Mature and immature platelets during the first week after birth and incidence of patent ductus arteriosus

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Abstract

Background: Thrombocytopenia is a risk factor for patent ductus arteriosus. Immature and mature platelets exhibit distinct haemostatic properties; however, whether platelet maturity plays a role in postnatal, ductus arteriosus closure is unknown. Methods: In this observational study, counts of immature and mature platelets (=total platelet count – immature platelet count) were assessed on days 1, 3, and 7 of life in very low birth weight infants (<1500 g birth weight). We performed echocardiographic screening for haemodynamically significant patent ductus arteriosus on day 7. Results: Counts of mature platelets did not differ on day 1 in infants with (n = 24) and without (n = 45) haemodynamically significant patent ductus arteriosus, while infants with significant patent ductus arteriosus exhibited lower counts of mature platelet on postnatal days 3 and 7. Relative counts of immature platelets (fraction, in %) were higher in infants with patent ductus arteriosus on day 7 but not on days 1 and 3. Receiver operating characteristic curve analysis unraveled associations between both lower mature platelet counts and higher immature platelet fraction (percentage) values on days 3 and 7, with haemodynamically significant ductus arteriosus. Logistic regression analysis revealed that mature platelet counts, but not immature platelet fraction values, were independent predictors of haemodynamically significant patent ductus arteriosus. Conclusion: During the first week of postnatal life, lower counts of mature platelets and higher immature platelet fraction values are associated with haemodynamically significant patent ductus arteriosus. Lower counts of mature platelet were found to be independent predictors of haemodynamically significant patent ductus arteriosus.

A patent ductus arteriosus in preterm infants is frequently associated with severe cardio-pulmonary complications, such as left ventricular volume overload, pulmonary oedema, and impairment of lung compliance.1–3 Thus, understanding the mechanisms that control ductal closure is pivotal in order to provide tailored care to at-risk neonates.4 Previously, Echtler et al. reported that platelets are recruited to the ducal endothelium after birth and contribute to ductal closure and subsequent vascular remodelling.5 However, the clinical significance of these animal experiments is still controversial. Low platelet counts are frequently observed among preterm infants, but several studies on their role in spontaneous or pharmacological ductus arteriosus closure produced ambiguous or conflicting results.6–8 We previously reported that lower platelet counts on the first postnatal day were not associated with a higher incidence of patent ductus arteriosus in 1430 very low birth weight infants.9 However, in infants who underwent pharmacological treatment with cycloxygenase inhibitors (ibuprofen/indomethacin) for haemodynamically significant patent ductus arteriosus, low platelet counts during the first postnatal week contributed to an increased rate of treatment failure.10 Collectively, these results suggest that the development of thrombocytopenia during the first week of postnatal life contributes to failure of ductus arteriosus closure.

The immature platelet fraction is a novel innovative tool to examine platelet production from peripheral blood samples.1 Immature platelet fraction values have been shown to predict the course of thrombocytopenia in preterm neonates and thus may prove useful in guiding platelet transfusion management.11,12 Furthermore, immature platelets have distinct functional features that might affect haemostasis independently from total platelet counts.13 To date, the proportion of mature and immature platelet counts and their possible association with haemodynamically significant patent ductus arteriosus have not been evaluated in...
preterm neonates. In this observational study, we investigated the number of mature and immature platelets during the first week of postnatal life and their possible association with the incidence of haemodynamically significant patent ductus arteriosus in very low birth weight preterm infants.

Methods

Patients, blood parameters, and clinical data

The study was conducted at the neonatal ICU, Department of Neonatology, Charité University Medical Center, Berlin, Germany (2009–2012). Blood sampling was performed at day 1, day 3 (±1 day), and day 7 (±2 days) after birth. Blood count parameters were measured using a Sysmex XE-2100 (Sysmex, Kobe, Japan) blood analyser as previously described. Immature platelet values are reported as absolute values per nanolitre (immature platelet count) and as percentage (immature platelet fraction). Mature platelet counts were obtained by using the following formula: mature platelet count = total platelet count – immature platelet count. The infants were grouped according to patent ductus arteriosus status (echocardiography on day 7, see below). Clinical data were obtained by chart reviews. All very low birth weight infants (birth weight <1500 g) admitted to the unit were eligible if no cardiac malformation or other genetic or syndromic disorders were present. Parental written consent for blood sampling and data collection was obtained after patient admission to the neonatal ICU. The study has been approved by the local ethics committee of Charité – Universitätsmedizin Berlin, Germany (EA1/229/08).

