

Original Article

Cite this article: Sallmon H, Metze B, Koehne P, Opgen-Rhein B, Weiss K, Will JC, Franke CV, Hansmann G, Koestenberger M, Bührer C, Berger F, Weber SC, and Cremer M (2020) Mature and immature platelets during the first week after birth and incidence of patent ductus arteriosus. *Cardiology in the Young* 30: 769–773. doi: [10.1017/S1047951120000943](https://doi.org/10.1017/S1047951120000943)

Received: 23 February 2020

Revised: 26 March 2020

Accepted: 2 April 2020

First published online: 28 April 2020

Keywords:



Preterm infant; very low birth weight; patent ductus arteriosus; platelets; IPF; thrombocytopenia

Author for correspondence:

Hannes Sallmon, MD, Department of Pediatric Cardiology, Charité Universitätsmedizin Berlin, Augustenburger Platz 1, Berlin 13353, Germany. Tel: +49 450 566 107; Fax: +49 450 566 927. E-mail: hannes.sallmon@charite.de

*These authors contributed equally to this work.

Mature and immature platelets during the first week after birth and incidence of patent ductus arteriosus

Hannes Sallmon^{1,2} , Boris Metze², Petra Koehne², Bernd Opgen-Rhein¹, Katja Weiss¹, Joachim C. Will¹, Christina Victoria Franke², Georg Hansmann³ , Martin Koestenberger⁴, Christoph Bührer², Felix Berger^{1,5,6}, Sven C. Weber^{1,*} and Malte Cremer^{2,*}

¹Department of Pediatric Cardiology, Charité University Medical Center, Berlin, Germany; ²Department of Neonatology, Charité University Medical Center, Berlin, Germany; ³Department of Pediatric Cardiology and Critical Care, Hannover Medical School, Hannover, Germany; ⁴Division of Pediatric Cardiology, Medical University Graz, Graz, Austria; ⁵Department of Congenital Heart Disease and Paediatric Cardiology, Deutsches Herzzentrum Berlin, Berlin, Germany and ⁶Deutsches Zentrum für Herz- und Kreislaufforschung (DZHK), Berlin, Germany

Abstract

Background: Thrombocytopenia is a risk factor for patent ductus arteriosus. Immature and mature platelets exhibit distinct haemostatic properties; however, whether platelet maturity plays a role in postnatal, ductus arteriosus closure is unknown. **Methods:** In this observational study, counts of immature and mature platelets (=total platelet count – immature platelet count) were assessed on days 1, 3, and 7 of life in very low birth weight infants (<1500 g birth weight). We performed echocardiographic screening for haemodynamically significant patent ductus arteriosus on day 7. **Results:** Counts of mature platelets did not differ on day 1 in infants with (n = 24) and without (n = 45) haemodynamically significant patent ductus arteriosus, while infants with significant patent ductus arteriosus exhibited lower counts of mature platelet on postnatal days 3 and 7. Relative counts of immature platelets (fraction, in %) were higher in infants with patent ductus arteriosus on day 7 but not on days 1 and 3. Receiver operating characteristic curve analysis unraveled associations between both lower mature platelet counts and higher immature platelet fraction (percentage) values on days 3 and 7, with haemodynamically significant ductus arteriosus. Logistic regression analysis revealed that mature platelet counts, but not immature platelet fraction values, were independent predictors of haemodynamically significant patent ductus arteriosus. **Conclusion:** During the first week of postnatal life, lower counts of mature platelets and higher immature platelet fraction values are associated with haemodynamically significant patent ductus arteriosus. Lower counts of mature platelet were found to be independent predictors of haemodynamically significant patent ductus arteriosus.

