

Evaluation of platelet function by Total Thrombus-Formation Analysis System (T-TAS®01) in term and preterm infants and its relationship with patent ductus arteriosus. A prospective observational pilot study

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Background - Newborns exhibit a pro-coagulant hemostatic profile despite platelet hyporeactivity and reduced coagulation factors. Assessing infant hemostasis, particularly in preterm infants, is challenging, with inconsistent findings regarding the relationship between platelet count and function in patients with patent ductus arteriosus (PDA).

Materials and methods - This study aims to assess platelet function using the Total Thrombus-Formation Analysis System (T-TAS®01) in term and preterm newborns. T-TAS®01 measures the Occlusion Start Time (OST), Occlusion Time (OT), and the Area Under the Curve (AUC) at the end of thrombus formation. The study includes term and preterm newborns below 30 weeks' gestational age (GA) admitted to the Neonatal Intensive Care Unit. Blood samples were collected from preterm newborns on the 1st day of life (T0), between 48-72 hours of life (T1), between the 7th and 10th day of life (T2), and from term newborns at T0 and T2. Secondary endpoints include the relationship between T-TAS®01 parameters and significant PDA in preterm newborns and the correlation between T-TAS®01 parameters, GA, and complete blood count (CBC).

Results - OST is delayed by 65.5 seconds in preterm infants at T0 ($p<0.001$) and by 46 seconds at T2 ($p=0.041$) compared to full-term newborns. OT is delayed by 164 seconds in preterm infants at T0 ($p=0.002$) and by 352 seconds at T2 ($p=0.002$). AUC at T0 is lower in preterm infants ($p=0.028$). There is no significant correlation between T-TAS®01 parameters and GA or CBC. Additionally, OST and OT are delayed, and AUC is reduced in preterm infants with PDA and hemodynamically significant PDA (hsPDA).

Discussion - T-TAS®01 is a reliable tool for evaluating platelet function in term newborns. However, measurements show higher variability in preterm infants, with significantly lower platelet activity observed in preterm infants with PDA and hsPDA.

Keywords: newborn, T-TAS®01, platelets, PDA, HsPDA.

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INTRODUCTION

Newborns carry a higher risk of bleeding when compared to adults and children, especially if born prematurely¹. About 25% of infants admitted to the Neonatal Intensive Care Unit (NICU) experience bleeding, particularly those with gestational age (GA) below 28 weeks².

Previous studies regarding platelet function showed that newborn platelets are hyporeactive compared to adults²⁻⁹. Moreover, platelet activation and responsiveness to agonists, as well as secretion of granule content, are reduced^{2,7,10,11}. However, adhesion is increased¹² due to a greater quantity of Von Willebrand factor (VWF), its multimeric form, and higher hematocrit (Ht) and mean corpuscular volume (MCV), which enhance the contact between platelets and endothelium^{3,13-15}.

In very low birth weight (VLBW) infants, platelets have a maximum *hyporeactivity* around 3-4 days of life, which tends to normalize at 10-14 days^{8,9}.

Moreover, newborns also have a reduced concentration of most coagulation factors, so prothrombin time (PT) and activated partial thromboplastin time (aPTT) are physiologically prolonged in the first days of life.

Though hyporeactive platelets and reduced coagulation factors would suggest defective hemostasis, it is now well accepted that hemostasis, even in preterm newborns, is balanced at birth due to the features mentioned above of primary hemostasis and a concurrent physiologic reduced concentration of anticoagulant factors. Furthermore, as demonstrated by several authors, VLBW infants show a trend towards a pro-coagulant profile after the first days of life¹⁶⁻¹⁹.

Patent ductus arteriosus (PDA) is a very common finding in preterm newborns with an incidence that increases with decreasing GA, occurring in 33% of VLBW infants and up to 65% of all extremely low birth weight (ELBW) infants²⁰⁻²². Platelet numbers and functions are also thought to have a role in the pathophysiology of PDA since Echtler *et al.* reported their contribution to its closure and subsequent vascular remodeling in mice²³. However, the clinical significance of these findings in human preterm infants is still controversial²⁴.

The association between platelet count and PDA has been investigated within the past decade with uneven findings²⁴. Recent reviews suggest that thrombocytopenia and platelet dysfunction contribute to the failure of

spontaneous and pharmacological PDA closure in preterm infants²⁵⁻²⁸. However, platelet contribution to PDA seems to have only moderate clinical significance. Regarding platelet function, Kahvecioglu *et al.* found a significantly longer PFA-100 closure time in preterm patients with PDA²⁹.

Our study aims to explore platelet function in term and preterm newborns through the Total Thrombus-Formation Analysis System (T-TAS[®]01) and to evaluate the potential correlation between T-TAS[®]01 parameters and PDA closure.

MATERIALS AND METHODS

This is an observational, prospective, monocentric, non-pharmacological cohort study carried out in the NICU of Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico of Milan, Italy.

The procedures followed in this study conformed to the tenets of the Declaration of Helsinki and were approved by the local Ethics Committee (Milan Area 2, Italy) with approval number 622-2021, and written informed consent was obtained from parents or legal guardians.

All full-term (GA ≥ 37 weeks) and preterm (GA ≤ 30 weeks) infants admitted to the NICU from February 26th to September 1st, 2021, were enrolled. Exclusion criteria were chromosomal diseases, alloimmune or autoimmune neonatal thrombocytopenia, metabolic diseases inducing bone marrow aplasia, and platelet transfusion before recruitment. Furthermore, concerning preterm infants alone, we excluded newborns diagnosed with congenital heart disease or pulmonary hypertension.

The primary endpoint was to detect any difference in platelet function parameters among full-term and ≤ 30 weeks' GA newborns and the correlation between T-TAS[®]01 (Fujimori Kogyo Co., Tokyo, Japan) parameters and platelet count, mean platelet volume (MPV), and Ht in the newborn.

The secondary endpoint evaluated the relationship between platelet function assessed with T-TAS[®]01 and hemodynamically significant PDA (hsPDA) and PDA in preterm infants and the correlation between T-TAS[®]01 parameters and GA and complete cell count (CBC).

