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SHORT COMMUNICATION

C-reactive protein, platelets, and patent ductus arteriosus

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Abstract

The association between inflammation, platelets, and patent ductus arteriosus (PDA) has not been studied so far. The purpose of this study was to evaluate whether C-reactive protein (CRP) is related to low platelet count and PDA. This was a retrospective study of 88 infants with a birth weight ≤1500 g and a gestational age ≤30 weeks. Platelet count, CRP, and an echocardiogram were assessed in all infants. The subjects were matched by sex, gestational age, and birth weight. Differences were compared using the χ^2, *t*-test, or Mann–Whitney *U*-test, as appropriate. Significant variables were entered into a logistic regression model. The association between CRP and platelets was evaluated by correlation and regression analysis. Platelet count (167 000 vs. 213 000 μl^{-1}, $p = 0.015$) was lower and the CRP (0.45 vs. 0.20 mg/dl, $p = 0.002$) was higher, and the platelet count correlated inversely with CRP ($r = -0.145$, $p = 0.049$) in the infants with vs. without PDA. Only CRP was independently associated with PDA in a logistic regression model (OR 64.1, 95% confidence interval 1.4–2941, $p = 0.033$).

Keywords

C-reactive protein, patent ductus arteriosus, platelets

History

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Introduction

The persistence of a patent ductus arteriosus (PDA) in preterm infants is still a major challenge. A significant contribution has been the elucidation of the role played by platelets in PDA closure and the report that a thrombocytopenia was a predictor of PDA [1]. Since this publication, several studies have yielded discordant results [2]. The controversy has not yet been resolved because the discordant results can be explained by differences in birth weight, gestational age, and male sex prevalence between the studies [3].

Similarly, it has been reported that sepsis [4] and chorioamnionitis [5] were associated with the persistence of a PDA. These findings suggest that inflammation plays an important role in the pathogenesis of PDA. We asked whether low-grade sterile inflammation represents an interface between platelets and PDA resembling the process described for atherosclerotic lesion development. Studies have shown that elevations in C-reactive protein (CRP) levels have a direct role on vascular endothelial cells and platelets dysfunction [6]. We have previously report higher CRP levels in preterm infants with PDA than that in the infants without PDA [7].

To the best of our knowledge, the possible association between CRP, platelets, and PDA has not been studied so far. The aim of this study was to evaluate whether CRP has a relation with low platelet count and PDA persistency.

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Methods

We reviewed the records of all newborns admitted to Hospital Privado, from 2009 to 2014. Inclusion criteria were gestational age ≤30 weeks, birth weight ≤1500 g, and whether they had received a blood examination and an echocardiogram on day 3 ± 1 and day 7 ± 1 of life. Exclusion criteria were malformations, death before day 7 of life, sepsis, chorioamnionitis (defined by maternal fever, purulent or foul-smelling amniotic fluid or vaginal discharge, uterine tenderness, and maternal leukocytosis), necrotizing enterocolitis, bleeding, shock (defined as the need for catecholamine treatment), intraventricular hemorrhage, multiple gestation, small for gestational age, and maternal preeclampsia. The subjects were matched by sex, gestational age, and birth weight (in 100 g strata).

On day 3 ± 1 of life, all infants underwent an echocardiographic examination using an ACUSON Sequoia 512 (Siemens, Erlangen, Germany) Doppler echocardiography devise. Clinically significant PDA was defined by a transductal diameter ≥ 1.5 mm and left atrium to aortic ratio ≥1.5, with unrestrictive (<1 m/s) left-to-right transductal flow on pulse wave Doppler and clinical signs of pulmonary overcirculation (increasing ventilation, and oxygenation problems). Significant PDA was treated with ibuprofen (10 mg/kg IV followed by two doses of 5 mg/kg IV 24 and 48 h later). Successful response was defined as absence of ductal shunt 48 h later. If the patient failed to respond to two courses of ibuprofen, PDA was surgically treated. If the ductus arteriosus was clinically significant and with a diameter >2 mm, primary surgical ligation was indicated.

Blood specimens were analyzed in an automated hematology analysis system (Sysmex XE 2100, Sysmex Corporation, Kobe, Japan). Platelets were manually evaluated if automated count was

<150 000/μl. High-sensitivity CRP concentrations were measured on a Roche/Hitachi modular P analyzer (Roche Diagnostics, Indianapolis, Indiana, USA).

Data were presented in frequencies and percentages with 95% confidence interval (95%CI), mean ± standard deviation (SD), or medians with interquartile range (IQR). Differences were compared by using the χ^2, t test, or Mann–Whitney U test as appropriate. Significant univariate variables were entered into a logistic regression model. The association between CRP and platelets was evaluated by correlation and regression analysis. Logarithmic transformation was performed when required. Statistical significance was set at $p < 0.05$.