A patent ductus arteriosus in preterm infants is frequently associated with severe cardio-pulmonary complications, such as left ventricular volume overload, pulmonary oedema, and impairment of lung compliance.^{1–3} Thus, understanding the mechanisms that control ductal closure is pivotal in order to provide tailored care to at-risk neonates.⁴ Previously, Echter et al. reported that platelets are recruited to the ductal endothelium after birth and contribute to ductal closure and subsequent vascular remodelling.⁵ However, the clinical significance of these animal experiments is still controversial. Low platelet counts are frequently observed among preterm infants, but several studies on their role in spontaneous or pharmacological ductus arteriosus closure produced ambiguous or conflicting results.^{6–8} We previously reported that lower platelet counts on the first postnatal day were not associated with a higher incidence of patent ductus arteriosus in 1430 very low birth weight infants.⁹ However, in infants who underwent pharmacological treatment with cyclooxygenase inhibitors (ibuprofen/indomethacin) for haemodynamically significant patent ductus arteriosus, low platelet counts during the first postnatal week contributed to an increased rate of treatment failure.¹⁰ Collectively, these results suggest that the development of thrombocytopenia during the first week of postnatal life contributes to failure of ductus arteriosus closure.

The immature platelet fraction is a novel innovative tool to examine platelet production from peripheral blood samples.⁶ Immature platelet fraction values have been shown to predict the course of thrombocytopenia in preterm neonates and thus may prove useful in guiding platelet transfusion management.^{11,12} Furthermore, immature platelets have distinct functional features that might affect haemostasis independently from total platelet counts.¹³ To date, the proportion of mature and immature platelet counts and their possible association with haemodynamically significant patent ductus arteriosus have not been evaluated in

© The Author(s), 2020. Published by Cambridge University Press. This is an Open Access article, distributed under the terms of the Creative Commons Attribution licence (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted re-use, distribution, and reproduction in any medium, provided the original work is properly cited.

preterm neonates. In this observational study, we investigated the number of mature and immature platelets during the first week of postnatal life and their possible association with the incidence of haemodynamically significant patent ductus arteriosus in very low birth weight preterm infants.

Methods

Patients, blood parameters, and clinical data

The study was conducted at the neonatal ICU, Department of Neonatology, Charité University Medical Center, Berlin, Germany (2009–2012). Blood sampling was performed at day 1, day 3 (± 1 day), and day 7 (± 2 days) after birth. Blood count parameters were measured using a Sysmex XE-2100 (Sysmex, Kobe, Japan) blood analyser as previously described.^{11,12} Immature platelet values are reported as absolute values per nanolitre (immature platelet count) and as percentage (immature platelet fraction). Mature platelet counts were obtained by using the following formula: mature platelet count = total platelet count – immature platelet count. The infants were grouped according to patent ductus arteriosus status (echocardiography on day 7, see below). Clinical data were obtained by chart reviews. All very low birth weight infants (birth weight < 1500 g) admitted to the unit were eligible if no cardiac malformation or other genetic or syndromic disorders were present. Parental written consent for blood sampling and data collection was obtained after patient admission to the neonatal ICU. The study has been approved by the local ethics committee of Charité – Universitätsmedizin Berlin, Germany (EA1/229/08).

Diagnosis of haemodynamically significant patent ductus arteriosus

Infants were examined for a haemodynamically significant patent ductus arteriosus on postnatal day 7 (± 2 days) as previously described.^{9,14,15} Echocardiographic evaluation included shunt direction of a patent ductus arteriosus in colour Doppler mode (high upper parasternal short axis) as well as the minimal internal ductal diameter detected within three to five measurements taken in B-mode. The left atrium to aortic root ratio was measured by M-mode (parasternal long axis). Doppler measurement of the resistance index (anterior cerebral artery) was performed at the same time. A patent ductus arteriosus with left-to-right shunt was considered haemodynamically significant if a respiratory set back with a supplemental oxygen requirement > 30% and/or mechanical ventilation, a left atrium to aortic root ratio ≥ 1.4 in the echocardiogram, ductal diameter ≥ 2.5 mm, and/or a decreased end-diastolic flow in the anterior cerebral artery with a resistance index ≥ 0.85 in the cerebral ultrasound were present. There has been no change in the clinical and echocardiographic criteria used to determine haemodynamic significance of a patent ductus arteriosus during the study period.

Statistics

Values are expressed as absolute number of patients or percentage values unless stated otherwise. Demographic characteristics are given as median and range. Comparisons between groups were made by Kruskal–Wallis or Mann Whitney U tests as appropriate for continuously scaled data and by chi-square test for categorical data. *p*-Values < 0.05 were considered statistically significant. Statistical analyses, including receiver operating characteristic curve analysis and linear regression analysis, were carried out using IBM SPSS Statistics 22 software.

