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Asymptomatic late thrombocytosis is a common finding in very preterm infants even in the absence of erythropoietin treatment

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Abstract

Objectives: Thrombocytosis is more prevalent in pediatric than in adult patients and is associated with complications or worsened outcomes after vascular events. This study aimed to determine the prevalence of thrombocytosis in very preterm infants who had not received human recombinant erythropoietin treatment (rHuEPO) and its relationship with other hematological parameters and clinical complications.

Methods: We performed a retrospective study of hematological and clinical data of very preterm infants who were admitted to our unit in their first 48 hours of life and stayed for longer than 1 week.

Results: Thrombocytosis was prevalent (32.6% of patients) in very preterm infants ($\leq$32 weeks of gestational age, n = 193) who had not received rHuEPO. The platelet count was positively correlated with calendar age. Infants with thrombocytosis were significantly more premature (28.0 $\pm$ 2.1 versus 29.6 $\pm$ 2.2 weeks) and had a lower birth weight (1036 $\pm$ 304 versus 1303 $\pm$ 304) than those without thrombocytosis. Thrombocytosis was associated with retinopathy of prematurity after adjusting for gestational age and comorbidities, but not with other prematurity-associated complications.

Conclusions: Late asymptomatic thrombocytosis is common in very preterm infants at approximately 1 month of postnatal age and it may be associated with retinopathy of prematurity.

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Thrombocytosis, very preterm infant, retinopathy of prematurity, blood count, human recombinant erythropoietin, platelet

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Introduction

Thrombocytosis is defined as a platelet count $\geq 500 \times 10^3/\mu L$ and can be classified as primary (essential) and secondary (reactive). This condition is more frequent in children, with an estimated incidence of 3% to 13% at pediatric hospitals[1] and it is a reactive process in most cases.[2] Thrombocytosis has been especially described in low birth weight infants between 2 and 4 weeks of age.[1,2]

Platelet production in adults is controlled by thrombopoietin (TPO), depending on a negative feedback mechanism. Although TPO appears to be the major regulator of thrombopoiesis and megakaryopoiesis in fetuses and neonates,[3] the exact regulatory mechanisms are not equivalent or fully understood. Human recombinant erythropoietin (rHuEPO) treatment has been described as a risk factor for development of thrombocytosis in very preterm infants (VPIs), and may be due to structural and functional similarities between erythropoietin (EPO) and TPO.[4]

Adult patients with thrombocytosis are at risk for thromboembolic and hemorrhagic complications,[5] and have poorer outcomes after an acute vascular event. Neonatal thrombocytosis appears to be rarely associated with complications, although few studies have analyzed clinical characteristics of affected patients.[6,7] Therefore, the present study aimed to determine the prevalence of thrombocytosis in a cohort of VPIs who had not received rHuEPO and its possible relationship with other hematological parameters and clinical complications.

Material and methods

Patients and methods

We performed a retrospective study by reviewing the medical records of all VPIs (≤ 32 weeks of gestational age) who were admitted to Hospital Sant Joan de Déu in Barcelona in their first 48 hours of life and stayed for longer than 1 week, between November 2011 and December 2014. We excluded children with major congenital malformations, congenital infection, or chromosomal abnormalities. The severity of illness, final outcome (including death), and having received blood transfusions were not exclusion criteria. Data for platelets, red blood cells, hemoglobin, hematocrit, mean corpuscular volume, leukocytes, and days of life at the time of extraction were collected. The platelet count was measured using ethylenediaminetetraacetic acid-anticoagulated whole blood on an automatic cell counter (Advia20120i; Siemens, Erlangen, Germany). Thrombocytosis was defined as a platelet count $\geq 500 \times 10^3/\mu L$ and classified as mild ($500–699 \times 10^3/\mu L$), moderate ($700–899 \times 10^3/\mu L$), severe ($900–999 \times 10^3/\mu L$), or extreme ($\geq 1 \times 10^6/\mu L$) according to the maximum number of platelets.[7] Clinical variables were extracted from the medical records. The study protocol was not specifically assessed by an ethics committee, due to its design as a retrospective analysis of routine clinical data. Individuals were anonymized by the assignation of a correlative number for the purpose of the study, so that identification data were not available.

Statistical analysis

All collected data were analyzed with SPSS v17.0 (SPSS Inc., Chicago, IL, USA). Continuous variables were compared by the Student's t test and qualitative variables by the chi-square test between the groups with and without thrombocytosis. Relationships between continuous variables were examined by Pearson's correlation. A p value < 0.05 was considered significant.

