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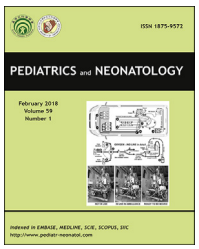

Original Article

Influence of platelet count, platelet mass index, and platelet function on the spontaneous closure of ductus arteriosus in the prematurity

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Key Words
patent ductus arteriosus;
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collagen-ADP;
hemoglobin;
hematocrit

Background: This study aims at evaluating the influence of platelet count, platelet mass index, and platelet function on the spontaneous closure of ductus arteriosus in prematurity.
Methods: All preterm babies were divided into two groups, including Group 1 with "open PDA" and Group 2 with "closed PDA". The variables of platelet count, mean platelet volume, platelet mass index, and platelet function were analyzed and compared between two groups of patients to identify the factors that significantly influenced spontaneous closure of ductus arteriosus.
Results: Twenty-four patients were in the "open PDA" group, whereas 36 patients were in the "closed PDA" group. Mean GA and BW were 27.6 ± 1.8 (23.1—30.4) and 28 ± 1.6 (23.4—30.6) weeks and 1009 ± 270 (585—1480) g and 1035 ± 298 (505—1500) g in "open PDA" and "closed PDA" groups, respectively ($p > 0.05$). The incidence of "Collagen-ADP > 130 s" was significantly higher in the "open PDA" group, and the levels of hemoglobin and hematocrit were significantly lower in the "open PDA" group ($p < 0.05$). Multivariate logistic regression analysis showed that respiratory distress syndrome (OR: 9, CI: 1.5—51.8) and collagen-ADP > 130 s (OR: 5.7 CI: 1.55—21.3) are two independent factors associated with ductal patency.

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Conclusion: This is the first study in the English literature providing evidence of the influence of platelet dysfunction on the spontaneous closure of ductus arteriosus in prematurity. Longer collagen-ADP duration is identified as a risk factor of ductal closure.

1. Introduction

Patent ductus arteriosus is an important clinical problem associated with exacerbation of respiratory distress syndrome (RDS), pulmonary hemorrhage, prolonged use of assisted ventilation, bronchopulmonary dysplasia (BPD), intraventricular hemorrhage (IVH), renal dysfunction, necrotizing enterocolitis (NEC), periventricular leukomalacia (PVL), cerebral palsy and mortality in prematurity.[1] Classical hypothesis considering ductal closure includes fetal patency maintained by low oxygen tension and vasodilator prostanoids including prostaglandin E2 (PGE2) and prostacyclin (PGI2). Increase in PaO_2 and decrease in PGE2 and PGI2 after birth induce constriction of smooth muscle cells in the ductus arteriosus (DA) and result in functional closure of DA. This contraction leads to a local hypoxia and triggers cell death and release of hypoxia inducible growth factors, which results in vascular remodeling and anatomic DA closure.[2] Recently, an alternative hypothesis was proposed by Echtler et al., who demonstrated the recruitment of platelets to the luminal aspect of the murine DA immediately after birth. Induced dysfunction of platelet adhesion or transgenic defects of platelet biogenesis resulted in persistent DA.[3] The studies on the platelet count published in recent years have conflicting results. Echtler et al. performed a retrospective study in preterm infants born at 24–30 weeks gestation and reported that thrombocytopenia on the first day of life was a significant risk factor for failure of DA closure.[3] Dizdar et al. evaluated premature infants with hemodynamically significant PDA (n = 154) and a control group without PDA (n = 207) and found a statistically significant correlation between hemodynamically significant PDA and thrombocytopenia.[4] In contrast, Bas-Suarez et al. reported that thrombocytopenia on the first 2 days of life was not related to the presence of PDA on day 3.[5] Simon et al. showed a marginal but significant association between low platelet counts and PDA in the preterm babies in the first day of life in their meta-analysis; however, they pointed out that the relationship between PDA and thrombocytopenia was unclear.[6]

In some studies it was proposed that impaired platelet function rather than platelet number might contribute to PDA.[7,8] These conflicting results of the effect of platelet count on the ductal patency led us to investigate the influence of platelet function on spontaneous ductal closure in preterm babies. In this study we aimed at investigating the platelet function (evaluated by PFA-100), platelet count, mean platelet volume, and platelet mass index on spontaneous ductal closure in preterm babies.