Table 1. Demographic characteristics of the study population

	All VLBW	With hsPDA	Without hsPDA	<i>p</i> -Values
n (%)	69	24 (34.8)	45 (65.2)	–
Female, n (%)	23 (33.3)	6 (25.0)	17 (37.8)	0.28
BW (g)				< 0.01
Median	970	657	1160	
Mean	973	720	1108	
Range	382–1495	383–955	382–1495	
GA (weeks)				< 0.01
Median	28 2/7	24 6/7	28 6/7	
Mean	27 5/7	25 0/7	29 2/7	
Range	23 2/7–33 2/7	23 2/7–31 1/7	25 2/7–33 2/7	

BW = birth weight; GA = gestational age; hsPDA = haemodynamically significant patent ductus arteriosus; VLBW = very low birth weight.

Results

Clinical data and incidence of haemodynamically significant patent ductus arteriosus

A total of 69 very low birth weight infants were included in the study. Of 69 infants, 24 were diagnosed with a haemodynamically significant patent ductus arteriosus (34.8%). Infants with haemodynamically significant patent ductus arteriosus were more immature at birth (25 0/7 versus 29 2/7, *p* < 0.01) and exhibited a lower birth weight (mean 1108 g versus 702 g, *p* < 0.01) while gender distribution did not differ significantly between groups (Table 1).

Mature and immature platelets during the first week of life

The mature platelet counts on postnatal day 1 did not differ significantly between infants with and without haemodynamically significant patent ductus arteriosus (*p* = 0.068). However, mature platelet counts were significantly lower in infants with haemodynamically significant patent ductus arteriosus on days 3 and 7 of life (*p* = 0.036 and < 0.01, respectively, Fig 1). Infants with and without haemodynamically significant patent ductus arteriosus did not show any significant differences in immature platelet fraction (percentage) values on days 1 and 3 of life (*p* = 0.46 and 0.07, respectively), while infants with haemodynamically significant patent ductus arteriosus exhibited higher immature platelet fraction (percentage) values on postnatal day 7 (*p* < 0.01). Of note, the absolute immature platelet counts were similar between groups at all time points (Fig 1).

Receiver operating characteristic curve analysis (platelet count, immature platelet fraction, gestational age, and birth weight)

Receiver operating characteristic curve analyses were performed in order to further characterise the relationship between mature and immature platelets, birth weight, gestational age, and haemodynamically significant patent ductus arteriosus (Table 2; Fig 2). Significant associations were found for gestational age, birth weight, and mature platelet counts on days 3 and 7, which all showed an area under the curve > 0.5, indicating a negative correlation between these variables and the incidence of a haemodynamically significant patent ductus arteriosus. Immature platelet fraction (percentage) values on days 3 and 7 of life exhibited an area under the curve < 0.5 and, thus, a positive