We collected data on maternal demographics and medical history during pregnancy, such as information on multiple gestations, exposure to non-steroidal

anti-inflammatory drugs (NSAIDs) and steroids for prenatal prophylaxis of hyaline membrane disease, intrauterine growth restriction, maternal diabetes, hypertension, chorioamnionitis, congenital infections³⁰⁻³⁵. We also collected data on perinatal period as GA (gestational age), sex, birth weight (BW), and whether the baby was small for gestational age (SGA), adequate for gestational age (AGA), or large for gestational age (LGA), defined as below the 10th percentile, between the 10th and 90th percentile, or above the 90th percentile, respectively³⁶. PDA was defined as any detectable blood flow across the ductus by color Doppler. While hsPDA was defined as a ductus with diameter ≥ 1.5 mm; left-right shunt $>70\%$ of the cardiac cycle; maximum speed through the ductus <2 m/s; left atrium to aorta ratio >1.5 ; LVO (left ventricular output) ≥ 250 mL/kg/min; end-diastolic flow in the left pulmonary artery ≥ 0.2 m/s and/ or absent or reverse diastolic flow in descending aorta³⁷.

Platelet function was measured by T-TAS®01 during the first ten days of life. According to the timeline (Online Supplementary Figure S1), blood samples were collected from preterm newborns at the day of life (DOL) 1st (To), at 48-72 hours (T1), and between DOL 7th and 10th (T2), and from term newborns at To and T2. At the same time of the collection, platelet count, MPV, and Ht were measured (Online Supplementary Figure S1). Blood was drawn through nonheparinized venous or arterial lines or direct venipuncture or capillary puncture from heel prick by other scheduled blood tests for routine or emergency evaluations to minimize distress. According to the study design, at least 2 whole blood samples (400 μ L) were performed for each patient.

The TTAS machine was located in the NICU, and the tests were performed at the bedside by properly trained NICU personnel.

Platelet thrombus formation was assessed using a T-TAS®01 device containing 330 μ L of whole blood stored at room temperature in benzylsulfonyl-D-arg-pro-4-amidinobenzylamide (BAPA) T-TAS®01 tubes. The blood sample was collected from the tube within 4 hours and then loaded into a microchip consisting of 26 collagen-coated capillaries. The instrument applied a pressure resulting in an 18 μ L/min flow with an initial shear stress of approximately 2000 s⁻¹. The capillaries

clogged when platelets adhered to the collagen and aggregated in the microchip.

The T-TAS®01 instrument provided the following parameters, as shown in Online Supplementary Figure S2, where a pressure versus time curve is plotted: 1. Occlusion Start Time (OST) is the time required to reach the Occlusion Start Pressure (OSP) equal to the baseline pressure plus 10 kPa; 2. Occlusion Time (OT) is the time needed to achieve platelet thrombus formation, and it is measured when the Occlusion Pressure (OP) is reached. OP is the baseline pressure plus 60 kPa; 3. Area Under the Curve (AUC) is the area under the pressure-time curve at the end of thrombus formation or after 10 minutes of flow, whichever is applicable. This area represents the stability of the thrombus.

Heart ultrasounds were performed in newborns with GA ≤ 30 weeks by an expert neonatologist who performed echocardiography at To, T1, and T2 (Online Supplementary Figure S1). A Canon Aplio 700 (Canon Medical Systems, Tokyo, Japan) ultrasound machine with a phased array probe (4-10Mhz) was used for the scans.

Statistical analysis

Descriptive statistics were obtained for all variables. Mean and standard deviations were used for continuous variables that were normally distributed, while the median and interquartile ranges were used for variables with asymmetric distribution. Categorical variables are presented as absolute numbers and proportions.

A descriptive analysis of the T-TAS®01 parameters obtained at different sampling moments was performed. Comparisons between groups were assessed using an independent t-test or Mann-Whitney U test for continuous variables and Fisher's Exact test for categorical variables. Patients who never reached an OST value were excluded from all the analyses. OT values greater than 10 minutes (600 seconds) are not recorded by T-TAS®01. For infants with OT greater than 10 minutes, the variable's value was equal to 601 seconds. This value represents an underestimate of the true unobserved occlusion time. AUC was calculated for patients who successfully reached OST and OT values.

Spearman's correlation index was used to investigate the correlation between T-TAS®01 parameters with platelet count, MPV, and Ht values and between T-TAS®01 parameters and GA. Differences in T-TAS®01 parameters

Table I - Clinical and demographic data of preterms (P) and full-terms (T) enrolled newborns

Clinical and demographic data		P (No.=26)	T (No.=29)	p-value
Gender (%)	Female	11 (42.3)	8 (27.6)	0.389
	Male	15 (57.7)	21 (72.4)	
GA weeks, mean (SD)		28.15 (1.54)	38.34 (1.23)	<0.001
BW grams, mean (SD)		1,036.92 (265.24)	3,310.17 (403.88)	<0.001
AGA_LGA_SGA (%)	AGA	21 (80.8)	25 (86.2)	0.175
	LGA	0 (0.0)	2 (6.9)	
	SGA	5 (19.2)	2 (6.9)	
IUGR (%)	No	20 (76.9)	28 (96.6)	0.076
	Yes	6 (23.1)	1 (3.4)	
Twins (%)	No	12 (46.2)	29 (100.0)	<0.001
	Yes	14 (53.8)	0 (0.0)	
Chorionic (%)	BC/DA	3 (11.5)		
	MC/DA	11 (42.3)		
TTTS (%)	No	7 (50.0)		
	Yes	7 (50.0)		
Gestational diabetes (%)	No	25 (96.2)	23 (79.3)	0.143
	Yes	1 (3.8)	6 (20.7)	
Hypertension (%)	No	25 (96.2)	29 (100.0)	0.956
	Yes	1 (3.8)	0 (0.0)	
Chorioamnionitis (%)	No	17 (65.4)	29 (100.0)	0.002
	Yes	9 (34.6)	0 (0.0)	
NSAIDs (%)	No	26 (100.0)	29 (100.0)	
TORCH (%)	No	26 (100.0)	29 (100.0)	
Antenatal corticosteroids (%)	two doses	24 (92.3)	0 (0.0)	<0.001
	one dose	1 (3.8)	0 (0.0)	
	No	1 (3.8)	29 (100.0)	

p-values from: t-test (mean (SD)); Mann-Whitney U test (median [IQR]) and Fisher's exact test (No. [%]). AGA: adequate for gestational age; BC: bi-chorionic; BW: birth weight; DA: diamniotic; GA: gestational age; IUGR: intrauterine growth restriction; LGA: large for gestational age; MC: monochorionic; NSAIDs: nonsteroidal anti-inflammatory drugs; SD: standard deviation; SGA: small for gestational age; TORCH: toxoplasmosis; others: syphilis, hepatitis B, rubella, cytomegalovirus and herpes simplex; TTTS: Twin-to-twin transfusion syndrome.

