

## CLINICAL STUDY

# Association between neutrophil/lymphocyte ratio with ductus arteriosus patency in preterm newborns

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**OBJECTIVE:** The aim of this study was to assess the influence of neutrophil to lymphocyte (N/L) ratio and platelet indices on patent ductus arteriosus (PDA) in preterm infants.

**BACKGROUND:** PDA is a common problem with potentially serious associated morbidities in preterm neonates.

**METHODS:** Premature infants with hemodynamically significant PDA (n = 47) and a control group without PDA (n = 50) who were hospitalized in the neonatal intensive care unit were retrospectively evaluated. The characteristics, perinatal factors, N/L ratio, platelet counts and other platelet indices of the infants in both groups during the first 3 days of life were recorded.

**RESULTS:** Platelet counts were significantly lower in the patient group than in the control group (p = 0.0343). There was a marked positive correlation between body weight and N/L ratio in preterm infants with PDA (p = 0.0001). PDA was associated with low platelet count.

**CONCLUSION:** Our results showed that N/L ratio is positively correlated with body weight in PDA group. These data suggest that platelet counts and N/L ratio might be useful predictors for the early diagnosis and evaluation of the clinical course of PDA in preterm infants (*Tab. 2, Ref. 28*). Text in PDF [www.elis.sk](http://www.elis.sk).

**KEY WORDS:** correlation, ductus arteriosus, neutrophil to lymphocyte ratio, platelet count, preterm infant.

**Introduction**

Patent ductus arteriosus (PDA) is one of the most common problems in preterm infants leading to potentially serious effects on several organ systems (1, 2). Its incidence varies inversely with gestational age and may be as high as 60 % among those with extremely low gestational age and in very low birth weight infants (3–6). In full-term infants, is the postnatal closure of ductus arteriosus divided in two phases: the “functional” closure of the lumen caused by the smooth muscle constriction within 18 to 24 hours after birth; and “anatomical” occlusion occurs over the next few days or weeks. Increase in arterial pO<sub>2</sub> and a drop in circulating PGE<sub>2</sub> and blood pressure within the lumen of the ductus (caused by the drop in pulmonary vascular resistance) promotes constriction of the ductus (7–10). However, the precise mechanism responsible for ductal constriction is still controversial.

A growing body of evidence from recent studies suggests that platelets play a complex role in acute and chronic inflammatory diseases and in tumor cell activation and growth beyond hemosta-

sis and wound repair (11–13). Besides the platelet count, different platelet parameters such as mean platelet volume (MPV), platelet distribution width (PDW) and plateletcrit (PCT) have also been investigated because these parameters may influence the reactivity of platelets (14–17).

The neutrophil/lymphocyte (N/L) ratio in the complete blood count, which is inexpensive and easy-to-perform test, is used as an inflammatory indicator especially in patients with cardiovascular disease (18–21). A higher neutrophil percentage or a lower lymphocyte percentage is thought to be associated with an increased inflammatory response (11–14, 18). The goal of this study was to determine a possible association between platelet indices and N/L ratio and the presence and/or closure of PDA in preterm newborns.

**Material and methods**

We retrospectively evaluated records of premature infants who were between 25 and 37 weeks of gestation (due date from last menstrual period). These infants had hemodynamically significant PDA diagnosed in the neonatal intensive care unit (NICU) of Gaziantep University Hospital between January 2015 and December 2015. Echocardiographical examination is routinely performed between 3rd–5th day of life. Hemodynamically significant PDA was defined as one with an internal ductal diameter of  $\geq 1.5$  mm and/or with a left atrium/aortic root ratio  $\geq 1.5$  echocardiographically. Infants with congenital heart disease, persistent pulmonary hypertension and gross congenital abnormalities were excluded. Patient characteristics including gestational age, gender, birth weight, presence of

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**Tab. 1. Comparison of neonatal demographic and maternal factors between infants with PDA and control group without PDA.**