2. Methods

This was a prospective study on the patients who were admitted to the NICU of Ankara University School of Medicine Children's Hospital, Ankara, Turkey between September 2013 and September 2014. Sixty premature infants, with GA $\leq$ 30 weeks and BW $\leq$ 1500 g, were grouped into two groups, including group 1 with "open PDA" and group 2 with "closed PDA", assessed by Doppler echocardiography in the 72–96 h of life. "Open PDA" was defined as the ductus with an internal diameter $\geq$1.5 mm and/or with a left atrium (LA)/aortic root (Ao) ratio $\geq$1.5. Consecutive echocardiographic examinations were conducted and patients with persistently open PDA were medically treated and/or surgically ligated. Patient characteristics including GA, BW, gender, maternal age, mode of delivery, prenatal steroid use, 1–5 min APGAR scores, need of resuscitation, presence of RDS, NEC, ROP, IVH, BPD, duration of NCPAP (nasal continuous positive airway pressure), mechanical ventilation, intravenous fluid intake, the day of full enteral feeding, and death were recorded for all premature infants. Complete blood count was obtained from the patients during the first 3 days of life, mostly within the first 24 h. Nadir value was determined only for those with more than one platelet count available for assessment in the first 3 days of life. Blood samples were carefully taken from the peripheral or umbilical vein and collected in ethylene diamine tetra acetic acid (EDTA) tubes. Platelet counts, hemoglobin, hematocrit, white blood count, and MPV were analyzed by the ADVIA 2120i Hematology System (Siemens Healthcare Diagnostic Inc., IL, USA). Platelet mass was determined by multiplying the platelet count with the mean platelet volume (MPV). Blood collected into a siliconized glass tube containing buffed sodium citrate on 3rd day of life was tested on the PFA-100 with both collagen/epinephrine and collagen/adenosine diphosphate cartridges using the PFA-100 Analyzer (Siemens Healthcare Global, Innovance PFA-200 System, Marburg, Germany). The PFA-100 is a simple, rapid, in vitro method of assessing primary hemostasis that measures the closure time required for a platelet plug to occlude an aperture in a membrane coated with collagen in the presence of ADP or epinephrine. Because the method is user-friendly for the laboratory operator, it is widely used to study the platelet function disorder. The prolongation of closure time indicates the dysfunction of platelets.[9–11] Demographical factors, platelet count, mean platelet volume, platelet mass index (platelet count $\times$ MPV), and platelet function assessed by PFA-100 were compared between two groups of patients. Accordingly, the cut-off values of collagen-

epinephrine and collagen-ADP were 160 s and 130 s, respectively.[12] The study was approved by the institutional review board of Ankara University and informed consent was obtained from the parents of all patients.

2.1. Statistical analysis

A comparison between the groups was performed using the *t*-test and/or Mann–Whitney *U*-test for non-parametric continuous variables in independent-samples and chi-square or Fisher's exact tests as appropriate for categorical variables. The data were presented as mean and standard deviation and/or median (minimum–maximum) for continuous variables as well as percentage and distribution of frequency for categorical variables. Parameters were compared between the groups and statistical significance was assessed by logistic regression to determine the independent predictive value of a specific parameter. Logistic models were summarized in p values and adjusted odds ratios (OR) with estimated 95% confidence intervals (CI). Receiver operating characteristic curve was used to determine the cut-off for collagen-ADP values. Statistical analysis was performed with Statistical Package for Social Sciences (SPSS) version 15 for Windows (SPSS Inc., St. Louis, MO) and statistical significance was set at a two-sided p value of 0.05.

3. Results

One hundred and five preterm infants with GA $\leq$ 30 weeks and BW $\leq$ 1500 g were admitted to our NICU during the study period. Forty-five patients were excluded from study due to exclusion criteria, and the remaining 60 patients were included to the statistical analysis (Fig 1).

Mean GA and BW were 27.8 $\pm$ 1.7 (23.1–30.6) weeks and 1024 $\pm$ 285 (505–1500) g, respectively. Patients were divided into two groups: Group 1 with "open PDA" (n = 24, 40%) and group 2 with "closed PDA" (n = 36, 60%). Fifteen patients in "Open PDA" group needed to be treated with intravenous or oral ibuprofen or oral paracetamol, and two unresponsive cases were treated with surgical ligation (Fig 1). Mean GA and median BW were 27.6 $\pm$ 1.8 vs. 28 $\pm$ 1.6 weeks and 1025 (585–1480) g vs. 1117 (505–1500) g in "open PDA" and "closed PDA" groups, respectively ($p > 0.05$). Male gender, respiratory distress syndrome, retinopathy of prematurity (ROP), duration of NCPAP, BiPAP (bilevel positive airway pressure) and mechanical ventilation rates were higher in the "open PDA" group ($p < 0.05$). Intravenous fluid intake and the day of full enteral feeding were longer in the "open PDA" group ($p < 0.05$) (Table 1). There were no significant differences in terms of maternal age, mode of delivery, prenatal steroid use, 1- and 5-min APGAR scores, need for resuscitation between groups. Incidence of other clinical morbidities, such as IVH, any stage of NEC, ROP requiring laser treatment, BPD and death did not differ between the two groups of patients. Mean hemoglobin and hematocrit levels were lower in the "open PDA" group. Although there were no differences in platelet count, MPV, and platelet mass index in both groups of patients, more patients had Collagen-ADP >130 s in the "open PDA" group ($p < 0.05$). Collagen-epinephrine parameters did not differ between two groups of patients (Table 2). The application of cut-off value of 131 s of Collagen-ADP to predict the ductal patency showed 61.3% sensitivity and 72.7% specificity.