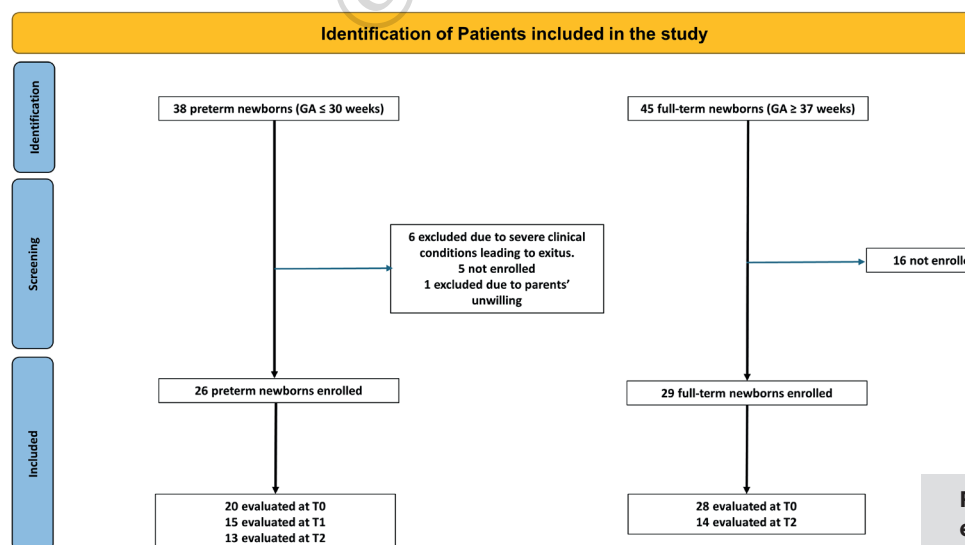


Figure 1 - Flowchart of enrollment of newborns

between preterm and full-term infants over time were studied using mixed effect models with group and time as covariates and subject considered as a random effect. p-values less than or equal to 0.05 are considered statistically significant. All analyses were performed with software R V4.0.0 or higher (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

A total of 55 infants were included in the study, 26 of whom were born preterm, and 29 were born at term (Figure 1). Clinical and demographic data are presented in Table I. In contrast, Table II shows the mean values of platelet function analyzed by T-TAS®01, platelet count,

MPV, and Ht in both groups at scheduled time points. Platelet count was significantly lower in the preterm group at T0 ($p=0.019$), although it was still within normal ranges in both groups. However, this difference was not observed at T2 due to the increase in platelet count in preterm infants.

MPV values were significantly higher in preterm infants at T0 and T2 (p-values 0.006 and 0.043, respectively), whereas Ht was lower in preterm infants at T0 ($p=0.001$) compared to term newborns.

Analyses were performed only for neonates who began to form platelet thrombi. In the preterm group, 20 of 26 values were included at T0, 15 of 20 at T1, and 13 of

Table II - Median values of platelet function analyzed by T-TAS®01, platelet count, MPV, and Ht in the preterm (P) and full-term (T) infant groups at times T0, T1, and T2

	T0			T1	T2		
	P (No.=20)	T (No.=28)	p	P (No.=15)	P (No.=13)	T (No.=14)	p
PLT count ($\times 10^9/L$) (median [IQR])	204 [152-273]	264 [230-318]	0.019	211 [168-297]	259 [177-361]	244 [209-289]	0.765
MPV fl (median [IQR])	10.4 [10-10.7]	9.9 [9.4-10.2]	0.006	10.9 [10.5-11]	11.9 [10.6-12.2]	10.5 [10.2-10.9]	0.043
Ht % (median [IQR])	43.7 [38.3-48.1]	51 [46.8-55.4]	0.001	46.9 [39.5-49.7]	40.8 [37.4-46.1]	46. [37.4-51.4]	0.264
PDA (%)							
No	2 (10)			5 (33.3)	12 (92.3)		
Yes	18 (90)			10 (66.7)	1 (7.7)		
HsPDA (%)							
No	15 (75)			12 (80)	13 (100)		
Yes	5 (25)			3 (20)			
OST (%)	20 (100)			15 (100)	13 (100)		
OST seconds (median [IQR])	152.0 [131-203.5]	86.5 [68.8-111.3]	<0.001	244.0 [145.5-346.5]	149 [100-373]	103 [83.3-125]	0.041
OT (%)							
No	7 (35)	4 (14.3)	0.182	10 (66.7)	9 (69.2)	1 (7.1)	0.003
Yes	13 (65)	24 (85.7)		5 (33.3)	4 (30.8)	13 (92.9)	
OT seconds (median [IQR])	436 [365.3-601]	272.0 [232.3-368]	0.002	601 [468-601]	601 [453-601]	249 [206.8, 361.5]	0.002
AUC (median [IQR])	334 [251-410.5]	414.3 [371.3-493.6]	0.028	394 [316.2-434.2]	386.2 [321.9-442.3]	445.5 [405.2; 461.8]	0.461
Sample (%)							
Capillary	1 (5)	3 (10.7)	<0.001	7 (46.7)	11 (84.6)	7 (50)	0.092
Arterial	5 (25)	3 (10.7)		2 (13.3)	1 (7.7)	0 (0)	
CVC	14 (7)	4 (14.3)		5 (33.3)	0 (0)	1 (7.1)	
Peripheral vein	0 (0)	18 (64.3)		1 (6.7)	1 (7.7)	6 (42.9)	

p-values from: Mann-Whitney U test for continuous variables, Fisher's exact test for categorical variables. T0: within 24 hours since birth; T1: between 48 and 72 hours of life; T2: at 7-10 days of life; PLT: platelets; MPV: mean platelet volume; Ht: hematocrit; PDA: patent ductus arteriosus; HsPDA: hemodynamically significant patent ductus arteriosus; OST: occlusion start time; OT: occlusion time; AUC: area under the curve; CVC central venous catheter; IQR: interquartile range.

21 at T2. All term neonates started platelet thrombi formation at T0 and T2.