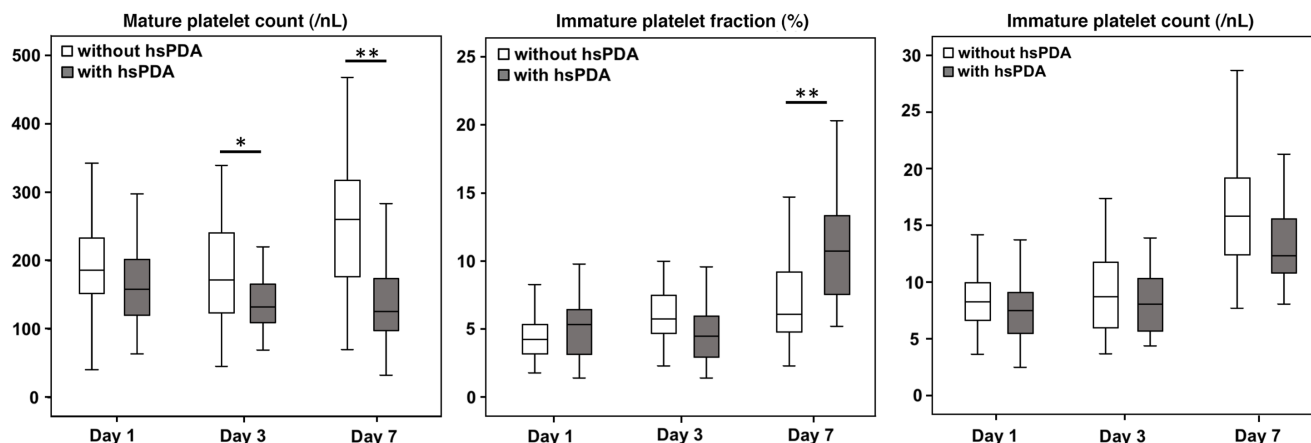


Figure 1. Boxplot: Mature platelet count and immature platelets (percentage and absolute count) in very low birth weight infants with and without haemodynamically significant patent ductus arteriosus. The boxplots display median, and 25th and 75th percentiles among the different groups. hsPDA = haemodynamically significant patent ductus arteriosus. Mature platelet and immature platelet counts are given per nanolitre. * $p < 0.05$, ** $p < 0.01$.

correlation with haemodynamically significant patent ductus arteriosus (Table 2; Fig 2).

Logistic regression analysis

In addition, logistic regression analysis was performed to investigate whether mature platelet counts and immature platelet fraction values on postnatal day 7 are independent risk factors for haemodynamically significant patent ductus arteriosus. While gestational age, birth weight, and mature platelet counts on day 7 were identified as independent risk factors for haemodynamically significant patent ductus arteriosus, immature platelet fraction (percentage) on postnatal day 7 was not found to be an independent predictor of haemodynamically significant patent ductus arteriosus ($p = 0.585$; Table 3).

Discussion

The role of platelets in ductal closure has first been demonstrated in murine models.⁵ Observational studies on the clinical significance of this finding in preterm infants have yielded conflicting results. However, two recent meta-analyses indicate a moderate association between low platelet counts in preterm infants and failure of spontaneous and pharmacologically induced ductus arteriosus closure.^{7,8} We previously demonstrated in 1430 very low birth weight infants that low platelet counts within the first 24 hours after birth were not associated with patent ductus arteriosus, while low platelet counts before and during cyclooxygenase inhibitor treatment were moderately associated with pharmacological ductus arteriosus treatment failure.^{9,10} Interestingly, a recent randomised controlled trial could not demonstrate any positive effect of platelet transfusions on patent ductus arteriosus in preterm infants.¹⁶ We and others therefore hypothesised that impaired platelet function, rather than platelet number, contributes to spontaneous or pharmacologically induced ductus arteriosus closure in preterm infants.^{17–19} Two recent studies support this hypothesis: Collagen-adenosine-diphosphate closure times > 130 seconds (platelet function analyser) were independently associated with ductal patency.²⁰ Moreover, impaired platelet function due to inflammation may contribute to failure of ductal closure in preterm infants with infection.²¹

The immature platelet fraction is a modern laboratory parameter that assesses the number of newly released platelets from peripheral blood samples. Immature platelet fraction measurements have been reported in preterm neonates and may prove useful in guiding platelet transfusion therapy.^{11,12,22} Similar to the reticulocyte count in anaemia, the immature platelet fraction increases in response to thrombocytopenia, if megakaryopoiesis and thrombopoiesis are intact.⁶ A recent study found an association between immature platelet fraction (percentage) and bleeding risk in children with immune thrombocytopenic purpura, independently from platelet counts.²³ The latter results support previous findings on immature platelets having distinct functional properties which affect their haemostatic potential.¹³

Based on these observations, we hypothesised that, due to their distinct functional properties, mature and immature platelets are independently associated with incidence of haemodynamically significant ductus arteriosus in preterm infants. Here, for the first time, we show that lower absolute counts of mature platelets on days 3 and 7, but not on day 1 after birth, are associated with ductal patency.^{9,10}

In addition, we found higher immature platelet fraction (percentage) values in infants with haemodynamically significant patent ductus arteriosus as compared to those without. However, logistic regression analysis revealed that only mature platelet counts on postnatal day 7, in contrast to immature platelet fraction values, were independently associated with haemodynamically significant patent ductus arteriosus in our cohort. Our results suggest that lower counts of mature platelet contribute to a higher incidence of haemodynamically significant patent ductus arteriosus. In contrast, immature platelet fraction values, which can be regarded as a physiological response to thrombocytopenia in preterm infants, do not independently affect ductal closure. Of note, we found similar results indicating a role for lower mature platelet counts in failed spontaneous ductal closure when comparing all infants with patent ductus arteriosus (not only haemodynamically significant ones) to those without a patent ductus arteriosus (data not shown).

