, 2012). In addition, the NLR and the platelet to lymphocyte ratio (PLR), have been reported as useful systemic inflammatory markers and prognostic

indicators of adverse cardiovascular events and cancer and neonatal sepsis (Can *et al.*, 2018). To the best of our knowledge, there are no reports concerning the NLR and the PLR in neonatal thrombocytopenia in our setting.

From this study the total white blood cell count was significantly higher among apparently healthy thrombocytopenic new-borns when compared with non-thrombocytopenic new-borns (p = 0.0289). The reason for the significantly higher white blood cell count

observed among thrombocytopenic neonate in this study is unclear. However, this is in agreement with the report of Esan (2016) who stated that at birth, there is an increase in white blood cell counts in both term and preterm new-borns without evidence of disease.

There was no significant difference in the neutrophil and lymphocyte counts between thrombocytopenic and non-thrombocytopenic new-borns from our study. However, studies have suggested that new-borns at birth with possible bacterial infection often present with normal neutrophil counts with a shift to the left (Esan, 2016). Therefore, the absence of any significant difference in the neutrophil counts between thrombocytopenic and non-thrombocytopenic new-borns from our study, may be attributed to our study population of new-borns at birth and may not rule out possible infection.

The platelet count and platelet-to-lymphocyte ratio (PLR) among thrombocytopenic new-born from our study were significantly lower than the non-thrombocytopenic new-borns ( $p < 0.0001$ ). Platelet counts may be reduced via various mechanisms which includes increased platelet destructions, decreased platelet productions and platelet consumption based upon the causative process (Lee *et al.*, 2009). Reduced platelet count and concomitant increase in mean platelet volume may indicate the release of large platelets into circulation following platelet destruction or utilization (Kotwal, 2011).

The platelet-lymphocyte ratio (PLR) is calculated as platelet count divided by absolute lymphocyte counts (Smith *et al.*, 2008). From our study, the PLR was significantly lower among thrombocytopenic new-borns. This may be attributed to the low platelet counts observed among the thrombocytopenic new-borns. An increased Platelet-lymphocyte ratio is a non-specific marker of systemic inflammatory response (Cakmak *et al.*, 2015). During chronic inflammatory processes, megakaryocytic series proliferate increasingly while lymphocyte counts tend to decrease due to severe apoptosis (Nikolsky *et al.*, 2007). This is in contrast with the significantly lower platelet-lymphocyte ratio among thrombocytopenic new-borns observed

from our study. Can *et al.* (2018) have reported that the PLR was significantly higher in the neonates with early onset sepsis compared with the controls, and it was significantly positively correlated. Also, the PLR has been reported to be associated with adult cardiovascular events. This inflammation affected the PLR and finally resulted in the raised PLR because of inflammation. In our study group, the low platelet count and high leucocyte count have caused the lower PLR among neonates with thrombocytopenia compared with non-thrombocytopenia.

The Neutrophil-Lymphocyte ratio (NLR) among thrombocytopenic and non-thrombocytopenic new-borns from our study was not significantly different ( $p > 0.05$ ). NLR is a routinely available marker of the systemic inflammatory response derived by dividing absolute neutrophil and absolute lymphocyte counts (Walsh *et al.*, 2005) and is reliable in the evaluation of the extent of stress or systemic inflammation which may express the severity of affliction (Zahorec, 2001). Sentuk *et al.* (2016) in their study attributed significant increase in NLR to systemic bacterial infection and inflammation. Systemic bacterial infection or sepsis may be characterised by a decrease in the number of lymphocytes and an increase in the number of neutrophils. This is in contrast with the findings from our study which did not observe any significant difference. The reason for our finding is unclear, However, Esan (2016) had reported that new-borns with bacterial infection may present with normal or below normal neutrophil counts with a shift to the left.

The Mean Platelet Volume was significantly higher among thrombocytopenic new-borns when compared the non-thrombocytopenic new-borns ( $p = 0.0008$ ). Reports have suggested that thrombocytopenia following infections may result in increased mean platelet volume as septicaemia related destruction of platelets increases production and the release into the peripheral blood of larger and younger platelets (Reddy *et al.*, 2014). This may explain the observed increase in mean platelet volume among thrombocytopenic new-borns from our study.



## Conclusion

The significantly higher leucocyte counts and mean platelet volume among the thrombocytopenic new-borns may suggest subclinical and early on-going infectious process, and platelet destruction may be the possible mechanism for the thrombocytopenia.

## Disclaimer

The views expressed in this article are that of the authors and not an official position of any hospital or institution.

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## Previous Publication

This article has not been submitted to any journal except the one being considered now.

## Conflict of Interest

There is no conflict of interest regarding this work among the authors.

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