The study was approved by the Institutional Ethics Review Board.

Results

A total of 138 infants were eligible, 42 were excluded (2 died, 1 data missing, 11 chorioamnionitis, sepsis, or necrotizing enterocolitis, 14 small for gestational age or preeclampsia, 5 shock, 7 intraventricular hemorrhage, and 2 congenital heart anomalies other than PDA). After matching the subjects, 8 additional patients were excluded, with 88 patients remaining for the study. Mean birth weight was 1111.9 ± 296.6 g (range 610–1499 g) with a mean gestational age of 28.4 ± 1.8 weeks (range 24–30 weeks); 7/44 (15.9% 95%CI 6.6–30) underwent primary surgical ligation on day 3 of life, and 10/44 (22.7% 95%CI 11.4–37.8) had a clinically nonsignificant PDA; 27/44 (61.3% 95%CI 45.4–75.6) received ibuprofen, and 3/27 (11.1% 95%CI 2.3–29.1) received surgical ligation after second unsuccessful cycle of intravenous ibuprofen. No patient received antibiotics or catecholamines.

Demographic, clinical, and laboratory characteristics of the infants with and without PDA are compared in Table I.

The median platelet count was significantly lower and the median CRP was significantly higher in the infants with PDA. On day 7 of life, before the PDA treatment neither the median platelet count nor the median CRP were statistically significant when compared to the infants with and without PDA [282 000 (210 000–375 000) platelets /μl vs. 287 000 (153 000–429 000) platelets μl^{-1}, $p = 0.815$ and a CRP of 0.42 (0.16–0.41) mg/dl vs. 0.35 (0.14–0.45) mg/dl, $p = 0.268$).

There were significant differences in median CRP between the patients with PDA and those without PDA [2.37 (IQR 0.31–0.80) mg/dl vs. 0.36 (IQR 0.10–0.32) mg/dl, respectively, $p = 0.004$], in the subset of the infants without invasive ventilation [41 infants, 18 (43.9% 95% CI 28.5–60.2) with PDA and 23 (56.1% 95% CI 39.7–71.5)] without PDA in the first 3 days of life.

Platelet count and plasma levels of CRP on day 3 of life were correlated inversely in the patients with PDA, Pearson's $r = -0.298$, $R^2 = 0.089$, $p = 0.049$, whereas in the patients without PDA platelet counts were not correlated with CRP (Pearson's $r = -0.151$, $R^2 = 0.023$, $p = 0.329$), as shown in Figure 1.

Only CRP was significantly and independently associated with PDA in a logistic regression model (OR 64.1, 95% CI 1.4–2941, $p = 0.033$).

Discussion

In this study, we found that a lower platelet count correlated significantly with higher CRP levels in the infants with PDA. Our design was intended to minimize the influence of preeclampsia, intrauterine growth restriction, chorioamnionitis, birth weight, gestational age, male gender, and neonatal sepsis on the variables under study [8].

Little evidence is available to document that mechanical ventilation is an antecedent of systemic inflammation in preterm infants [9]. With or without a cause–effect relationship, mechanical ventilation can obscure the relationship between inflammation and PDA. To minimize such confounding we carried out separate analysis in a subsample of infants without invasive ventilation in the first 3 days of life. We found that even in this invasive ventilation free group of infants, PDA was significantly associated with elevated concentrations of CRP.

The rationale of the association between low platelet count and PDA lies in the fact that platelet adhesion and aggregation are key steps in ductal closure after initial constriction. Completion of these steps could be partially inhibited by low platelet counts [10]. The relevance of this finding is seriously questioned by the fact that the incidence of PDA is not improved by platelet transfusions [11]. Moreover, the incidence of PDA does not increase in preterm infants with immune thrombocytopenia [12].

It has been proposed that impaired platelet function rather than platelet number may contribute to PDA [9]. Echler *et al*. have demonstrated in mice that defective platelet function was associated with insufficient ductal closure [1].

Based on our findings, we speculate that low-grade sterile systemic inflammation, probably caused by oxidative stress [13], might be involved in PDA pathogenesis. This inflammation could increase cyclooxygenase 1 expression and induce cyclooxygenase

Table I. Demographic, clinical, and laboratory characteristics of the preterm infants with and without patent ductus arteriosus on day 3 of life.