Our study is limited by its relatively low sample size. Thus, we were unable to assess a potential association between low mature platelet counts and treatment failure as only 24 infants underwent pharmacological treatment for patent ductus arteriosus in our cohort. In addition, the current study did not address a potential

Table 2. ROC curve analyses in VLBW and ELBW infants

	AUC	95% CI
MPC d1	0.625	0.481–0.769
MPC d3	0.664	0.531–0.796
MPC d7	0.854	0.762–0.946
IPF (%) number d1	0.429	0.277–0.581
IPF (%) number d3	0.338	0.203–0.473
IPF (%) number d7	0.202	0.093–0.310
IPC (#) number d1	0.574	0.423–0.726
IPC (#) number d3	0.463	0.308–0.617
IPC (#) number d7	0.606	0.459–0.753
Birth weight	0.833	0.308–0.933
Gestational age	0.935	0.459–0.996

Statistical significance is indicated by bold values.

AUC = area under the curve; CI = confidence interval; d = day of life; ELBW = extremely low birth weight; IPC (#) = immature platelet count; IPF (%) = immature platelet fraction (percentage); MPC = mature platelet count; ROC = receiver operating characteristic; VLBW = very low birth weight.

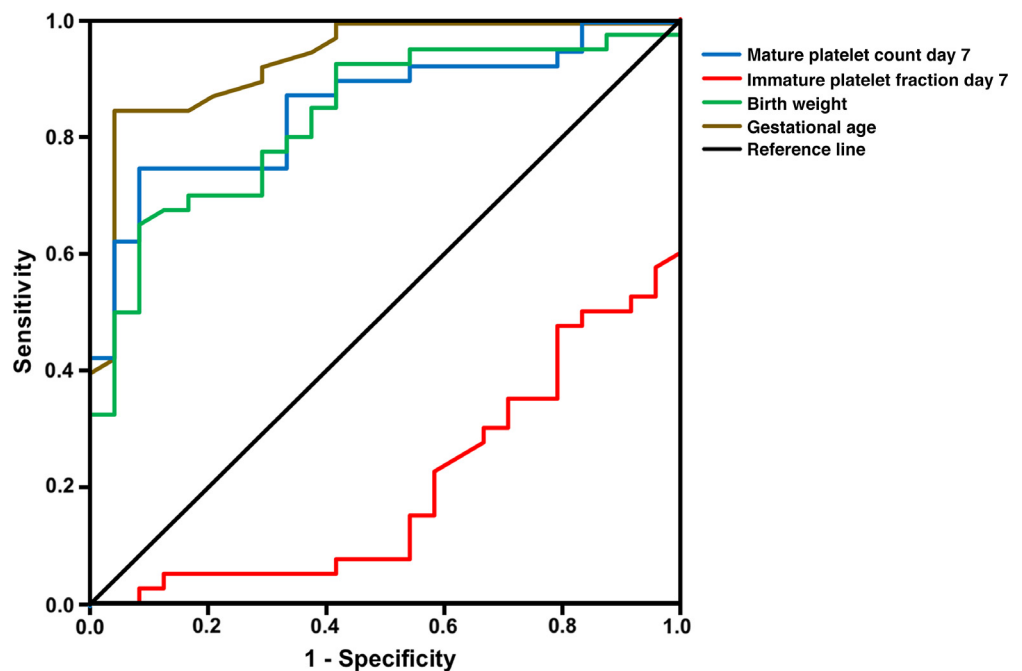
Table 3. Logistic regression analysis for prediction of haemodynamically significant patent ductus arteriosus

	Regression coefficient (B)	Standard error	Wald	Df	Significance level (p-values)	Exponent (B)
Birth weight	0.007	0.003	4.276	1	0.041	1.007
Gestational age	−1.789	0.554	10.44	1	0.001	0.167
IPF day 7	−0.037	0.147	0.62	1	0.803	0.964
MPC day 7	−0.018	0.008	5.38	1	0.020	0.982

66/69 (95.7%) of cases with a complete dataset were included in this analysis. Statistical significance is indicated by bold values.