In preterm infants, 65% (13 of 20) achieved occlusion at T0 compared to 85.7% in term infants ($p=0.182$). However, at T2, 30.8% (4 of 13) of preterm infants achieved occlusion compared to 92.9% (13 of 14) of term infants ($p=0.003$).

OST was delayed by 65.5 seconds at T0 ($p<0.001$) and 46 seconds at T2 ($p=0.041$) in preterm versus term infants. OT was delayed by 164 seconds at T0 and 352 seconds at T2 ($p=0.002$). AUC values between term and preterm infants differed by 80.3 points at T0 ($p=0.028$) and 59.3 points at T2 ($p=0.46$). Data for T1 are reported for preterm infants (Table II).

Table III reports estimates from mixed effects models of the differences between the two groups and time points for OT, OST, and AUC. The models confirmed a

lower mean OT and OST in term infants ($p < 0.001$ and $p=0.002$, respectively) and a higher mean AUC ($p=0.001$). No significant differences over time were found for any of the three parameters.

Figure 2 shows the distributions of OST, OT, and AUC for preterm and term infants at T0 and T2, visually representing what is shown in Tables II and III.

No significant correlations were found between T-TAS[®]01 parameters and GA or between T-TAS[®]01 parameters and CBC (platelet count, MPV, Ht).

Comparative analysis of T-TAS[®]01 parameters at T0 and T1 was performed between infants with and without PDA and infants with and without hsPDA (Table IV).

Due to the small number of patients in some groups, the evaluation can only be descriptive. Neonates with PDA or hsPDA generally have longer OST and OT at T0 and T1

Table III - Mixed effect models estimate the mean difference in T-TAS[®]01 parameters between preterm and full-term newborns and between time points

Parameter	Term	Estimate	Conf. low	Conf. high	p-value
OT seconds	FT vs PT	-164.3	-239.2	-89.4	<0.001
	T2 vs T0	16.5	-45.7	78.7	0.594
OST seconds	FT vs PT	-93.0	-149.3	-36.7	0.002
	T2 vs T0	21.1	-29.2	71.4	0.403
AUC	FT vs PT	76.2	33.4	119.1	0.001
	T2 vs T0	-0.7	-44.3	42.9	0.974

T0: within 24 hours since birth; T2: at 7-10 days of life; FT: full-term; PT: preterm; OST: occlusion start time; OT: occlusion time; AUC: area under the curve.

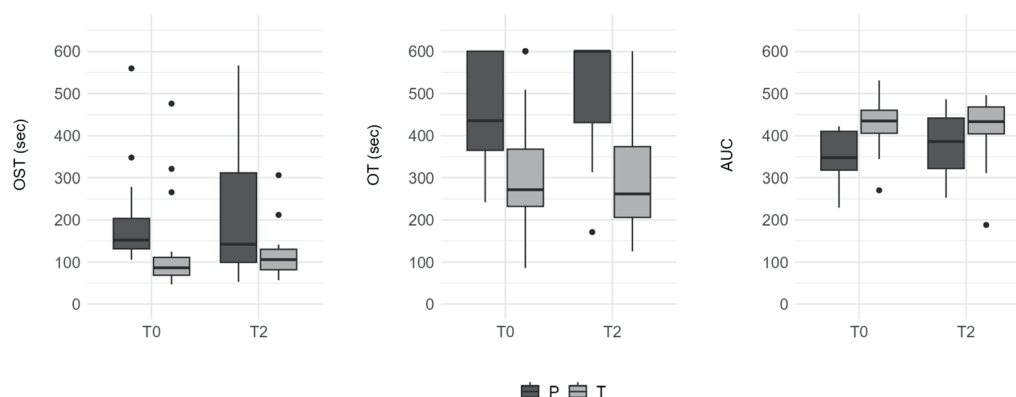


Figure 2 - T-TAS[®]01 parameters' distributions in preterm infants at the established timeline

OST: occlusion start time; OT: occlusion time; AUC: area under the curve.

Table IV - Comparative analysis of T-TAS®O1 and CBC parameters between newborns with or without PDA or HsPDA at different time points

		T0		T1		T2	
TTAS	PDA	No.	Median [IQR]	No.	Median [IQR]	No.	Median [IQR]
OST seconds	No	2	122 [114; 130]	5	181 [140; 234]	12	198.5 [121; 388.8]
	Yes	18	164 [132.2; 204.5]	10	268.5 [174.2; 388]	1	53
OT seconds	No	2	342.5 [322.2; 362.8]	5	601 [295; 601]	12	601 [564; 601]
	Yes	18	486 [374; 601]	10	601 [579.2; 601]	1	171
AUC	No	2	372.2 [353.1; 391.4]	2	408.6 [384.8; 432.3]	3	344.8 [299; 386.2]
	Yes	11	347.8 [284.6; 383.7]	3	345.9 [263.6; 386.4]	1	486.2
PLT ×10 ⁹ /L	No	2	184 [167; 201]	5	173 [164; 180]	12	293 [176; 361]
	Yes	18	204 [158; 278]	10	234 [185; 320]	1	211
MPV fl	No	2	9.9 [9.9; 9.9]	5	10.9 [10.7; 11]	11	12.0 [10.4; 12.2]
	Yes	18	10.4 [10.1; 10.8]	10	10.9 [10.3; 11]	1	11.7
Ht %	No	2	50.2 [49.5; 50.9]	5	49.6 [44.8; 49.8]	12	39.6 [36.1; 46]
	Yes	18	43.4 [38.2; 46.7]	10	45.1 [38.6; 49.2]	1	46.9

TTAS	HsPDA	No.	Median [IQR]	No.	Median [IQR]	No.	Median [IQR]
OST seconds	No	15	138 [124.5; 172.5]	12	257.5 [173.5; 403.2]	13	149 [100; 373]
	Yes	5	279 [255; 348]	3	121 [96.5; 193.5]	0	
OT seconds	No	15	383 [325; 579.5]	12	601 [520; 601]	13	601 [453; 601]
	Yes	5	601 [472; 601]	3	601.0 [423.0; 601]	0	
AUC	No	11	347.8 [320; 411.6]	4	353.5 [304.7; 384.8]	4	386.2 [321.9; 442.3]
	Yes	2	292 [260.7; 323.3]	1	427	0	
PLT ×10 ⁹ /L	No	15	218 [172; 283]	12	195 [171; 259]	13	259 [177; 361]
	Yes	5	173 [153; 197]	3	334 [238; 336]	0	
MPV fl	No	15	10.2 [9.9; 10.4]	12	10.9 [10.8; 11.1]	12	11.8 [10.6; 12.2]
	Yes	5	10.7 [10.7; 10.8]	3	10.0 [9.9; 10.5]	0	
Ht %	No	15	46.1 [41.0; 48.9]	12	47.5 [42.6; 49.7]	13	40.8 [37.4; 46.1]
	Yes	5	38.4 [36.8; 43.3]	3	38.0 [35.4; 49.4]	0	

