Platelet Counts and Patent Ductus Arteriosus in Preterm Infants: A Systematic Review and Meta-Analysis

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Key Words

Platelets · Ductus arteriosus · Meta-analysis

Abstract

Background: Several cohort studies have shown an association between low platelet counts in the first day(s) of life and patent ductus arteriosus (PDA) in preterm infants. However, these results have not been confirmed by other studies. **Objective:** To perform a meta-analysis of all the studies addressing the relationship between platelet counts in the first day(s) of life and PDA in preterm infants. **Methods:** PubMed/MEDLINE and EMBASE were searched from their inception until December 2014. Results from 11 cohort studies involving 3,479 preterm infants (gestational age <32 weeks) were pooled using random-effects modeling. **Results:** Meta-analysis showed a significant positive association between PDA and platelet counts $<150 \times 10^9/l$ [6 studies, risk ratio (RR) = 1.215, 95% CI: 1.027–1.436], between PDA and platelet counts $<100 \times 10^9/l$ (5 studies, RR = 1.255, 95% CI: 1.034–1.525), and between significant PDA (SPDA) and platelet counts $<100 \times 10^9/l$ (5 studies, RR = 1.254, 95% CI: 1.021–

1.540). The association between SPDA and platelet counts $<150 \times 10^9/l$ was not statistically significant (6 studies, RR = 1.289, 95% CI: 0.925–1.795). Pooled standard differences in mean platelet counts between infants with and without PDA/SPDA were not statistically different. **Conclusion:** This meta-analysis reveals a marginal but significant association between low platelet counts in the first day(s) of life and PDA/SPDA in very preterm infants. This association needs to be confirmed in prospective studies.

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Introduction

Despite decades of clinical and basic science progress, the persistence of a patent ductus arteriosus (PDA) in very preterm infants is still presenting important challenges in terms of pathobiology, assessment, clinical consequences, and management [1–6]. Recently, Echtler et al. [7] demonstrated that activated platelets adhered to and accumulated in the lumen of the constricted mice ductus arteriosus (DA) within minutes after birth. More-

Table 1. Characteristics of the studies included in the meta-analysis

First author [Ref.]	Year	n	GA cohort, weeks	BW cohort, g	% males	PDA assessment, day of life	% PDA	% SPDA	Range PL assessment, days	% PL <150	% PL <100
Echtler [7]	2010	123	28.0	1,036	48.8	3–5	70.7	23.6	1	15.4	0.0
Fujioka [11]	2011	118	27.9	1,059	57.6	3–5	29.7	23.7 ^a	1	16.1	6.8 ^a
Shah [12]	2011	497	25.8	825	52.6	3	34.0	NR	3	27.2 ^a	8.7 ^a
Dwarakanath [19] ^b	2011	148	26.0	733	50.0	NR	60.8	45.9 ^c	3	41.2	NR
Dizdar [9]	2012	361	28.6	1,054	46.5	3–5	NR	42.7	3	23.82	5.5 ^a
Sallmon [13]	2012	1,350	28.3	1,060	48.7	4–5	56.3	26.7	1	16.44	3.4
Dani [10]	2012	163	27.1	931	49.7	1–2	NR	82.8	1	32.5	19.6 ^a
Brunner [18]	2013	322	29.0	1,170	50.6	2–7	50.9	47.2	1	NR	2.8 ^d
Bas-Suárez [15]	2014	194	27.9	1,008	55.7	2–4	NR	54.6	2	11.3	6.7
Chen [14]	2014	77	29.5	1,153	49.4	3–6	NR	44.2	1	NR	NR
CPS ^e	2015	126	28.4	1,096	51.2	2–4	69.8	64.3	2	28.57	14.28

In the study of Shah et al. [12], all the patients received prophylactic indomethacin. In the study of Echtler et al. [7], a nonreported number of patients received prophylactic indomethacin. GA = Gestational age (mean or median); BW = birth weight total cohort (g, mean or median); PL = platelets ($\times 10^9/l$); NR = data not reported. ^a Data not reported in the original article but obtained after request to authors. ^b Abstract. ^c Based on number of patients treated with COX inhibitors. ^d Patients with platelet count $<50 \times 10^9/l$ were excluded. ^e Unpublished multicenter study, Carbamoyl Phosphate Synthetase (CPS) Polymorphisms as Risk Factor for Necrotizing Enterocolitis.

over, mice with defective platelet adhesion or biogenesis had high rates of PDA [7]. Thus, platelets may be crucial for mice DA closure by promoting thrombotic sealing of the constricted DA and luminal remodeling [7]. However, whether this is also the case in human infants remains controversial [1, 8].