	Control (n=50)	PDA (n=47)	p
Gestational age (weeks)	32.52±2.22	31.85±2.94	0.2067
Gender			0.9265
Male (n %)	24 (48.0)	23 (48.9)	
Female (n %)	26 (52.0)	24 (51.1)	
Birth weight (total, g)	1858.90±583.58	1756.04±777.54	0.4613
VLBW (≤1500 g, n=12)	1114.92±308.71	1054.45±282.00	0.3173
Birth weight (>1500 g, n=38)	2093.84±431.84	2275.74±593.63	0.0863
Maternal history			
Preeclampsia/eclampsia, n (%)	11 (22.0)	10 (21.3)	1.0000
EMR, n (%)	8 (16.0)	3 (6.4)	0.2015
Chorioamnionitis, n (%)	2 (4.0)	3 (6.4)	0.6712
Multiple births, n (%)	23 (46.0)	34 (72.3)	<b>0.0152</b>
Mother's age (years)	28.92±5.14	30.76±5.67	0.0970
Accompanying diseases, n (%)			
RDS	5 (10.0)	10 (21.3)	0.1632
NEC, stage 2–3	7 (14.0)	4 (8.5)	0.5262
Sepsis, early-onset	4 (8.0)	3 (6.4)	1.0000
Pneumonia	7 (14.0)	11 (23.4)	0.2990
Ibuprofen treatment	–	24 (51.1)	
Paracetamol treatment	–	7 (14.9)	
PDA ligation	–	1 (2.1)	
PDA closure with drug treatment	–	23 (48.9)	
PDA closure without drug treatment	–	8 (17.0)	
Hemoglobin (g/dl)	16.16±2.24	15.66±3.11	0.3637
Platelet count (x 10 <sup>3</sup> /mm <sup>3</sup> )	254.58±83.19	220.47±72.50	0.0343
MPV (fl)	10.02±0.94	10.00±0.89	0.9147
PCT (%)	0.25±0.08	0.22±0.07	0.0529
PDW (%)	11.15±1.77	11.27±2.14	0.7636
Neutrophils (x 10 <sup>3</sup> /mm <sup>3</sup> )	6.13±3.61	6.05±5.38	0.9313
Lymphocytes (x 10 <sup>3</sup> /mm <sup>3</sup> )	3.69±1.47	3.54±1.50	0.6201
Neutrophils/ Lymphocytes ratio	1.94±1.26	2.06±2.06	0.7431
ECHO finding			
Ductal diameter (mm)	–	2.53±1.34	

Data show mean±SD values. PDA, patent ductus arteriosus; MPV, mean platelet volume; PDW, platelet distribution width; RDS, respiratory distress syndrome; EMR, early membrane rupture; VLBW, very low birth weight; PCT, plateletcrit. PCT is calculated according to the following formula (28): PCT = platelet count x MPV/10 000.

accompanying disease [respiratory distress syndrome (RDS), necrotizing enterocolitis (NEC), sepsis, and pneumonia], maternal risk factors including preterm prolonged rupture of membranes (EMR), chorioamnionitis and preeclampsia/eclampsia were reviewed for all infants. The control group consisted of preterm infants (the same range of gestational weeks) but without hemodynamically significant PDA on routine echocardiography in the NICU.

Complete blood counts were obtained both from the patients and controls during the first 3 days of life, mostly within the first 24 h. Blood samples were taken from the umbilical vein and collected in ethylenediaminetetraacetic acid tubes. Platelet, neutrophil, lymphocyte counts and the other platelet indices including MPV, PDW and PCT of the infants in both groups were performed using the Coulter Counter model LH (Coulter electronics, Hialeah, FL, USA) and recorded. Ibuprofen or paracetamol treatment was started in infants with hemodynamically relevant PDA unless they had any contraindications. Treatment was withheld if platelet count was <50,000/mm<sup>3</sup>. Within 24–48 h of completion of a course of medical treatment, echocardiography was repeated to evaluate the closure of ductus arteriosus. The study was undertaken in compliance with the guidelines of the Declaration of Helsinki, and it was approved by the local Ethics Committee.