T0: within 24 hours since birth; T1: between 48 and 72 hours of life; T2: at 7-10 days of life; PLT: platelets; MPV: mean platelet volume; Ht: hematocrit; PDA: patent ductus arteriosus; hsPDA: hemodynamically significant patent ductus arteriosus; OST: occlusion start time; OT: occlusion time; AUC: area under the curve; IQR: interquartile range.

with lower AUC values. In the PDA and hsPDA groups, the platelet count varies more at different time points, while MPV remains stable and Ht is lower than newborns without PDA. The rate of ductal occlusion at T2 was found to be 92%.

DISCUSSION

Although we can currently investigate secondary hemostasis at the bedside and by laboratory tests, this is not true for primary hemostasis^{1,8,12,16,38-44}. Indeed, reference values of tests studying hemostasis are lacking in neonates, especially preterm newborns⁵.

This study is the first one to analyze newborn platelet

function by T-TAS®O1. According to current literature, our results show that platelet function is defective in preterm newborns compared to term infants^{2,4,7,8,10-14,45}. OST and OT were longer in preterm infants than full-term infants at T0 and T2, and AUC was significantly reduced in preterm infants at T0.

Regarding CBC values, our analysis shows that full-term infants have a higher platelet count (mean $264 \times 10^9/L$) on the first day of life but lower mean platelet volume and higher hematocrit at birth and T2 than premature babies. These findings agree with current literature since platelet count increases progressively with GA^{1-4,6}. Wiedmeier *et al.* showed that in newborns with GA ≤ 32 weeks, the 5th

percentile platelet count was 104,200/ μ L, suggesting a lower average number of platelets in infants with severe prematurity than in adults⁴⁶. MPV in neonates is similar to adults' (7-9 fl) and decreases gradually with platelet age^{2,3,7}.

Corticosteroids are known to increase platelet count, and exogenous hypercortisolism might affect platelet function⁴⁷. Thus, administering antenatal corticosteroids to mothers in most premature newborns might affect platelet count and function. The extent of this effect is difficult to assess, as literature is scarce, but this issue should still be considered when interpreting results.

Platelet count has been considered a reassuring element of platelet function and bleeding prevention for a long time to decide on platelet transfusion. However, recent studies have demonstrated that platelet function rather than platelet count correlates with bleeding, and it can also guide platelet transfusions in premature newborns⁴⁸⁻⁵⁰.

Since 2010, several studies have investigated the relationship between platelet count and PDA, with conflicting results^{27,51-55}. Thus, many authors hypothesized that thrombocytopenia associated with PDA is the epiphenomenon of the patient's hemodynamic stability rather than an influencing factor on ductal liability to closure²².

Unlike previous studies, we found no clear relationship between platelet count and PDA^{23,25,27,51-56}. Among values expressed in CBC, only Ht shows a variation between newborns with and without PDA at different time points, which is enhanced in newborns with hsPDA (Table 4). This condition can be interpreted as a positive influence of Ht on platelet function.

In 2015, a review concluded that a marginal but significant association between platelet count in the first week of life and PDA could be hypothesized²⁵. Demir *et al.* found a correlation between hsPDA and MPV or platelet mass but not with platelet count or platelet distribution width (PDW) in preterms⁵⁷. Subsequently, a Turkish study of 100 VLBW infants concluded that hsPDA was significantly associated with a higher PDW in the first days of life⁵⁸. A second meta-analysis found that the number of platelets, the plateletcrit, and the platelet mass of the first three days of life could identify subjects who would have had hsPDA. However, the authors suggested investigating the link between PDA and thrombocytopenia and platelet

function through prospective studies²⁶. A recent review has confirmed the existence of an association between thrombocytopenia and PDA/hsPDA. The results of the study indicate a significant association between platelet count less than 150,000/ μ L and lower than 100,000/ μ L and PDA or hsPDA. However, it is noteworthy that there is no association with platelet counts lower than 50,000/ μ L. In addition, the results of the review indicate an association between reduced platelet mass, lower mean platelet count, increased PDW, and hsPDA⁵⁶. Anyway, platelet transfusions do not seem to reduce the hsPDA incidence and instead are associated with a higher incidence of IVH⁵⁹.

Recently, studies have investigated the relationship between clotting function and PDA²⁴. Turkish research based on PFA-100 found a significant platelet dysfunction expressed as longer closure time in preterms with PDA²⁹. Another Italian protocol investigated thromboelastography (TEG), concluding that this test did not predict spontaneous PDA closure. Still, increased fibrinolysis was found in infants who did not respond to the pharmacological treatment of hsPDA³⁹.

As suggested by reviews, we analyzed premature infants, looking for a relationship between T-TAS[®]01 parameters and PDA^{25,29,56}. However, we did not reach statistical significance due to the small sample size of infants having a closed ductus arteriosus (DA) at T0 or T1. Nevertheless, our data suggest a lower platelet activity in newborns with PDA and hsPDA. This is a fact that, if confirmed, would support the hypothesis that a worse platelet function is associated with patency of the ductus²⁹.

Finally, infants with hsPDA have a delayed onset of OST at T0 compared to infants with non-hsPDA (Table IV), which would agree with the hypothesis that platelet function may be reduced in these patients.

The T-TAS[®]01 method is an intriguing alternative to traditional laboratory investigations as it is an in vitro tool replicating the reactions in vivo. This includes the flow of blood and the response of platelets upon coming into contact with the collagen of the sub-endothelium. Moreover, the instrument requires a small amount of blood compared to other platelet function analyzers previously used^{1,2,4,45,60}. The blood sample is withdrawn directly from the infant and not from the umbilical cord, as it was often done in previous studies due to the greater availability^{5-7,61}.

This is relevant because the platelet function of cord blood differs from newborn blood samples⁶². For this reason, to date, the study of platelet function is usually limited to the research field without clinical use because of ethical reasons.