Results

There were 895 blood counts from 193 VPIs who fulfilled the inclusion criteria during the study period. Approximately two thirds of the patients had four or less blood tests during admission (26 [13.5%] had one; 44 [22.8%] had two; 36 [18.7%] had three; and 20 [10.4%] had four tests), with a range between one and 19 blood tests. Children with thrombocytosis had had significantly more blood tests during admission than those without thrombocytosis ($p < 0.001$, Table 1). Characteristics of the population are shown in Table 1. VPIs who had thrombocytosis were more premature ($p < 0.001$) and had a lower birth weight ($p < 0.001$) than those without thrombocytosis. However, the proportion of VPIs with intrauterine growth restriction was not different between the groups. There was no significant difference in the incidence of thrombocytosis by sex (28.3% in boys and 37.9% in girls, $p = 0.188$).

A total of 125 (14.0%) platelet counts were $> 500{,}000$, 25 (2.8%) were $> 700{,}000$, and 184 (20.5%) were $< 150{,}000/\mu L$ (Figure 1). Measurements of red blood cell levels progressively decreased with days of life (erythrocyte count: $r = -0.207$, $p < 0.0001$; hemoglobin concentration: $r = -0.567$, $p < 0.0001$; hematocrit: $r = -0.565$, $p < 0.0001$) as did the leukocyte count. In contrast, the platelet count was positively correlated with calendar age ($r = 0.355$, $p < 0.0001$). Therefore, VPIs with high platelet counts were older at the time when the test was performed than those without high platelet counts (39.5 ± 23.3 versus 21.0 ± 28.2 days, $p < 0.0001$). No VPIs had high platelet counts in the first week of life. The 95th percentile of the number of platelets at that time point was $< 500{,}000/\mu L$ at all gestational ages (Table 2).

Sixty-three (32.6%) patients had $\geq 500{,}000$ platelets/μL in at least one of the blood tests. They were classified according to the maximum platelet number. Of these, 42 (66.7%) had mild thrombocytosis, 16 (25.4%) had moderate thrombocytosis, and three (4.8%) had severe thrombocytosis. There were no cases of extreme thrombocytosis. Approximately one quarter (15/63, 23.8%) of patients who presented with thrombocytosis had a low platelet count at some point (Figure 2). The proportion of thrombocytopenic VPIs was similar (36/130, 27.7%) to that of those who had no thrombocytosis.

Red blood cell estimators and the platelet count showed weak inverse correlations (red blood cell count: $r = -0.253$, $p < 0.0001$; hemoglobin concentration: $r = -0.380$, $p < 0.0001$; hematocrit: $r = -0.371$, $p < 0.0001$). VPIs who received a red blood cell transfusion had a significantly higher prevalence of thrombocytosis than those who did not (63.5% versus 36.5%, $p = 0.004$).

With regard to clinical outcomes, the incidence of complications of prematurity (requirement for oxygen at 28 days, persistence of patent ductus arteriosus, retinopathy of prematurity [ROP], late-onset sepsis) was significantly higher in VPIs with thrombocytosis than in those without thrombocytosis (all $p < 0.05$). When introduced into a logistic regression model, thrombocytosis was not significantly associated with the outcome, with the exception of ROP. The association of thrombocytosis

Table 1. Comparison of perinatal and prematurity-associated morbidities between patients with and without thrombocytosis.