### Statistical analyses

Data were expressed as mean±standard deviation (SD) or percentage. Statistical analysis was performed using GraphPad Instat version 3.05 (GraphPad Software Inc., San Diego, CA, USA). For comparisons of the differences between mean values of two groups, the unpaired Student's t test was used. The Chi-square test for independence and the Fisher's exact tests were used for calculation of the significance of differences in genotype and allele frequencies. The Pearson's test was used to identify the correlations. All statistical tests and p values were two-sided, and p < 0.05 was considered statistically significant.

### Results

Comparison of the demographic features, antenatal and neonatal history, and accompanying diseases between the study groups are presented in Table 1. There were no differences between the study groups with regards to gestational age, birth weight and gender. When compared based on the maternal history, multiple births were significantly higher in the PDA group than in the control group (p = 0.0152). In 8 patients (17 %) platelet counts were <150,000/mm<sup>3</sup> in the PDA group, while this ratio was 6 %

**Tab. 2. Correlations between the birth weight, N/L ratio, and platelet indices in preterm infants with PDA.**

Parameters	Correlation coefficient (r)	Coefficient of determination (r <sup>2</sup> )	p
BW (total) ↔ Platelet count (total)	0.1231	0.0152	0.4096
BW (total) ↔ MPV (total)	0.1236	0.0153	0.4076
BW (total) ↔ PCT (total)	0.1494	0.0223	0.3162
BW (total) ↔ PDW (total)	-0.0252	0.0006	0.8663
BW (total) ↔ N/L ratio	0.6310	0.3982	<b>0.0001</b>
VLBW (≤1500 g) ↔ Platelet count (VLBW)	0.3291	0.1083	0.1565
VLBW (≤1500 g) ↔ MPV (VLBW)	-0.2857	0.0816	0.2221
VLBW (≤1500 g) ↔ PCT (VLBW)	0.2806	0.0788	0.2307
VLBW (≤1500 g) ↔ N/L ratio	0.5366	0.2879	0.1098
BW(>1500 g) ↔ Platelet count (>1500 g)	0.1430	0.0205	0.4767
BW(>1500 g) ↔ MPV (>1500 g)	-0.1708	0.0292	0.3944
BW(>1500 g) ↔ PCT (>1500 g)	0.0925	0.0086	0.6462
BW(>1500 g) ↔ N/L ratio	0.6436	0.4142	<b>0.0012</b>

BW, body weight; MPV, mean platelet volume; PCT, plateletcrit; PDW, platelet distribution width; N/L, Neutrophils/Lymphocytes; VLBW, very low birth weight

(3 infants) in the control group ( $p = 0.1140$ ). Platelet count was significantly lower in the PDA group than in the control group ( $p = 0.0343$ ). MPV, PDW and PCT values were similar between the two groups.

Totally 31 patients out of 47 with PDA were treated with ibuprofen (24 infants, 51.1 %) and paracetamol (7 infants, 14.9 %), respectively. Closure of the ductus was reached in 23 patients (48.9 %) with single or repeated courses of drug treatment. Only one infant underwent surgical ligation (2.1 %) after failure of three treatment courses. None of the patients experienced any re-openings during follow-up. As a result, we found diminished platelet counts in the PDA group, but no association with the other platelet indices were observed (Tab. 1).

Correlations between the birth weight and platelet indices and N/L ratio in preterm infants with PDA were also assessed in this study (Tab. 2). Significant correlations were observed between body weight and N/L ratio. This correlation was present in preterms with >1500 g body weight, but not with VLBW ( $\leq 1500$  g). This could be related to the low numbers of preterms ( $n = 12$ ) in the VLBW group.

