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

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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 × 10⁹/l [6 studies, risk ratio (RR) = 1.215, 95% CI: 1.027–1.436], between PDA and platelet counts <100 × 10⁹/l (5 studies, RR = 1.255, 95% CI: 1.034–1.525), and between significant PDA (SPDA) and platelet counts <100 × 10⁹/l (5 studies, RR = 1.254, 95% CI: 1.021–1.540). The association between SPDA and platelet counts <150 × 10⁹/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.

Key Words
Platelets · Ductus arteriosus · Meta-analysis

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-
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, multivariable analysis in a cohort of 123 infants born at 24–30 weeks of gestation. They reported that a platelet count between 101 and 140 × 10⁹/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.

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 outcomes.
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 influ-
ence on the summary effect, we deleted studies one at a time. Het-
erogeneity was assessed with the Q statistic and quantified using
the I² statistic. Because of the small number of studies, a funnel plot
analysis to assess publication bias was not conducted. Meta-regres-
sion, 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 publica-
tion. A p value less than 0.05 (0.10 for heterogeneity) was consid-
ered statistically significant. The study is reported according to the
PRISMA checklist.

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 addi-
tional 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 Carbam-
yl 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 ≤1,500 g [20, 21]. Data on
platelet counts of these patients were collected by two in-
vestigators (G.C. and L.v.Z.). The main characteristics of
the studies are shown in table 1. All the studies were co-
hort 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 pro-
vided data on closed DA, small PDA, and SPDA. We in-
cluded 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.

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

<table>
<thead>
<tr>
<th>First author [Ref.]</th>
<th>Significant risk factors on multivariable analysis</th>
<th>Odds ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shah [12]</td>
<td>absolute platelet nadir</td>
<td>0.73</td>
<td>0.55–0.98</td>
</tr>
<tr>
<td></td>
<td>highest quintile platelet nadir</td>
<td>0.43</td>
<td>0.2–0.91</td>
</tr>
<tr>
<td></td>
<td>gestational age</td>
<td>0.84</td>
<td>0.7–0.99</td>
</tr>
<tr>
<td></td>
<td>antenatal betamethasone</td>
<td>0.52</td>
<td>0.34–0.81</td>
</tr>
<tr>
<td></td>
<td>preeclampsia</td>
<td>0.37</td>
<td>0.14–0.99</td>
</tr>
<tr>
<td></td>
<td>respiratory distress syndrome</td>
<td>2.73</td>
<td>1.37–5.40</td>
</tr>
<tr>
<td></td>
<td>fluid intake</td>
<td>1.09</td>
<td>1.00–1.19</td>
</tr>
<tr>
<td></td>
<td>platelet distribution width &gt;17</td>
<td>3.030</td>
<td>1.571–5.847</td>
</tr>
<tr>
<td></td>
<td>respiratory distress syndrome</td>
<td>2.534</td>
<td>1.602–4.009</td>
</tr>
<tr>
<td>Sallmon [13]</td>
<td>male sex</td>
<td>1.64</td>
<td>1.32–2.04</td>
</tr>
<tr>
<td></td>
<td>sepsis</td>
<td>2.82</td>
<td>1.47–5.40</td>
</tr>
<tr>
<td></td>
<td>gestational age</td>
<td>not shown</td>
<td></td>
</tr>
<tr>
<td>Dani [10]</td>
<td>platelet count &lt;100 × 10⁹/l</td>
<td>4.50</td>
<td>1.39–14.61</td>
</tr>
<tr>
<td></td>
<td>gestational age</td>
<td>1.25</td>
<td>1.12–1.39</td>
</tr>
<tr>
<td>Bas-Suárez [15]</td>
<td>gestational age</td>
<td>0.778</td>
<td>0.633–0.951</td>
</tr>
<tr>
<td></td>
<td>respiratory distress syndrome</td>
<td>5.19</td>
<td>1.76–15.27</td>
</tr>
<tr>
<td></td>
<td>arterial hypotension</td>
<td>2.99</td>
<td>1.338–6.721</td>
</tr>
</tbody>
</table>

* Univariable odds ratio.
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 $1.138 (95\% \text{ CI}: 0.991–1.3067, p = 0.067)$, when the study of Echtler et al. [7] was excluded, to $1.291 (95\% \text{ CI}: 1.106–1.507, p = 0.001)$, 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 $1.220 (95\% \text{ CI}: 0.886–1.681, p = 0.223)$, when the study of Shah et al. [12] and Fujioka et al. [11] were not reported in the articles but provided by the authors.

**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.
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 × 10⁹/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 × 10⁹/l (RR = 1.072, 95% CI: 0.787–1.459, Z = 0.580, p = 0.562). 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 × 10⁹, 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 × 10⁹ (95% CI: −46.74 to −12.98, p = 0.001).

**Fig. 3.** Forest plot for studies examining the effect of platelet counts below 150 × 10⁹/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 × 10⁹/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.
Data on the rate of SPDA in infants with platelet counts above or below 150 × 10⁹/l were available from 6 cohorts (fig. 3). Meta-analysis could not detect a significant association between SPDA and platelet counts below 150 × 10⁹/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 Echtler 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 × 10⁹/l were available from 5 cohorts (fig. 4). There was a significant positive association between SPDA and platelet counts below 100 × 10⁹/l. In sensitivity analyses, excluding one study at a time, the summary RR ranged from 1.177 (95% CI: 0.979–1.414, p = 0.052), 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 × 10⁹/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 × 10⁹/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 × 10⁹, 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 × 10⁹/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 × 10⁹/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 × 10⁹/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 × 10^9/l (Spearman’s correlation coefficient, ρs = 0.4276, p = 0.2499) or 100 × 10^9/l (ρs = 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 × 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 × 10^9/l [29]. In addition, the clinical relevance of mild (100–150 × 10^9/l) or even moderate (50–100 × 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 × 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 × 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 × 10⁹/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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