The limitation of our analysis is related to the small sample size. Furthermore, T-TAS®01 processed the sample for a maximum time of 10 minutes. In order to analyze OT data for all patients, the variable's value was set equal to 601 seconds for those who exceeded this time. This value represents an underestimation of the true unobserved occlusion time. Therefore, the differences we observe between pre-term and full-term will underestimate the true differences.

Finally, it is possible that the variability in T-TAS®01 parameters, especially for OST at T1 and T2, could be attributed to the different blood sources used for the analysis and the sampling methods, such as arterial, venous, or capillary. However, research has indicated that platelet function in VLBW infants undergoes rapid changes in the first few days of life, likely due to medications administered to premature infants in the NICU, such as ampicillin⁷⁻⁹.

CONCLUSIONS

Our study is the first to examine platelet function in neonates using a new point-of-care device. We found a significant difference in platelet function between term and preterm infants. Our results also suggest that a decrease in platelet function may affect ductal patency. However, further studies are needed to determine the relationship between platelet function and bleeding risk in preterm infants and to assess the relationship between clotting ability and ductus arteriosus occlusion. In addition, ductus arteriosus occlusion appears to be influenced by more effective primary hemostasis. Understanding platelet function could help clinicians manage cardiocirculatory adaptation.

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AUTHOR CONTRIBUTIONS

EC, GR, NP, GC, SG, and GF contributed to the study's conception and design. EC, VC, GR, NP, GC, SG, and GF

contributed to the study's methodology, investigation, and data curation. SG, GC, NP, IP, and GF contributed to the study's validation, formal analysis, and resources. NP performed the statistical analysis. EC, GR, NP, GC, SG, and GF wrote the original draft preparation of the manuscript. The co-first EC and VC authorship and co-last SG and GF authorship contributed equally and have the right to list their name as first or last in their *Curriculum Vitae*. EC, VC, GR, NP, IP, MF, GC, SG, and GF wrote, reviewed, and edited the manuscript. GF and GC contributed equally to the visualization of the manuscript. GC and GF contributed to the supervision and project administration of the study.

The Authors declare no conflicts of interest.

REFERENCES

- Haley KM, Recht M, McCarty OJ. Neonatal platelets: mediators of primary hemostasis in the developing hemostatic system. *Pediatr Res* 2014; 76: 230-237. doi: 10.1038/pr.2014.87.
- Davenport P, Sola-Visner M. Hemostatic challenges in neonates. *Front Pediatr* 2021; 9: 627715. doi: 10.3389/fped.2021.627715.
- Margraf A NC, Sperandio M. Ontogeny of platelet function. *Blood Adv* 2019; 26; 3: 692-703. doi: 10.1182/bloodadvances.2018024372.
- Saxonhouse MA, Sola MC. Platelet function in term and preterm neonates. *Clin Perinatol* 2004; 31: 15-28. doi: 10.1016/j.clp.2004.03.009.
- Baker-Groberg SM, Lattimore S, Recht M, McCarty OJ, Haley KM. Assessment of neonatal platelet adhesion, activation, and aggregation. *J Thromb Haemost* 2016; 14: 815-827. doi: 10.1111/jth.13270.
- Israels SJ RM, Michelson AD Neonatal platelet function. *Semin Thromb Hemost* 2003; 29: 363-372. doi: 10.1055/s-2003-42587.
- Sola-Visner M. Platelets in the neonatal period: developmental differences in platelet production, function, and hemostasis and the potential impact of therapies. *Hematology Am Soc Hematol Educ Program* 2013; 2012: 506-511. doi: 10.1182/asheducation-2012.1.506.
- Strauss T, Sidlik-Muskatell R, Kenet G. Developmental hemostasis: primary hemostasis and evaluation of platelet function in neonates. *Semin Fetal Neonatal Med* 2011; 16: 301-304. doi: 10.1016/j.siny.2011.07.001.
- Michelson A. Platelet function in the newborn. *Semin Thromb Hemost* 1998; 24: 507-512. doi: 10.1055/s-2007-996049.
- Ferrer-Marin F, Sola-Visner M. Neonatal platelet physiology and implications for transfusion. *Platelets* 2022; 33: 14-22. doi: 10.1080/09537104.2021.1962837.
- Ferrer-Marin F, Stanworth S, Josephson C, Sola-Visner M. Distinct differences in platelet production and function between neonates and adults: implications for platelet transfusion practice. *Transfusion* 2013; 53: 2814-2821; quiz 2813. doi: 10.1111/trf.12343.
- HvasAM, FavalaroEJ. Platelet function testing in pediatric patients. *Expert Rev Hematol* 2017; 10: 281-288. doi: 10.1080/17474086.2017.1293518.
- Bednarek FJ, Bean S, Barnard MR, Frelinger AL, Michelson AD. The platelet hyporeactivity of extremely low birth weight neonates is age-dependent. *Thromb Res* 2009; 124: 42-45. doi: 10.1016/j.thromres.2008.10.004.
- Deschmann E, Sola-Visner M, Saxonhouse MA. Primary hemostasis in neonates with thrombocytopenia. *J Pediatr* 2014; 164: 167-172. doi: 10.1016/j.jpeds.2013.08.037.