Multivariate logistic regression analysis showed an independent association of ductus patency and RDS (OR: 9 CI: 1.5–51.8) and collagen-ADP > 130 s (OR: 5.7 CI: 1.55–21.3).

4. Discussion

Our study demonstrated that higher collagen-ADP duration, as a platelet functional parameter, could be highlighted as an independent risk factor for ductal patency. Echtler et al. demonstrated that activated platelets adhered to and

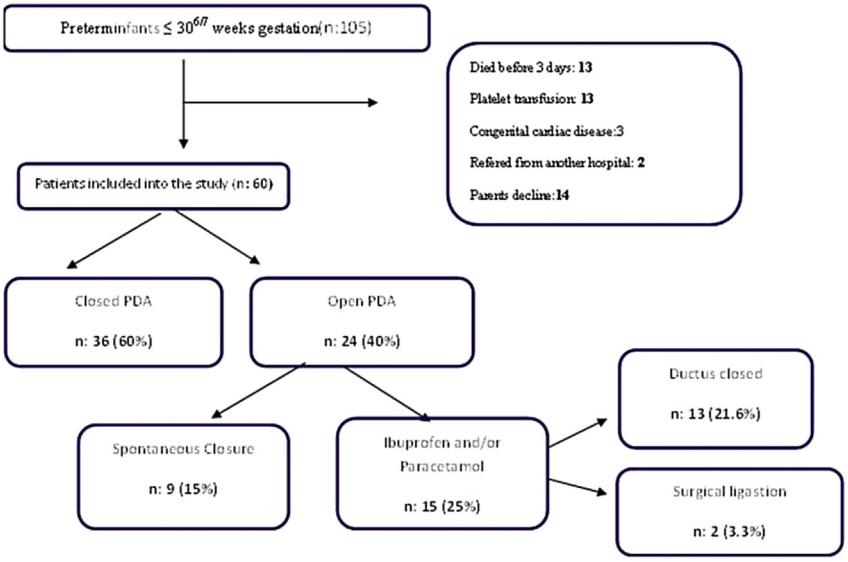

Figure 1 Flow chart of the study population.

Table 1 Demographical and clinical characteristics of Open PDA and Closed PDA groups.

	Open PDA (n:24)	Closed PDA (n:36)	p*
Male, n (%)	16 (66.7)	11 (30.6)	**0.006**
Gestational age, weeks, mean ± SD (min–max)	27.6 ± 1.8 (23.1–30.4)	28 ± 1.6 (23.4–30.6)	0.408
Birth weight, g, mean ± SD (min–max)	1009 ± 270 (585–1480)	1035 ± 298 (505–1500)	0.695
RDS, n (%)	22 (91.7)	21 (58.3)	**0.005**
CPAP, hours median (min–max)	108 (0–840)	16 (0–768)	**0.001**
BiPAP, hours, median (min–max)	24 (0–144)	0 (0–120)	**0.001**
MV, hours, median (min–max)	120 (0–1008)	0 (0–288)	**0.001**
ROP, n (%)	6 (37.5)	2 (6.5)	**0.013**
Intravenous fluid, day, median (min–max)	14,5 (5–48)	9,5 (3–37)	**0.029**
Full enteral feeding, day, median (min–max)	17 (9–38)	11 (6–38)	**0.015**

*Statistically significant p values are highlighted.
Abbreviations: PDA; patent ductus arteriosus, CPAP; continuous positive airway pressure, BiPAP; bilevel positive airway pressure, MV; mechanical ventilation, ROP; retinopathy of prematurity.