Echtler et al. [7] also performed a retrospective, multi-variable analysis in a cohort of 123 infants born at 24–30 weeks of gestation. They reported that a platelet count between 101 and $140 \times 10^9/l$ on the first day of life was an independent predictor of PDA. Since the publication of this study, several additional cohorts have been reported with discordant results [9–15]. We performed a meta-analysis of all the studies reported to date addressing the association between platelet counts in the first day(s) of life and the presence of a PDA in preterm infants, in an attempt to clarify this issue.

Methods

A protocol was developed prospectively that detailed the specific objectives, criteria for study selection, approach to assessing study quality, clinical outcomes, and statistical methodology.

Sources

A comprehensive literature search was undertaken using the PubMed/MEDLINE, and EMBASE databases from their inception

to December 1, 2014. The search terms involved various combinations of the following keywords: ‘ductus arteriosus’, ‘patent ductus arteriosus’, ‘PDA’, ‘platelet(s)’, ‘platelet count(s)’, and ‘thrombocytopenia’. We searched the Science Citation Index and Google Scholar for articles citing the studies identified by our electronic searches.

Study Selection

Three investigators (S.R.S., M.P.B.-S., and E.V.) independently evaluated studies for inclusion and any disagreements were resolved by discussion. Studies were included if they had a comparison group, examined preterm infants, and reported primary data that could be used to measure the association between PDA and platelet counts.

Data Extraction and Assessment of Study Quality

Two investigators (S.R.S. and M.P.B.-S.) independently extracted data on study design, study quality, demographics (table 1), rate of PDA and/or significant PDA (SPDA), and platelet counts. A third reviewer (E.V.) checked the data extraction for completeness and accuracy. In cases in which necessary data were missing from the studies, additional information was requested from the authors. Methodological quality was assessed using the Newcastle-Ottawa Scale (NOS) for cohort studies [16]. This scale uses a star rating system (range: 0–9 stars) scoring three aspects of the study: selection (0–4), comparability (0–2), and outcomes (0–3).

Statistical Analysis

Studies were combined and analyzed using Comprehensive Meta-Analysis v. 2.0 software (Biostat Inc., Englewood, N.J., USA). The effect measures estimated were risk ratio (RR) for dichotomous outcomes, and standard mean difference for continuous

Table 2. Risk factors for the development of PDA/SPDA

First author [Ref.]	Significant risk factors on multivariable analysis	Odds ratio	95% CI
Echtler [7]	platelet count $<150 \times 10^9/l$	13.13	3.47–49.60
Shah [12]	absolute platelet nadir	0.73	0.55–0.98
	highest quintile platelet nadir	0.43	0.2–0.91
	gestational age	0.84	0.70–0.99
	antenatal betamethasone	0.52	0.34–0.81
	preeclampsia	0.37	0.14–0.99
	respiratory distress syndrome	2.73	1.37–5.40
Dizdar [9]	fluid intake	1.09	1.00–1.19
	platelet count $<150 \times 10^9/l$	2.083	1.252–3.465
	platelet distribution width >17	3.030	1.571–5.847
Sallmon [13]	respiratory distress syndrome	2.534	1.602–4.009
	male sex	1.64	1.32–2.04 ^a
	sepsis	2.82	1.47–5.40 ^a
Dani [10]	gestational age	not shown	
	platelet count $<100 \times 10^9/l$	4.50	1.39–14.61
Bas-Suárez [15]	gestational age	1.25	1.12–1.39
	respiratory distress syndrome	0.778	0.633–0.951
	arterial hypotension	5.19	1.76–15.27
		2.99	1.338–6.721

^a Univariable odds ratio.

data. DerSimonian and Laird random-effect models were used to derive random-effect estimates and 95% CI for all outcomes. To identify any study that may have exerted a disproportionate influence on the summary effect, we deleted studies one at a time. Heterogeneity was assessed with the Q statistic and quantified using the I^2 statistic. Because of the small number of studies, a funnel plot analysis to assess publication bias was not conducted. Meta-regression, using random effects (method of moments estimator), was performed to explore the following sources of heterogeneity in the relationship between PDA/SPDA and platelet counts: number of patients, cohort mean or median gestational age and birth weight, percent males, rate of PDA/SPDA, range of days in which platelet counts were assessed, thrombocytopenia rate, and year of publication. A p value less than 0.05 (0.10 for heterogeneity) was considered statistically significant. The study is reported according to the PRISMA checklist [17].