15. Del Vecchio A, Latini G, Henry E, Christensen RD. Template bleeding times of 240 neonates born at 24 to 41 weeks gestation. *J Perinatol* 2008; 28: 427-431. doi: 10.1038/jp.2008.10.
16. Raffaelli G, Tripodi A, Cavallaro G, Cortesi V, Scalabrino E, Pesenti N, et al. Thromboelastographic profiles of healthy very low birthweight infants serially during their first month. *Arch Dis Child Fetal Neonatal Ed* 2020; 105: 412-418. doi: 10.1136/archdischild-2019-317860.
17. Raffaelli G, Tripodi A, Manzoni F, Scalabrino E, Pesenti N, Amodeo I, et al. Is placental blood a reliable source for the evaluation of neonatal hemostasis at birth? *Transfusion* 2020; 60: 1069-1077. doi: 10.1111/trf.15785.
18. Tripodi A, Raffaelli G, Scalabrino E, Padovan L, Clerici M, Chantarangkul V, et al. Procoagulant imbalance in preterm neonates detected by thrombin generation procedures. *Thromb Res* 2020; 185: 96-101. doi: 10.1016/j.thromres.2019.11.013.
19. Tripodi A, Ramenghi LA, Chantarangkul V, De Carli A, Clerici M, Groppo M, et al. Normal thrombin generation in neonates in spite of prolonged conventional coagulation tests. *Haematologica* 2008; 93: 1256-1259. doi: 10.3324/haematol.12566.
20. Hamrick SEG, Sallmon H, Rose AT, Porras D, Shelton EL, Reese J, Hansmann G. Patent ductus arteriosus of the preterm infant. *Pediatrics* 2020; 146: e20201209. doi: 10.1542/peds.2020-1209.
21. Conrad C, Newberry D. Understanding the pathophysiology, implications, and treatment options of patent ductus arteriosus in the neonatal population. *Adv Neonatal Care* 2019; 19: 179-187. doi: 10.1097/ANC.0000000000000590.
22. 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. doi: 10.1016/j.clp.2015.11.008.
23. Echter K, Stark K, Lorenz M, Kerstan S, Walch A, Jennen L, et al. Platelets contribute to postnatal occlusion of the ductus arteriosus. *Nat Med* 2010; 16: 75-82. doi: 10.1038/nm.2060.
24. Sallmon H, Timme N, Atasay B, Erdevi O, Hansmann G, Singh Y, et al. Current controversy on platelets and patent ductus arteriosus closure in preterm infants. *Front Pediatr* 2021; 9: 612242. doi: 10.3389/fped.2021.612242.
25. 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. doi: 10.1159/000431281.
26. Ding R, Zhang Q, Duan Y, Wang D, Sun Q, Shan R. The relationship between platelet indices and patent ductus arteriosus in preterm infants: a systematic review and meta-analysis. *Eur J Pediatr* 2021; 180: 699-708. doi: 10.1007/s00431-020-03802-5.
27. Bas-Suarez MP, Gonzalez-Luis GE, Saavedra P, Villamor E. Platelet counts in the first seven days of life and patent ductus arteriosus in preterm very low-birth-weight infants. *Neonatology* 2014; 106: 188-194. doi: 10.1159/000362432.
28. González-Luis G, Ghirardello S, Bas-Suárez P, Cavallaro G, Mosca F, Clyman RI, et al. Corrigendum: Platelet counts and patent ductus arteriosus in preterm infants: an updated systematic review and meta-analysis. *Front Pediatr* 2021; 9: 694606. doi: 10.3389/fped.2021.694606. Erratum for: *Front Pediatr* 2021; 8: 613766. PMID: 34095038.
29. Kahvecioglu D, Erdevi O, Akduman H, Ucar T, Alan S, Kahir U, 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. doi: 10.1016/j.pedneo.2017.01.006.
30. Borse V, Shanks A. Twin-to-twin transfusion syndrome. [Updated 2022 Oct 10]. In: StatPearls [Internet]. Treasure Island (FL), USA: StatPearls Publishing; 2022. Available at: <https://www.ncbi.nlm.nih.gov/books/NBK563133/>. PMID. Accessed on 28/01/2021.
31. Chew L, Verma R. Fetal Growth Restriction. [Updated 2023 Aug 8]. In: StatPearls [Internet]. Treasure Island (FL), USA: StatPearls Publishing; 2023. Available at: <https://www.ncbi.nlm.nih.gov/books/NBK562268/>. PMID. Accessed on 28/01/2021.
32. Quintanilla Rodriguez B, Mahdy H. Gestational Diabetes. [Updated 2023 Aug 8]. In: StatPearls [Internet]. Treasure Island (FL), USA: StatPearls Publishing; 2023. Available at: <https://www.ncbi.nlm.nih.gov/books/NBK545196/>. PMID. Accessed on 28/01/2021.
33. Karrar SA, Hong PL. Preeclampsia [Updated 2023 Feb 13]. In: StatPearls [Internet]. Treasure Island, FL, USA: StatPearls Publishing; 2023. Available at: <https://www.ncbi.nlm.nih.gov/books/NBK570611/>. PMID. Accessed on 26/05/2021.
34. Kachikis A, Eckert LO, Walker C, Bardaji A, Varricchio F, Lipkind HS, et al. Chorioamnionitis: Case definition & guidelines for data collection, analysis, and presentation of immunization safety data. *Vaccine* 2019; 37: 7610-7622. doi: 10.1016/j.vaccine.2019.05.030.
35. Jaan A, Rajnik M. TORCH Complex. [Updated 2023 Jul 17]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available at: <https://www.ncbi.nlm.nih.gov/books/NBK560528/>. Accessed on 28/01/2021.
36. Osuchukwu OO, Reed DJ. Small for Gestational Age. [Updated 2022 Nov 14]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available at: <https://www.ncbi.nlm.nih.gov/books/NBK563247/>. Accessed on 28/01/2021.
37. Gillam-Krakauer M, Mahajan K. Patent Ductus Arteriosus. 2023 Aug 8. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. PMID: 28613509. Available at: <https://www.ncbi.nlm.nih.gov/books/NBK430758/>. Accessed on 28/01/2021.
38. Amelio GS, Raffaelli G, Amodeo I, Gulden S, Cortesi V, Manzoni F, et al. Hemostatic Evaluation With Viscoelastic Coagulation Monitor: A Nicu Experience. *Front Pediatr* 2022; 10: 910646. doi: 10.3389/fped.2022.910646.
39. Ghirardello S RG, Crippa BL, Gulden S, Amodeo I, Consonni D, Cavallaro G, Schena F, Mosca F. The Thromboelastographic Profile at Birth in Very Preterm Newborns with Patent Ductus Arteriosus. *Neonatology* 2020; 117: 316-323. doi: 10.1159/000507553.
40. Manzoni F, Raffaelli G, Cortesi V, Amelio GS, Amodeo I, Gulden S, et al. Viscoelastic coagulation testing in Neonatal Intensive Care Units: advantages and pitfalls in clinical practice. *Blood Transfus* 2023; 21: 538-548. doi: 10.2450/2023.0203-22.
41. Katsaras G, Sokou R, Tsantes AG, Piovani D, Bonovas S, Konstantinidi A, et al. The use of thromboelastography (TEG) and rotational thromboelastometry (ROTEM) in neonates: a systematic review. *Eur J Pediatr* 2021; 180: 3455-3470. doi: 10.1007/s00431-021-04154-4.
42. Konstantinidi A, Sokou R, Parastatidou S, Lampropoulou K, Katsaras G, Boutsikou T, et al. Clinical Application of Thromboelastography/Thromboelastometry (TEG/TEM) in the Neonatal Population: A Narrative Review. *Semin Thromb Hemost* 2019; 45: 449-457. doi: 10.1055/s-0039-1692210.
43. Parastatidou S, Sokou R, Tsantes AG, Konstantinidi A, Lampridou M, Ioakeimidis G, et al. The role of ROTEM variables based on clot elasticity and platelet component in predicting bleeding risk in thrombocytopenic critically ill neonates. *Eur J Haematol* 2021; 106: 175-183. doi: 10.1111/ejh.13534.
44. Sokou R, Tsantes AG, Konstantinidi A, Ioakeimidis G, Lampridou M, Parastatidou S, et al. Rotational Thromboelastometry in Neonates Admitted to a Neonatal Intensive Care Unit: A Large Cross-sectional Study. *Semin Thromb Hemost* 2021; 47: 875-884. doi: 10.1055/s-0041-1729964.
45. Del Vecchio A, Motta M, Romagnoli C. Neonatal platelet function. *Clin Perinatol* 2015; 42: 625-638. doi: 10.1016/j.clp.2015.04.015.
46. Wiedmeier SE, Henry E, Sola-Visner MC, Christensen RD. Platelet reference ranges for neonates, defined using data from over 47,000 patients in a multihospital healthcare system. *J Perinatol* 2009; 29: 130-136. doi: 10.1038/jp.2008.141.
47. Signorello MG, Ravera S, Leoncini G. Oxidative stress induced by cortisol in human platelets. *Int J Mol Sci* 2024; 25: 3776. doi: 10.3390/ijms25073776.
48. Deschmann E, Saxonhouse MA, Feldman HA, Norman M, Barbian M, Sola-Visner M. Association of bleeding scores and platelet transfusions with platelet counts and closure times in response to adenosine diphosphate (CT-ADPs) among preterm neonates with thrombocytopenia. *JAMA Netw Open* 2020; 3: e203394. doi: 10.1001/jamanetworkopen.2020.3394.
49. Deschmann ESM, Feldman HA, Norman M, Barbian M, Sola-Visner M. Association between in vitro bleeding time and bleeding in preterm infants with thrombocytopenia. *JAMA Pediatr* 2019; 173: 393-394. doi: 10.1001/jamapediatrics.2019.0008.