accumulated in the lumen of the constricted ductus arteriosus in mice DA within minutes after birth. They proposed that platelets might be crucial for ductal closure in mice by promoting thrombotic sealing of the constricted ductus and luminal remodeling, and they corroborated their findings with a retrospective study in the preterm infants.[3] However, some clinical studies did not demonstrate a relationship between low platelet count and spontaneous closure of DA.[13–15] The incidence of ductal closure was not related to the circulating platelet counts in preterm infants in our study. Shah et al. found that the ductal closure was not related with low platelet number in preterm babies. They noticed that low circulating platelet number is not responsible for persistent PDA. They adduced that severe thrombocytopenia due to alloimmune thrombocytopenia or Wiskott–Aldrich Syndrome, in term infants, was not associated with ductal closure. They also proposed that preterm infants with hypoxia were likely to have delayed ductal closure.[13] Although recent meta-analysis showed that low platelet count in the first day of life is a modest but significant risk factor for ductal patency, there was high variability of statistical results among studies.[6] A randomized, controlled trial of platelet transfusions failed to alter the incidence of PDA in thrombocytopenic premature infants.[14] Larger thrombocytes are enzymatically more active than the smaller ones.[16] Under normal function of platelet and endothelium, platelet plug formation may be influenced more by the platelet mass than by the platelet count because larger platelets may function better to form an effective platelet plug.[17] Platelet mass index can be applied to guide platelet transfusions.[18,19] Higher mean platelet volume (MPV) has been found to be a risk factor for occurrence of complications, such as RDS, BPD and neonatal sepsis in prematurity.[17,20,21] Dani et al. failed to demonstrate any influence of MPV on ductal patency.[22] Dizdar et al. could not find any correlation between MPV and ductal closure.[4] In our study, MPV and platelet mass index were not significantly different between two groups of patients. We also found a positive correlation between low hemoglobin level and ductal patency. Chen et al. assessed the influence of hemoglobin and perinatal factors on ductal patency and found that lower hemoglobin after birth was a risk factor; and they suggested that an increase in hemoglobin might increase the PaO_2 and promoted the ductal closure.[23] Collagen-ADP duration was found to be positively correlated to ductal

Table 2 Comparison of hematologic parameters of two groups.

	Open PDA (n:24)	Closed PDA (n:36)	p*
Hb, g/dl, mean ± SD	13.7 ± 2.76	15.6 ± 2.4	**0.006**
Hct, %, mean ± SD	41.4 ± 8.4	47.3 ± 7.3	**0.006**
WBC, median (min–max)	6550 (2700–31,800)	7350 (3000–61,400)	0.428
Platelet, median (min–max)	181,000 (64,000–277,000)	189,500 (52,000–580,000)	0.512
MPV, fL, median (min–max)	8.2 (7–9.8)	8 (7–10.2)	0.492
Platelet mass index, median (min–max)	1448 (467–2246)	1473 (530–4872)	0.597
Collagen-epinephrine, median (min–max)	250 (128–300)	174 (65–300)	0.219
Collagen-epinephrine, n, (%)			
≤160	7 (29.2)	14 (38.9)	0.439
>160	17 (70.8)	22 (61.1)	
Collagen-ADP, median (min–max)	146 (70–300)	109 (53–300)	**0.05**
Collagen-ADP, n (%)			
≤130	6 (27.3)	17 (54.8)	**0.046**
>130	16 (72.7)	14 (45.2)	

*Statistically significant p values are highlighted.
Abbreviations: Hb; hemoglobin, Hct: hematocrit, WBC; white blood cell, MPV; mean platelet volume.

patency in our study. We suggest that impaired platelet function may play an important role in the pathogenesis of ductal patency in prematurity. Decreased expression of P-selectin and procaspase activating compound-1 (PAC-1) and decreased platelet adhesion cause impaired platelet function in preterm infants.[9] In preterm infants, platelet deposition on extracellular matrix under shear conditions was less than it was in term infants, despite comparable levels of vWF antigen and ristocetin cofactor activity, which causes impaired platelet adhesion.[24,25] Agonist-induced secretion of platelet granules is reduced in the preterm infants due to immature signal transduction pathways.[26]

We found no correlation between "open PDA" and collagen-epinephrine. The most likely explanation may be the presence of fewer α2-adrenergic receptors in neonates, which results in profound hyporesponsiveness to epinephrine.[9,10,24]

Our study has some limitations. First, the size of the study population was small and came from a single center. Second, PFA-100 analysis may be not so sensitive to platelet dysfunction as to platelet aggregometry. However, the blood volume required for aggregometry is relatively large; thus, PFA-100 has been recommended as a screening test for platelet function in premature infants.[27,28]

In conclusion, longer collagen-ADP duration is highlighted as a risk factor for ductal closure.

To the best of our knowledge, this is the first study in the English literature that provides clinical evidence for the effect of platelet dysfunction on spontaneous closure of DA.

Conflict of interest

The authors declare that there is no conflict of interest.

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