Letter to the Editor

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Evaluation of PFA-100 closure times in cord blood samples of healthy term and preterm neonates

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To the Editor,

The investigation of neonatal platelet function remains an issue of ongoing research with conflicting results so far. The *in vitro* hyporesponsiveness of neonatal platelets, especially those of preterms, assessed using aggregometry and flow cytometry, is well established in both cord blood and peripheral neonatal blood [1]. However, the *in vitro* assessment of primary hemostasis via a platelet function analyzer (PFA) demonstrated enhanced primary hemostasis in healthy neonates compared to adults [2–5]. Despite the fact that neonatal platelet function has been evaluated via PFA-100 in cord blood samples, differences in study design, methods, population heterogeneity and the small number of samples complicate the establishment of reference ranges in neonates so far. In our study, we report PFA-100 closure times (CTs) in a large homogeneous population of healthy term and preterm neonates selected with strict inclusion and exclusion criteria with precise and strict preanalytical procedure for samples handling.

PFA-100 is a system of easy, fast, accurate and quantitative *in vitro* measurement of platelet function. Anticoagulated citrated whole blood is inserted in a cartridge with a membrane with an aperture of 147 μm (diameter), which is coated either with collagen and epinephrine (COL/EPI) or adenosine 5′-phosphate (COL/ADP). Platelets, in response to these agents, undergo all stages of primary hemostasis which results in clot formation and occlusion of the aperture. The time in seconds required for full obstruction of the blood flow is defined as CT [6].

One hundred and four full-term (≥37 weeks' gestation) and 14 preterm neonates (<37 weeks' gestation) born at Aretaieio Hospital, National and Kapodistrian University of Athens and monitored till discharge, were included in our study. Demographic and clinical data are summarized in Table 1. The exclusion criteria included: cord blood Hct < 35%, platelet count < 100×10^9/L, major chromosomal anomaly, family history of bleeding disorder or platelet dysfunction, cord blood pH < 7.25, Apgar score < 8 at 1st or 5th min, BW < 10th centile, intrauterine growth restriction, history of gestational diabetes or pregnancy-induced hypertension. Blood was collected from the umbilical cord vein immediately after clamping. Specimens were drawn smoothly with a 21-gauge needle into 3.2% sodium-citrate tubes and tested within 4 h according to the manufacturer's instructions. Two CTs (with COL/EPI and COL/ADP agonists) were determined for each subject in duplicate and the mean value was calculated; the correlation between the first and the second measurement was high ($r = 0.92$ for COL/EPI and $r = 0.89$ for COL/ADP, $p < 0.0001$ for both correlations). The method's CV was found as 12.54% for COL/EPI and 8.49% for COL/ADP. The Hospital Ethics Review Committee approved the study and mothers signed an informed consent. The SAS-9.4 software was used to perform the statistical analysis. The results are presented as median

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Table 1: Demographic and clinical data along with statistical comparisons.

Variable	Term (n = 104)	Preterm (n = 14)	p-Value [OR (95% CI)]
Birth weight, g	3360 (3170–3650)	2535 (2360–2730)	**<0.0001**
Centile	53 (36–70)	32 (21–57)	**0.0490**
Gestational age, weeks	39 (39–40)	36 (35–37)	**<0.0001**
Temperature at birth, °C	36 (36–37)	36 (36–36)	0.1453
Neonatal Hct (%)	45 (43–48)	45 (40–47)	0.4605
Neonatal RBC ($\times 10^6/\mu L$)	4.3 (4.1–4.7)	4.1 (3.9–4.6)	0.0958
Neonatal WBC ($\times 10^3/\mu L$)	13.5 (11.7–15.4)	10.5 (8.3–14)	0.0882
Neonatal PLT ($\times 10^9/L$)	196 (171–234)	204.5 (197–228)	0.8157
Neonatal MPV, fL	9.8 (8.4–10.5)	9.8 (9.1–10.5)	0.8800
Gender			
Male	48 (87.3%)	7 (12.7%)	0.7865 [0.9 (0.3–2.6)]
Female	56 (88.9%)	7 (11.1%)	
Type of delivery			
Vaginal	38 (95.0%)	2 (5.0%)	0.1354
Caesarean	66 (84.6%)	12 (15.4%)	[0.3 (0.1–1.4)]
Blood group			
O	39 (84.8%)	7 (15.2%)	0.3938
Non-O	65 (90.3%)	7 (9.7%)	[0.6 (0.2–1.8)]
Aspirin			
No	96 (88.9%)	12 (11.1%)	**<0.001**
Yes >7 d before labor	8 (100%)	0	
Yes <7 d before labor	0	2 (100%)	
LMWH			
No	97 (89.8%)	11 (10.2%)	0.0970
Yes	7 (70.0%)	3 (30.0%)	[3.8 (0.9–16.8)]
Levothyroxine			
No	90 (90.0%)	10 (10.0%)	0.3857
Yes	13 (81.3%)	3 (18.8%)	[2.1 (0.5–8.6)]
Ampicillin peripartum			
No	97 (91.5%)	9 (8.5%)	0.0056
Yes	7 (58.3%)	5 (41.7%)	[7.7 (2.0–29.3)]
Pethidine peripartum			
No	99 (87.6%)	14 (12.4%)	1
Yes	5 (100%)	0	OR is NA
Epidural anesthesia			
No	28 (96.6%)	1 (3.4%)	0.183
Yes	76 (85.4%)	13 (14.6%)	[4.8 (0.6–38.3)]
General anesthesia			
No	103 (88.0%)	14 (12.0%)	1
Yes	1 (100%)	0	OR is NA