Df = degree of freedom; IPF = immature platelet fraction (percentage); MPC = mature platelet count; Wald = Wald chi-square test.

Figure 2. Receiver operating characteristic (ROC) curve analyses. ROC curve analysis for mature platelet counts and immature platelet fraction (percentage) on day 7 of life, birth weight, and gestational age comparing infants with and without haemodynamically significant patent ductus arteriosus (hsPDA). All ROC curves presented in this figure show an unequal division resulting in an area under the curve (AUC) of > 0.5 or < 0.5 (for confidence intervals, see Table 2). An AUC > 0.5 (gestational age, birth weight, and mature platelet day 7) indicates a negative and an AUC < 0.5 (IPF day 7) a positive correlation between the variable and hsPDA incidence. IPF = immature platelet fraction (percentage); MPC = mature platelet count.



association between inflammation, as seen in septic preterm neonates, mature and immature platelets, and ductus arteriosus. Furthermore, ductal closure is a process that is characterised by a complex interplay between molecular and morphological changes within the vessel wall, which, in addition to platelets, is triggered by vascular adhesion and migration of mononuclear blood cells to the muscular layer of the vessel wall.²⁴ Assessing those complex molecular and cellular processes requires a highly sophisticated experimental design and is beyond the scope of this paper. Thus, further research is required to elucidate the complex interplay between immature and mature platelets on ductal closure and to assess their specific functions and association with bleeding risk and thromboembolic complications in preterm infants.^{25,26}

Acknowledgement. We thank Silke Wilitzki for her expert assistance.

Financial Support. This research received no specific grant from any funding agency, commercial, or not-for-profit sectors.

Conflicts of Interest. Malte Cremer served as an advisory board member for Sysmex Deutschland GmbH, Hamburg, Germany.

Ethical Standards. The study was carried out in accordance with national and international recommendations, including the Declaration of Helsinki, and has been approved by the local ethics committee (EA1/229/08). Written informed parental consent was obtained.