Results

We identified 31 potentially relevant studies through electronic and hand searches. After careful review, 8 full articles [7, 9, 10, 12–15, 18], 1 letter to the editor [11], and 1 abstract [19] were eligible for the meta-analysis. An additional unpublished cohort of 126 preterm infants was

included in the meta-analysis. The infants in this cohort had been enrolled into a multicenter study, the Carbamoyl Phosphate Synthetase (CPS) Polymorphisms as Risk Factor for Necrotizing Enterocolitis (NCT00554866), for which the patient inclusion criteria were gestational age ≤ 30 weeks and birth weight $\leq 1,500$ g [20, 21]. Data on platelet counts of these patients were collected by two investigators (G.C. and L.v.Z.). The main characteristics of the studies are shown in table 1. All the studies were cohort studies and only one [18] was prospective. Each study was allocated more than 6 NOS stars.

From the included studies, 4 [11, 12, 18, 19] compared infants with and without PDA, i.e. infants with a small PDA were included in the PDA group. Four studies [9, 10, 14, 15] compared infants with and without SPDA, i.e. infants with a small PDA were included in the non-SPDA group. Finally, 2 studies [7, 13] and the CPS cohort provided data on closed DA, small PDA, and SPDA. We included these 2 studies in both the comparison of PDA versus non-PDA and the comparison of SPDA versus non-SPDA. The risk factors for PDA/SPDA identified by multivariable analysis in the different studies are shown in table 2.

Fig. 1. Forest plot for studies examining the effect of platelet counts below $150 \times 10^9/l$ in the first day(s) of life on PDA. Risk ratio >1 indicates increased risk of PDA. The data of Shah et al. [12] were not reported in the article but provided by the authors.

Fig. 2. Forest plot for studies examining the effect of platelet counts below $100 \times 10^9/l$ in the first day(s) of life on PDA. Risk ratio >1 indicates increased risk of PDA. The study of Brunner et al. [18] reported the PDA rate in infants with platelet counts above $100 \times 10^9/l$ and between 50 and $100 \times 10^9/l$. The data of Shah et al. [12] and Fujioka et al. [11] were not reported in the articles but provided by the authors.

Meta-Analysis

Data on the rate of PDA in infants with platelet counts above or below $150 \times 10^9/l$ were available from 6 cohorts (fig. 1). There was a significant positive association between PDA and platelet counts below $150 \times 10^9/l$. In sensitivity analyses, excluding one study at a time, the summary RR ranged from 1.138 (95% CI: 0.991–1.3067, $p = 0.067$), when the study of Echtler et al. [7] was excluded,

to 1.291 (95% CI: 1.106–1.507, $p = 0.001$), when the study of Sallmon et al. [13] was excluded. Data on the rate of PDA in infants with platelet counts above or below $100 \times 10^9/l$ were available from 5 cohorts (fig. 2). There was a significant positive association between PDA and platelet counts below $100 \times 10^9/l$. In sensitivity analyses, excluding one study at a time, the summary RR ranged from 1.220 (95% CI: 0.886–1.681, $p = 0.223$), when the study of

Fig. 3. Forest plot for studies examining the effect of platelet counts below $150 \times 10^9/\text{l}$ in the first day(s) of life on SPDA. Risk ratio >1 indicates increased risk of SPDA. The data of Dani et al. [10] were not reported in the article but provided by the authors.

Fig. 4. Forest plot for studies examining the effect of platelet counts below $100 \times 10^9/\text{l}$ in the first days of life on SPDA. Risk ratio >1 indicates increased risk of SPDA. The data of Dizdar et al. [9] and Dani et al. [10] were not reported in the articles but provided by the authors.

Sallmon et al. [13] was excluded, to 1.327 (95% CI: 1.162–1.516, $p < 0.001$), when the study of Shah et al. [12] was excluded. Data on the rate of PDA in infants with platelet counts above or below $50 \times 10^9/\text{l}$ were available from 3 cohorts [12, 13 and CPS]. Meta-analysis could not detect a significant association between PDA and platelet counts below $50 \times 10^9/\text{l}$ (RR = 1.072, 95% CI: 0.787–1.459, $Z = 0.441$, $p = 0.659$). Mean (SD) platelet counts in infants

with and without PDA were available from 3 cohorts [11, 12 and CPS]. The pooled standard difference (no-PDA minus PDA) in means was not statistically significant (-6.67×10^9 , 95% CI: -46.74 to -12.98 , $p = 0.754$). When the CPS cohort was removed from the analysis, the pooled standard difference in means became -29.86×10^9 (95% CI: -46.74 to -12.98 , $p = 0.001$).