50. Waller AK, Lantos L, Sammut A, Salgin B, McKinney H, Foster HR, et al. Flow cytometry for near-patient testing in premature neonates reveals variation in platelet function: a novel approach to guide platelet transfusion. *Pediatr Res* 2019; 85: 874-884. doi: 10.1038/s41390-019-0316-9.
51. Alyamac Dizdar E, Ozdemir R, Sari FN, Yurttutan S, Gokmen T, Erdevi O, et al. Low platelet count is associated with ductus arteriosus patency in preterm newborns. *Early Hum Dev* 2012; 88: 813-816. doi: 10.1016/j.earlhumdev.2012.05.007.
52. Dani C, Poggi C, Fontanelli G. Relationship between platelet count and volume and spontaneous and pharmacological closure of ductus arteriosus in preterm infants. *Am J Perinatol* 2013; 30: 359-364. doi: 10.1055/s-0032-1324702.
53. Kulkarni VV, Dutta S, Sundaram V, Saini SS. Preterm thrombocytopenia and delay of ductus arteriosus closure. *Pediatrics* 2016; 138: e20161627. doi: 10.1542/peds.2016-1627.
54. Sallmon H, Weber SC, Huning B, Stein A, Horn PA, Metze BC, et al. Thrombocytopenia in the first 24 hours after birth and incidence of patent ductus arteriosus. *Pediatrics* 2012; 130: e623-630. doi: 10.1542/peds.2012-0499.
55. Fujioka K, Morioka I, Miwa A, Morikawa S, Shibata A, Yokoyama N, Matsuo M. Does thrombocytopenia contribute to patent ductus arteriosus? *Nat Med* 2011; 17: 29-30; author reply 30-21. doi: 10.1038/nm0111-29.
56. Gonzalez-Luis G, Ghiradello S, Bas-Suarez P, Cavallaro G, Mosca F, Clyman RI, Villamor E. Platelet Counts and Patent Ductus Arteriosus in Preterm Infants: An Updated Systematic Review and Meta-Analysis. *Front Pediatr* 2020; 8: 613766. doi: 10.3389/fped.2020.613766.
57. Demir N, Peker E, Ece I, Agengin K, Bulan KA, Tuncer O. Is platelet mass a more significant indicator than platelet count of closure of patent ductus arteriosus? *J Matern Fetal Neonatal Med*. 2016; 29: 1915-1918. doi: 10.3109/14767058.2015.1067296.
58. Demirel G, Yilmaz A, Vatansever B, Tastekin A. Is high platelet distribution width in the first hours of life can predict hemodynamically significant patent ductus arteriosus in preterm newborns? *J Matern Fetal Neonatal Med* 2020; 33: 2049-2053. doi: 10.1080/14767058.2018.1536743.
59. Kumar JDS, Sundaram V, Saini SS, Sharma RR, Varma N. Platelet transfusion for PDA closure in preterm infants: a randomized controlled trial. *Pediatrics* 2019; 143: e20182565. doi: 10.1542/peds.2018-2565.
60. Cowman J, Quinn N, Geoghegan S, Mullers S, Oglesby I, Byrne B, et al. Dynamic platelet function on von Willebrand factor is different in preterm neonates and full-term neonates: changes in neonatal platelet function. *J Thromb Haemost* 2016; 14: 2027-2035. doi: 10.1111/jth.13414.
61. Israels SJ, Cheang T, McMillan-Ward EM, Cheang M. Evaluation of primary hemostasis in neonates with a new in vitro platelet function analyzer. *J Pediatr* 2001; 138: 116-119. doi: 10.1067/mpd.2001.109794.
62. Saxonhouse MA, Garner R, Mammel L, Li Q, Muller KE, Greywoode J, et al. Closure times measured by the platelet function analyzer PFA-100 are longer in neonatal blood compared to cord blood samples. *Neonatology* 2010; 97: 242-249. doi: 10.1159/000253755.