References

- Sallmon H, Koehne P, Hansmann G. Recent advances in the treatment of preterm newborn infants with patent ductus arteriosus. *Clin Perinatol* 2016; 43: 113–129.
- Hamrick SE, Hansmann G. Patent ductus arteriosus of the preterm infant. *Pediatrics* 2010; 125: 1020–1030.
- Vrancken SL, van Heijst AF, de Boode WP. Neonatal Hemodynamics: From Developmental Physiology to Comprehensive Monitoring. *Front Pediatr* 2018; 6: 87.
- Gillam-Krakauer M, Hagadorn JJ, Reese J. Pharmacological closure of the patent ductus arteriosus: when treatment still makes sense. *J Perinatol* 2019; 39: 1439–1441.
- Echtler K, Stark K, Lorenz M et al. Platelets contribute to postnatal occlusion of the ductus arteriosus. *Nat Med* 2010; 16: 75–82.
- Cremer M, Sallmon H, Kling PJ, Buhner C, Dame C. Thrombocytopenia and platelet transfusion in the neonate. *Semin Fetal Neonatal Med* 2016; 21: 10–18.
- Simon SR, van Zogchel L, Bas-Suarez MP, Cavallaro G, Clyman RI, Villamor E. Platelet counts and patent ductus arteriosus in preterm infants: a systematic review and meta-analysis. *Neonatology* 2015; 108: 143–151.
- Mitra S, Chan AK, Paes BA. The association of platelets with failed patent ductus arteriosus closure after a primary course of indomethacin or ibuprofen: a systematic review and meta-analysis. *J Matern Fetal Neonatal Med* 2017; 30: 127–133.
- Sallmon H, Weber SC, Huning B et al. Thrombocytopenia in the first 24 hours after birth and incidence of patent ductus arteriosus. *Pediatrics* 2012; 130: e623–630.
- Sallmon H, Weber SC, Dirks J et al. Association between platelet counts before and during pharmacological therapy for patent ductus arteriosus and treatment failure in preterm infants. *Front Pediatr* 2018; 6: 41.
- Cremer M, Paetzold J, Schmalisch G et al. Immature platelet fraction as novel laboratory parameter predicting the course of neonatal thrombocytopenia. *Br J Haematol* 2009; 144: 619–621.
- Cremer M, Weimann A, Schmalisch G, Hammer H, Buhner C, Dame C. Immature platelet values indicate impaired megakaryopoietic activity in neonatal early-onset thrombocytopenia. *Thromb Haemostasis* 2010; 103: 1016–1021.
- Lador A, Leshem-Lev D, Spectre G, Abelow A, Kornowski R, Lev EI. Characterization of surface antigens of reticulated immature platelets. *J Thromb Thrombolysis* 2017; 44: 291–297.
- Groves AM, Singh Y, Dempsey E, et al. Introduction to neonatologist-performed echocardiography. *Pediatr Res* 2018; 84 (Suppl 1): 1–12.
- van Laere D, van Overmeire B, Gupta S, et al. Application of NPE in the assessment of a patent ductus arteriosus. *Pediatr Res* 2018; 84 (Suppl 1): 46–56.
- Kumar J, Dutta S, Sundaram V, Saini SS, Sharma RR, Varma N. Platelet transfusion for PDA closure in preterm infants: a randomized controlled trial. *Pediatrics* 2019; 143:e20182565.
- Sallmon H, Weber SC, von Gise A, Koehne P, Hansmann G. Ductal closure in neonates: a developmental perspective on platelet-endothelial interactions. *Blood Coagul Fibrinolysis* 2011; 22: 242–244.
- Ali Engür M, Engür D. Platelet-rich plasma for patent ductus arteriosus: an orthopaedic surgeon's perspective. *Cardiol Young*. 2014;24(3):385–387.
- Sallmon H, Barikbin P, Koehne P, von Gise A, Hansmann G. Platelet-rich plasma for the treatment of patent ductus arteriosus: not quite ready for prime time. *Cardiol Young* 2015; 25: 139–140.
- Kahvecioglu D, Erdev O, Akduman H et al. Influence of platelet count, platelet mass index, and platelet function on the spontaneous closure of ductus arteriosus in the prematurity. *Pediatr Neonatol* 2018; 59: 53–57.
- Meinarde L, Hillman M, Rizzotti A, Basquiera AL, Tabares A, Cuestas E. C-reactive protein, platelets, and patent ductus arteriosus. *Platelets* 2016; 27: 821–823.
- MacQueen BC, Christensen RD, Henry E, et al. The immature platelet fraction: creating neonatal reference intervals and using these to categorize neonatal thrombocytopenias. *J Perinatol* 2017; 37: 834–838.
- McDonnell A, Bride KL, Lim D, Paessler M, Witmer CM, Lambert MP. Utility of the immature platelet fraction in pediatric immune thrombocytopenia: Differentiating from bone marrow failure and predicting bleeding risk. *Pediatr Blood Cancer* 2018; 65. doi: [10.1002/pbc.26812](https://doi.org/10.1002/pbc.26812).
- Waleh N, Seidner S, McCurnin D, et al. Anatomic closure of the premature patent ductus arteriosus: The role of CD14+/CD163+ mononuclear cells and VEGF in neointimal mound formation. *Pediatr Res* 2011; 70: 332–338.
- Ibrahim H, Schutt RC, Hannawi B, DeLao T, Barker CM, Kleiman NS. Association of immature platelets with adverse cardiovascular outcomes. *J Am Coll Cardiol* 2014; 64: 2122–2129.
- Matter RM, Ragab IA, Roushdy AM, Ahmed AG, Aly HH, Ismail EA. Determinants of platelet count in pediatric patients with congenital cyanotic heart disease: Role of immature platelet fraction. *Congenit Heart Dis* 2018; 13: 118–123.