## Discussion

Significant patency of ductus arteriosus, especially in preterm infants, is associated with life-threatening complications such as congestive heart failure, pulmonary edema and hemorrhage, prolonged requirement of ventilatory support, bronchopulmonary dysplasia, acute renal failure, intraventricular hemorrhage, and NEC (1, 2). Although several hypotheses have been introduced to date, the precise mechanisms of ductal closure were proposed to differ in preterm and full-term infants. In full-term infants, ductus closure is related with the decreasing blood flow both in the ductus lumen and vaso vasorum, leading to hypoxia in the vessel wall after birth. Platelet aggregation has a minor role in this setting. On the other hand, ductus arteriosus is much thinner in preterm infants and extracts the oxygen it needs from luminal blood after constriction of the ductus arteriosus. Decrease in the blood flow alone does not provide "sufficient" hypoxia and platelet aggregation is needed for permanent closure. Therefore, low platelet counts may result in increased risk of PDA in preterm infants (22).

Recently, Echtler et al (23) showed clear histological evidence of platelet accumulation within the lumen of the ductus arteriosus in newborn mice by using intravital microscopy, and identified that platelets are recruited to the constricting ductus arteriosus and initiate thrombotic occlusion of the ductus. They found a higher incidence of persistent ductus arteriosus in congenitally thrombocytopenic mice. Echtler et al (23) additionally evaluated premature infants in their clinic retrospectively to find out what is the clinical relevance of their findings and identified thrombocytopenia as an independent predictor for ductal patency. Similarly, Dani et al (16) demonstrated that ductus arteriosus is associated with low platelet count at birth, but not after 24–48 h of birth. In another study, Alyamac Dizdar et al (15) have studied 154 preterm infants born at 26–29 weeks of gestation and indicated that low platelet count and high PDW were independent risk factors of ductus arteriosus with hemodynamic relevance. A large-scale study of 1,350 infants with birth weights < 1500 g by Sallmon et al (24) found no relationship between platelet count on the first day of birth and patent ductus arteriosus. Sallmon et al (24) concluded that impaired platelet function due to immaturity and critical illnesses related with the patency of ductus arteriosus are connected with hemaodynamic relevance rather than the low platelet count. Similarly, Shah et al (14), and Olukman et al (25) could not find any relationship between low platelet counts and permanent ductal closure or ductal re-opening. In the study of Shah et al (14), the authors showed that full-term infants with severe thrombocytopenia, secondary to Wiskott–Aldrich syndrome or alloimmune thrombocytopenia do not have a higher incidence PDA. We have showed low platelet counts in infants with PDA indicating that platelet count may contribute to the development of PDA.

Like MPV, PDW is another marker which reflects the platelet activation in various clinical conditions. In the literature, there are several studies that have found a significant association between elevated MPV and PDW values and severe inflammatory processes. Wasiluk et al (26) demonstrated higher MPV and lower PDW values in term infants when compared with preterm infants. However, Alyamac Dizdar et al (15) have reported that PDA was independently associated with high PDW but not MPV. In contrast to these previous studies, we did not find any association between MPV and PDW values and the risk of ductal patency. Although

PCT reflect the total platelet mass like PDW, we found no association in PCT between the patients and the control groups.

N/L ratio has been recently defined as a novel potential inflammation marker in cardiovascular diseases (18–21). N/L ratio can easily be calculated using the peripheral blood count than measuring other inflammatory cytokines, such as IL-6, IL-1 $\beta$ , and TNF- $\alpha$  (27). To our knowledge, there was no study investigating the correlation between body weight and N/L ratio in PDA infants.

In conclusion, to the best of our knowledge, our study is the first that investigates all platelet indices including MPV, PDW and PCT and another easy to perform test as an inflammatory marker, the N/L ratio, in patients with PDA. Our results showed that low platelet count is associated with PDA. The findings of this study demonstrated that N/L ratio is positively correlated with body weight in PDA group. Since N/L ratio and birth weight are positively correlated, platelet counts and N/L ratio might have a prognostic role for the early diagnosis and clinical course of PDA in preterm infants.

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Received March 20, 2017.

Accepted April 17, 2017.