Fig. 5. Random-effects (methods of moments) meta-regression analysis of the relation between birth weight (**a**) and gestational age (**b**) and risk of PDA with platelet counts below $100 \times 10^9/l$ in the first day(s) of life.

Data on the rate of SPDA in infants with platelet counts above or below $150 \times 10^9/l$ were available from 6 cohorts (fig. 3). Meta-analysis could not detect a significant association between SPDA and platelet counts below $150 \times 10^9/l$. In sensitivity analyses, excluding one study at a time, the summary RR ranged from 1.093 (95% CI: 0.863–1.383, $p = 0.460$), when the study of Echter et al. [7] was excluded, to 1.404 (95% CI: 0.998–1.977, $p = 0.052$), when the study of Bas-Suárez et al. [15] was excluded. Data on the rate of SPDA in infants with platelet counts above or below $100 \times 10^9/l$ were available from 5 cohorts (fig. 4). There was a significant positive association between SPDA and platelet counts below $100 \times 10^9/l$. In sensitivity analyses, excluding one study at a time, the summary RR ranged from 1.177 (95% CI: 0.979–1.414, $p = 0.082$), when the study of Dizdar et al. [9] was excluded, to 1.302 (95% CI: 1.100–1.541, $p = 0.002$), when the study of Bas-Suárez et al. [15] was excluded. Data on the rate of SPDA in infants with platelet counts above or below $50 \times 10^9/l$ were available from 3 cohorts [12, 13 and CPS]. Meta-analysis could not detect a significant association between SPDA and platelet counts below $50 \times 10^9/l$ (RR = 1.123,

95% CI: 0.717–1.757, $Z = 0.506$, $p = 0.613$). Mean (SD) platelet counts in infants with and without SPDA were available from 5 cohorts [9, 10, 14, 15 and CPS]. The pooled standard difference (no-SPDA minus SPDA) in means was not statistically significant (11.69×10^9 , 95% CI: -16.23 to 39.62 , $p = 0.412$).

Random-effects meta-regression analysis showed a significant positive correlation between mean gestational age (fig. 5a) and mean birth weight (fig. 5b) of the infants included in each study and risk of PDA when platelet count was below $100 \times 10^9/l$. Thus, studies with more mature or larger infants showed higher RRs. In contrast, the studies which included a higher number of infants showed significantly lower risk of PDA with platelets below $150 \times 10^9/l$ ($Z = -2.460$, $p < 0.014$), and the studies including a higher percentage of males showed significantly lower RRs for SPDA when the platelet count was below $100 \times 10^9/l$ ($Z = -1.976$, $p = 0.048$). Meta-regression analyses did not show any significant effect of publication year, rate of PDA/SPDA, or rate of thrombocytopenia in the results of the meta-analysis. The range of postnatal age when platelet count was assessed (1–3 days; table 1) did

not correlate with the incidence of platelet counts below $150 \times 10^9/l$ (Spearman's correlation coefficient, $\rho_s = 0.4276$, $p = 0.2499$) or $100 \times 10^9/l$ ($\rho_s = 0.2951$, $p = 0.4366$) and random-effects meta-regression analysis did not show any significant effect of this range in the results of the meta-analysis.

Discussion

Our meta-analysis, which combines data from 11 study cohorts (3,479 preterm infants), shows that a low platelet count in the first day(s) of life is a modest but significant risk factor for PDA/SPDA. However, the low robustness of the association and the high degree of statistical heterogeneity among studies suggest that the link between platelet counts and DA closure might not be clinically relevant.

Term infants with conditions characterized by altered platelet number or function, such as Wiskott-Aldrich syndrome, alloimmune thrombocytopenia, or Glanzmann thrombasthenia, do not show an increased incidence of PDA [8, 22–24]. This argument has been used to question whether the findings of thrombotic closure of the mouse DA by Echtler et al. [7] can be extrapolated to humans [8]. It is important to note that in the full-term human newborn, postnatal DA constriction leads to narrowing of the lumen and compression of vasa vasorum, resulting in a hypoxic stimulus within the vessel wall. This hypoxia is necessary for DA remodeling and permanent anatomic closure [8, 25, 26]. The very preterm human DA and the term mouse DA are thin vessels and lack vasa vasorum. Therefore, they can still extract sufficient oxygen from residual luminal blood even when the DA is constricted [8, 25, 26]. Thus, thrombotic luminal occlusion might play a significant role in the very preterm human DA, as it does in the mouse. However, as an argument against this hypothesis, we found a positive correlation between the maturity of the study infants and the association between PDA and low platelet counts (fig. 5).