	Thrombocytosis n = 63	No Thrombocytosis n = 130	p	Platelet count ≥700,000 n = 19	Platelet count <700,000 n = 174	p
Mean ± standard deviation						
Gestational age at birth (weeks)	28.0 ± 2.1	29.6 ± 2.2	**<0.001**	27.6 ± 2.2	29.3 ± 2.3	**<0.001**
Birth weight (g)	1036 ± 304	1303 ± 304	**<0.001**	971 ± 223	1243 ± 385	**0.004**
Duration of central line (days)	11.6 ± 8.7	9.8 ± 8.3	0.159	10.6 ± 8.0	10.3 ± 8.5	0.886
Days on antibiotics	15.7 ± 15.1	9.8 ± 12.6	**0.005**	14.8 ± 12.6	11.4 ± 13.8	0.309
Length of stay	76.0 ± 27.9	47.9 ± 27.3	**<0.001**	79.3 ± 26.3	54.7 ± 30.0	**0.001**
Number of blood tests	6.4 ± 3.8	3.8 ± 3.6	**<0.001**	6.68 ± 3.6	4.41 ± 3.8	**0.013**
n (%)						
Intrauterine growth restriction	8 (12.7)	17 (13.1)	0.941	1 (5.3)	24 (13.8)	0.259
Oxygen at 28 days of life	23 (36.5)	25 (20.8)	**0.022**	8/19 (42.1)	40/164 (24.4)	0.086
Oxygen at 36 weeks postmenstrual	15 (23.8)	11 (9.3)	**0.008**	6 (31.6)	20 (12.3)	**0.036**
Patent ductus arteriosus	35 (55.6)	47 (36.2)	**0.011**	10 (52.6)	72 (41.4)	0.346
Medical treatment	27 (43.5)	34 (26.4)	**0.017**	7 (38.9)	54 (31.2)	0.506
Surgical treatment	7 (11.1)	10 (7.7)	0.432	2 (10.5)	15 (8.6)	0.518
Retinopathy of prematurity	34/63 (54.0)	25/107 (23.4)	**<0.001**	12/19 (63.2)	47/151 (31.1)	**0.006**
Laser surgery	2 (3.2)	4/113 (3.5)	1.000	0/19 (0.0)	6/151 (4.0)	0.486
Necrotizing enterocolitis	2 (3.2)	4 (3.1)	1.000	1 (5.3)	5 (2.9)	0.468
Sepsis (late onset)	17 (27.0)	18 (13.8)	**0.026**	6 (31.6)	29 (16.9)	0.109
Red blood cell transfusion	40 (63.5)	53 (41.1)	**0.004**	15 (78.9)	78 (45.1)	**0.005**
Platelet transfusion	4 (6.6)	14 (11.1)	0.322	0 (0.0)	18 (10.7)	0.132
Died	1 (1.6)	12 (9.2)	**0.064**	0 (0.0)	19 (10.6)	0.371
Intraventricular hemorrhage	13 (20.6)	23 (17.7)	0.623	2 (10.5)	34 (21.6)	0.536
Cystic periventricular leukomalacia	2 (3.2)	7 (5.4)	0.721	0 (0.0)	9 (5.6)	0.310

Results are expressed as mean ± standard deviation for continuous variables or number (percentage in brackets) for qualitative variables. Variables were compared with the Student's t test and chi-square test.

p values that were significant (<0.05) are highlighted in bold.

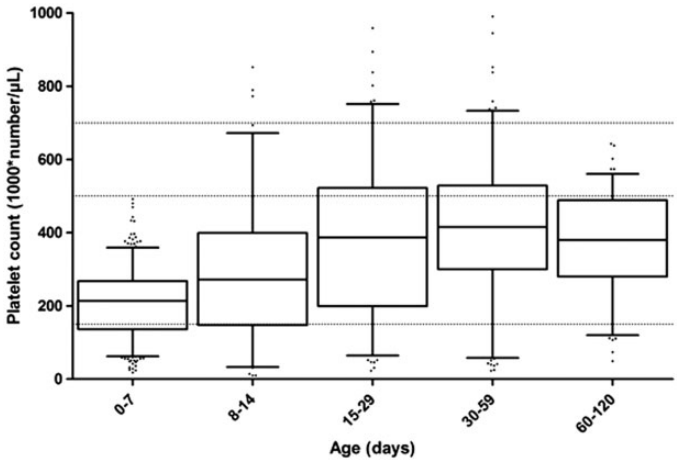

Figure 1. Boxplot showing the distribution of platelet counts by age. The boxes represent the 25th and 75th percentiles with the horizontal line at the mean and the whiskers extend to the 5th and 95th percentiles. Horizontal dash reference lines are shown at 150,000 platelets/μL (thrombocytopenia), 500,000 platelets/μL (thrombocytosis), and 700,000 platelets/μL (moderate to severe thrombocytosis).

Table 2. Percentiles of platelet counts (platelet number/μL) during the first week by gestational age group.

Gestational age (weeks)	Percentile							
	n	5	10	25	50	75	90	95
<25	46	117,000	117,000	199,750	244,000	317,000		
25–26.9	243	147,800	174,400	215,000	276,000	320,000	338,000	363,800
27–28.9	300	90,200	110,600	133,000	224,000	269,000	334,200	445,600
29–30.9	181	86,300	122,000	179,000	220,500	270,000	326,500	358,150
≥31	125	94,000	167,000	207,000	246,000	283,000	342,000	382,000

and ROP was still significant in logistic regression (Nagelkerke R square: 0.358, $p < 0.001$) after including the relevant covariates from univariate analysis (gestational age, late-onset sepsis, and oxygen at 28 days).