Results are presented as median and interquartile range (IQR) for the arithmetic parameters and as number of observations (percentage) for the categorical data; bold entries indicate statistically significant differences, for categorical parameters odds ratio (OR) and 95% confidence interval (CI) is also presented, excluding cases that it is not applicable (NA) due to zero cases in a group or due to more than two categories; LMWH, low molecular weight heparin; Aspirin, LMWH, Levothyroxin, Ampicillin peripartum, Pethidine peripartum, Epidural anesthesia, General anesthesia: these parameters refer to mother; When considering no administration of aspirin vs. administration of aspirin (irrelevant of intake timing) the following result is observed: p = 0.4056, OR = 2, 95% CI = 0.4–10.5.

value and the interquartile range (IQR) for the arithmetic parameters (COL/EPI, COL/ADP, weight, etc.) and as number of observations along with the relevant percentage for the categorical data. The t-test, Kruskal-Wallis test, chi-squared test, Fisher exact test and Spearman correlation coefficient were used as appropriate. The significance level for all comparisons was set to $p < 0.05$.

According to our results, COL/EPI CT ranges for healthy term neonates and for healthy preterms were: 76s–164s (median 111s) and 101s–178s (median 149s), respectively, (p = 0.0236) and accordingly COL/ADP ranges: 59s–85s (median 72) and 64s–86s (median 78s), respectively, (p = 0.2783). Therefore COL/EPI was significantly prolonged in preterms compared to term neonates while

COL/ADP was also prolonged but not statistically significantly, contrarily to the results of Saxonhouse et al. [7]. A slight platelet hyperreactivity and enhanced primary hemostasis in healthy term neonates compared to adult ones was confirmed according to our results as depicted in Table 2 [2, 3, 4, 7, 8]. The enhanced platelet-related primary hemostasis of term neonates is more obvious when COL/ADP CTs are compared. COL/ADP CTs in cord blood tend to remain shorter than the corresponding adult ones (Table 2) [2, 3, 4, 7, 8]. The shorter neonatal CTs could be possibly attributed to enhanced neonatal VWF (von Willebrand factor) activity mediated by high molecular VWF multimers [3, 5] as well as to the higher Hct in neonates [3]. The observation in our study that the difference in responsiveness between neonatal and adult platelets is more apparent with some agonists like ADP than with others like epinephrine raises several questions concerning neonatal platelet function that could be attributed to the decreased alpha-adrenergic receptors on neonatal platelets [1]. Additionally, higher Hct and subsequent release of ADP from neonatal RBCs might compensate for decreased ADP release from neonatal platelets and could also result in more profoundly shortened COL/ADP CTs compared to adults [3].

COL/EPI CT values of term neonates recorded in our study (76s–164s) were comparable to these reported by Boudewinjs et al. (49–168s), but slightly prolonged related to those reported by Roschitz et al. (50–112s), by Carcao et al. (61–108s) and by Saxonhouse et al. (65–112s) [2, 4, 5, 7]. The smaller number of term neonates included in these studies (70, 17 and 21, respectively) should be taken into consideration in the evaluation of their results. On the other hand, our COL/ADP CT reference ranges of term neonates seem to be similar with the results of most of the relevant studies depicted in Table 2.