Characteristic	With Patent Ductus Arteriosus ($n = 44$)	Without Patent Ductus Arteriosus ($n = 44$)	p Value
Male sex *N*[%(95%CI)]	22[50(34.6–65.4)]	22[50(34.6–65.4)]	1.000
Gestational age [weeks μ ± SD(range)]	28.4 ± 1.7[24–30]	28.4 ± 1.7[24–30]	1.000
Birth weight [g μ±SD(range)]	1080.4 ± 309.8(610–1495)	1143.5 ± 282.7(640–1499)	0.321*
Prenatal steroids *n*[%(95%CI)]	29[65.9(50.1–79.5)]	27[61.4(45.5–75.6)]	0.825
PPROM** *n*[%(95%CI)]	5[11.3(3.8–24.5)]	3[6.8(1.4–18.6)]	0.711
Cesarean section n[%(95%CI)]	30[68.1(52.3–81.3)]	28[63.6(47.7–77.6)]	0.822
Apgar score median (IQR)	8(5–8)	8(7–9)	1.000
Respiratory Distress Syndrome *n*[%(95%CI)]	42[95.5(84.6–99.4)]	40[90.9(78.3–97.5)]	0.672
Surfactant *n*[%(95%CI)]	40[90.9(78.3–97.5)]	34[77.3(63.7–90.8)]	0.145
itIMV*** n[%(95%CI)]	26[59(43.74.7)]	21[47.7(31.8–63.6)]	0.393
C-reactive protein (mg/dl) median (IQR)	0.45(0.27–0.96)	0.20(0.14–0.30)	0.002
Platelet count nadir (x/μl) median (IQR)	167 000(127 000–235 000)	213 000(150 250–283 250)	0.015
Thrombocytopenia (platelets ≤ 150 000/μl) *n*[%(95%CI)]	17[38.6(23.9–54)]	10[22.7(11.4–37.8)]	0.165

*Matched by 100 g strata. **Preterm premature rupture of membranes. ***Intratracheal intermittent mandatory ventilation.

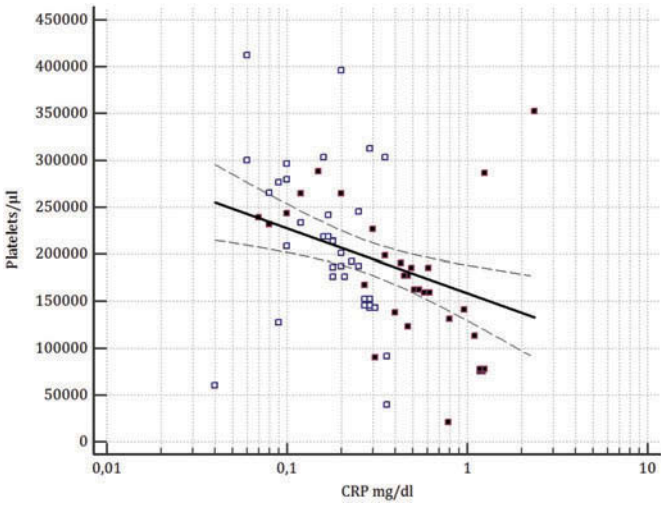

Figure 1. Correlation between platelet counts and C-reactive protein (CRP) plasma levels at day 3 of life in the infants with PDA ($r = -0.298$, $R^2 = 0.089$, $p = 0.049$).

2 to augment prostaglandin E_2 and prostacyclin, which keep the ductus open and prevent platelet aggregation [14]. Elevated CRP can either be a marker of inflammation or participate by itself in inflammatory reactions and platelet dysfunction [15,16]. In vitro, CRP is able to inhibit thrombin, ADP, and collagen-induced platelet aggregation [17,18]. Monomeric CRP has been shown to induce interleukin-8 through peroxynitrite signaling in human neutrophils. Activated platelets, via lysophosphatidylcholine, can dissociate pentameric to monomeric CRP [19], and inhibition of platelet function could compromise phospholipase A2 dependent dissociation of circulating pentameric CRP to monomeric CRP [20].

According to this approach, low platelet count and elevated CRP levels would be an epiphenomena related to inflammation. Therefore, the antenatal and neonatal efforts targeting inflammation may facilitate ductal closure.

The main limitations of our study include its retrospective design and the relatively small sample size. We tried to avoid selection and retrospective information bias by using strict definitions and cutoffs, and we attempted to homogenize the sample by applying strict exclusion criteria. Also the patients were matched to reduce the likelihood of making spurious associations. Although the sample was relatively small, it is sufficient to demonstrate a correlation coefficient of at least 0.3 between platelet count and CRP [minimal required sample size: 86 patients, for r 0.29, α 0.05, and 1-β 0.80, calculated with MedCalc Statistical Software version 13.3.3 (MedCalc Software bvba, Ostend, Belgium)].

To verify our findings, further prospective studies with a large number of patients are required.

Declaration of Interest

The authors report no conflicts of interest.

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