Discussion

Our findings support a high incidence of thrombocytosis in VPIs at approximately 1 month of postnatal age, despite not having received rHuEPO. Platelet values ranged from 23,000 to 991,000/μL, and 32.6% of patients had thrombocytosis. The percentiles of platelet counts per age group during the first week of life are in agreement with the literature.[2] Thrombocytosis appears to be a common finding in VPI[7] and our incidence is similar to other series, with reported ranges of 31% to 38%.[1,8,9] Matsubara et al described thrombocytosis in 38% of a sample of 24 low birth weight newborns with a considerably higher gestational age (33.9 ± 1.4 weeks) than our group.[1] During treatment

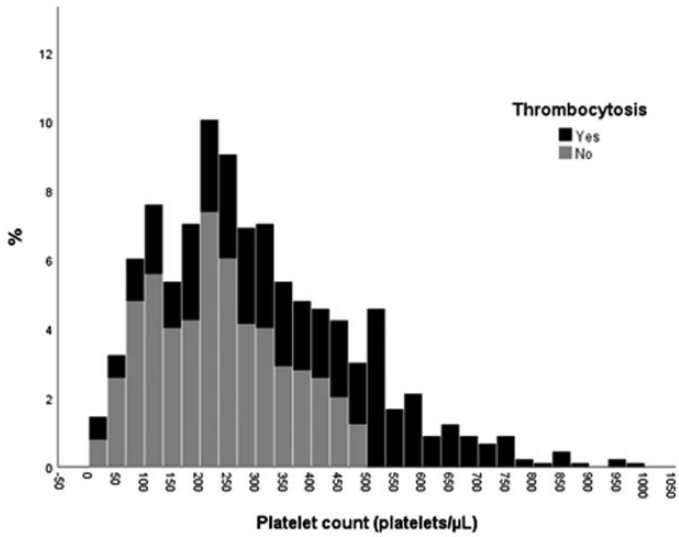

Figure 2. Histogram of platelet counts in increments of 50 platelets/μL. Counts of patients who developed thrombocytosis and those who did not are differentiated by color (black and grey, respectively).

with rHuEPO, 31% of 114 infants with a gestational age of 27.8 ± 2.4 weeks developed thrombocytosis.[8]

Little is known about the pathophysiology underlying thrombocytosis in neonates, especially if they are very preterm. Kinetics of the TPO pathway[3] might be involved because expression of the TPO receptor is low in platelets until 1 month after birth, leading to accumulation of high free TPO levels in blood.[10] Megakaryocytic precursors of VPI also display an *in vitro* increased sensitivity to TPO.[11] These facts are consistent with the temporal pattern of increased platelet counts in our VPIs between 20 and 40 days of life, as previously described by McPherson et al.[9]

EPO is another hemopoietic-stimulating factor with some structural and functional similarities to TPO.[4] We found a tendency for the erythrocyte number to decrease with days of life and a moderate inverse correlation between red blood cells and platelet count. Anemia is a common finding in VPI, secondary to iron deficiency, and is an inadequate response to endogenous EPO and increased losses with blood sampling. Although our population did not receive rHuEPO, our data support a role for increased levels of endogenous EPO due to anemia in development of a higher platelet count.

As previously described, most of the usual clinical complications of prematurity cannot be related to high platelet numbers[1,2,8] and their prevalence appears to differ according to the gestational age of the groups. We did not find any differences that were suggestive of occlusive events, such as cystic periventricular leukomalacia or a shorter duration of central catheter lines that could be attributed to unplanned withdrawal due to occlusion. Although major thrombotic events would probably have reflected in deterioration of the clinical course, minor thrombotic or hemorrhagic complications might have gone undetected. We do not have any data to speculate on any long-term effects of thrombocytosis.

There was a higher incidence of ROP in VPIs with thrombocytosis, and this was still significant after correcting for possible

confounding factors. There appears to be a complex role for platelets in the pathophysiology of ROP. Levels of serum vascular endothelial growth factor, a major factor in abnormal retinal vascular development, correlate with the platelet count.[12] However, there might also be involvement of local angiogenic factors contained in or scavenged by platelets.[13] This was the mechanism proposed by Vinekar et al.[13] who reported that thrombocytopenia was a risk factor for aggressive ROP. To the best of our knowledge, our study is the first to describe an association between thrombocytosis and the incidence of ROP. We did not find any differences in the prevalence of high platelet counts in VPIs who required laser treatment, but the sample size was too small to draw any further conclusion.

In conclusion, thrombocytosis is a frequent finding in VPIs after the first weeks of life. This appears to be a benign process, and is especially prevalent in the most immature and lighter infants, but it might play a role in the development of ROP. Neonatologists should be aware of the platelet number dynamics in these infants to avoid unnecessary tests, which could further contribute to anemia in this population.

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Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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