Regarding preterm neonates, and in accordance with previous results [7], we observe prolonged CTs in healthy preterm neonates compared to term infants, supporting the hyporeactivity of preterms' platelets. Still, CTs of preterm neonates remain shorter or similar to these reported in adults (Table 2). Platelet hyporeactivity has been well reported in preterm and very low birth weight neonates using different laboratory assessment methods [9].

COL/EPI was not correlated with neonatal weight, centile, gestational age and birth temperature, while COL/ADP was negatively correlated with gestational age (Spearman's $r = -0.184$, $p = 0.0475$) in the total neonatal population study. The inverse relationship between CT-ADP and gestational age using PFA-100 found in our study has been previously described [7]. As expected, COL/EPI and COL/ADP were significantly correlated ($r = 0.321$, $p = 0.0004$).

A significant negative correlation of COL/ADP with neonatal PLT count (Spearman's $r = -0.188$, $p = 0.0437$) was noted. There is evidence that low platelet count affects

Table 2: Ranges for COL/EPI and COL/ADP CTs for adults and neonates according to previous studies and present study.

	Article	n	COL/EPI CT	COL/ADP CT	Buffered sodium citrate concentration
Adults	Mammen et al., 1998 [8]	206	94–191	72–120	3.8%
	Carcao et al. [2]	39	82–142	67–111	3.2%
	Bock et al., 1999 [8]	309	82–150	62–100	3.2%
	Israels et al. [3]	21	89–133	74–108	3.2%
	Roschitz et al. [5]	25	84–150	64–98	3.2%
	Boudewijns et al. [4]	20	85–165	72–120	3.8%
	Haubelt et al., 2005 [8]	120	93–223	64–117	3.8%
	Cho et al. [8]	120	80–162	64–121	3.2%
	Saxonhouse et al. [7]	10	95–135	63–84	3.2%
Term neonates	Carcao et al. [2]	17	61–108	48–65	3.2%
	Israels et al. [3]	31	55–109	44–76	3.2%
	Roschitz et al. [5]	70	50–112	43–98	3.2%
	Boudewijns et al. [4]	80	49–168	40–92	3.8%
	Saxonhouse et al. [7]	21	65–112	47–56	3.2%
	Present study	**104**	**76–164**	**59–85**	**3.2%**
Preterm neonates	Saxonhouse et al. [7]	30	58–105	46–62	3.2%
	Present study	**14**	**101–178**	**64–86**	**3.2%**

CT, closure time (s); studies referring to adults were conducted in peripheral vein samples while studies referring to neonates were all conducted in cord blood samples; Israels et al. [3]: results based on a mixed population of term and preterm neonates. Bold values refer to the present study.

negatively and prolongs CTs in neonates [6]. This finding is supported by a recent study reporting that CT-ADP, but not CT-EPI, was prolonged in infants with platelet counts below 90×10^9/L [10]. The present study imposed a cut-off value of 100×10^9/L regarding PLT count. The exclusion of thrombocytopenic neonates indicates that the negative correlation between platelet count and CTs is genuine and does not appear only in extreme PLT count. No other association was found between CTs and other hematological parameters (Hct, RBCs, WBCs and MPV, $r<|0.06|$ and all $p>0.05$).

PFA-100 serves as a point-of-care test to assess platelet-related primary hemostasis. Establishing age-dependent reference ranges is imperative for the correct interpretation of the results as a normal CT value by adult reference ranges could possibly imply a bleeding tendency in a neonate. Although cord blood well approximates venous, the reported CT values may not apply to samples from peripheral blood. Possible differences between peripheral blood and cord blood CT values have a limited documentation [7] so far and have to be further investigated.

In the present study, we evaluated COL/EPI and COL/ADP CTs in the largest number, so far reported, of cord blood samples of well-characterized neonates. Based on our findings that CT with COL/ADP agonist has a much smaller range, implying that any values outside it could probably be clinically relevant, we suggest that CT measured by PFA-100 and COL/ADP as agonist could be a good way to identify abnormal platelet function in the newborn. This facilitates the interpretation of primary hemostasis in sick neonates or offspring of high-risk pregnancies, and along with further testing, allows prompt intervention and treatment of hemorrhagic complications.

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