One of the main difficulties in understanding the relationship between thrombocytopenia and PDA is the lack of agreement among neonatologists as to what defines these clinical entities. It is not clear whether the definition of neonatal thrombocytopenia as a platelet count $<150 \times 10^9/l$ is applicable to very preterm infants [27, 28]. In a large population study involving 47,291 neonates, the 5th percentile of all platelet counts for infants <32 weeks of gestation was found to be $104 \times 10^9/l$ [29]. In addition, the clinical relevance of mild (100 – $150 \times 10^9/l$) or even mod-

erate (50 – $100 \times 10^9/l$) thrombocytopenia in premature infants is still unclear [28]. In the only prospective randomized trial to address this issue, investigators found that platelet transfusions in the setting of mild-to-moderate thrombocytopenia did not alter the incidence of PDA in preterm infants or other short-term outcomes [30].

The definition of PDA is also under debate. Clear evidence is lacking for or against many of the current approaches to a PDA in preterm infants. These uncertainties have resulted in different definitions of what investigators have called 'significant PDA', i.e. one that causes symptoms or is worthy of treatment [3, 4, 31, 32]. We noted marked differences in the rates of PDA/SPDA reported in the studies included in our meta-analysis (table 1). This may reflect differences in the criteria used to define a PDA as clinically and/or hemodynamically significant [32]. However, meta-regression analysis showed no significant impact of the individual rates of PDA/SPDA on the results of the meta-analysis.

The majority of the studies included in our meta-analysis were retrospective. As a result, important risk factors might not have been accurately identified or recorded [33] and the results may be confused by the timing of assessment of platelet counts and PDA. As an example of this, the platelet counts were generally done at the discretion of healthcare providers and not at a fixed interval. Therefore, the rate of thrombocytopenia in the more stable patients could have been underestimated. In addition, the range of days in which platelet counts were determined varied between 1 and 3. It should be noted that the majority of preterm neonates who ultimately develop thrombocytopenia during the first week of life have either a low-normal platelet count (150 – $200 \times 10^9/l$) or mild thrombocytopenia at birth [34]. Over the next several days, the platelet count falls, reaching a nadir on day 4–5, before recovering to $>150 \times 10^9/l$ by 7–10 days. The relationship between the time of thrombocytopenia nadir and time of PDA closure is important since only 10–15% of preterm infants <28 weeks of gestation close their PDA between days 1 and 3; whereas, 30% close their PDA by day 7 [2, 3]. Only the studies of Shah et al. [12] and Bas-Suárez et al. [15] reported platelet counts after the third day of life, and they did not observe a relationship between low platelet counts on days 3–7 and PDA/SPDA.

It is also unclear whether any potential relationship between PDA and thrombocytopenia is really with thrombocytopenia itself or with some of its etiologies, such as intrauterine growth retardation or sepsis [34], which could affect DA closure. Rather than being responsible for PDA, it has been suggested that platelet counts

are merely a surrogate marker of clinical maturity and stability [12]. In the study by Shah et al. [12], the infants whose platelet counts never fell below the highest quintile ($>230 \times 10^9/l$) had the lowest incidence of PDA.

In conclusion, the present meta-analysis reveals a marginal but significant association between low platelet counts in the first day(s) of life and PDA/SPDA in very preterm infants. This association needs to be confirmed in prospective studies. In addition, any relationship between association and causation needs to be determined. It has been proposed that impaired platelet function, due to immaturity and critical illness, rather than platelet number, may contribute to PDA [13, 35]. Therefore, future studies should take into account not only the number but also the function of platelets [35]. However, platelet function tests require a large volume of blood and poorly simulate in vivo primary hemostasis [36]. Finally, further studies are warranted to evaluate the role of platelet counts in the response of PDA to treatment. Paradoxically, despite their known inhibitory effects on platelet

activation in vitro [7], both indomethacin and ibuprofen are effective at inducing PDA closure. Interestingly, Boo et al. [37] and – very recently – Ahamed et al. [38] reported an association between low platelet counts before starting indomethacin treatment and lack of response to the therapy. Nevertheless, this finding has not been confirmed in other studies [12, 15].

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Disclosure Statement

The authors report no competing interests.

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