Diagnosis of haemodynamically significant patent ductus arteriosus

Infants were examined for a haemodynamically significant patent ductus arteriosus on postnatal day 7 (±2 days) as previously described. Echocardiographic evaluation included shunt direction of a patent ductus arteriosus in colour Doppler mode (high upper parasternal short axis) as well as the minimal internal ductal diameter detected within three to five measurements taken in B-mode. The left atrium to aortic root ratio was measured by M-mode (parasternal long axis). Doppler measurement of the resistance index (anterior cerebral artery) was performed at the same time. A patent ductus arteriosus with left-to-right shunt was considered haemodynamically significant if a respiratory set back with a supplemental oxygen requirement >30% and/or mechanical ventilation, a left atrium to aortic root ratio ≥1.4 in the echocardiogram, ductal diameter ≥2.5 mm, and/or a decreased end-diastolic flow in the anterior cerebral artery with a resistance index ≥0.85 in the cerebral ultrasound were present. There has been no change in the clinical and echocardiographic criteria used to determine haemodynamic significance of a patent ductus arteriosus during the study period.

Statistics

Values are expressed as absolute number of patients or percentage values unless stated otherwise. Demographic characteristics are given as median and range. Comparisons between groups were made by Kruskal–Wallis or Mann Whitney U tests as appropriate for continuously scaled data and by chi-square test for categorical data. p-Values < 0.05 were considered statistically significant. Statistical analyses, including receiver operating characteristic curve analysis and linear regression analysis, were carried out using IBM SPSS Statistics 22 software.

Table 1. Demographic characteristics of the study population

|                      | All VLBW | With hsPDA | Without hsPDA | p-Values |
|----------------------|----------|------------|---------------|----------|
| n (%)                | 69       | 24 (34.8)  | 45 (65.2)     | –        |
| Female, n (%)        | 23 (33.3)| 6 (25.0)   | 17 (37.8)     | 0.28     |
| BW (g)               |          |            |               |          |
| Median               | 970      | 657        | 1160          |          |
| Mean                 | 973      | 720        | 1108          |          |
| Range                | 382–1495 | 383–955    | 382–1495      |          |
| GA (weeks)           |          |            |               |          |
| Median               | 28 2/7   | 24 6/7     | 28 6/7        | < 0.01   |
| Mean                 | 27 5/7   | 25 0/7     | 29 2/7        |          |
| Range                | 23 2/7–33 2/7 | 23 2/7–31 1/7 | 25 2/7–33 2/7 |          |

BW = birth weight; GA = gestational age; hsPDA = haemodynamically significant patent ductus arteriosus; VLBW = very low birth weight.

Results

Clinical data and incidence of haemodynamically significant patent ductus arteriosus

A total of 69 very low birth weight infants were included in the study. Of 69 infants, 24 were diagnosed with a haemodynamically significant patent ductus arteriosus (34.8%). Infants with haemodynamically significant patent ductus arteriosus were more immature at birth (25 0/7 versus 29 2/7, p < 0.01) and exhibited a lower birth weight (mean 1108 g versus 702 g, p < 0.01) while gender distribution did not differ significantly between groups (Table 1).

Mature and immature platelets during the first week of life

The mature platelet counts on postnatal day 1 did not differ significantly between infants with and without haemodynamically significant patent ductus arteriosus (p = 0.068). However, mature platelet counts were significantly lower in infants with haemodynamically significant patent ductus arteriosus on days 3 and 7 of life (p = 0.036 and < 0.01, respectively, Fig 1). Infants with and without haemodynamically significant patent ductus arteriosus did not show any significant differences in immature platelet fraction (percentage) values on days 1 and 3 of life (p = 0.46 and 0.07, respectively), while infants with haemodynamically significant patent ductus arteriosus exhibited higher immature platelet fraction (percentage) values on postnatal day 7 (p < 0.01). Of note, the absolute immature platelet counts were similar between groups at all time points (Fig 1).

Receiver operating characteristic curve analysis (platelet count, immature platelet fraction, gestational age, and birth weight)

Receiver operating characteristic curve analyses were performed in order to further characterise the relationship between mature and immature platelets, birth weight, gestational age, and haemodynamically significant patent ductus arteriosus (Table 2; Fig 2). Significant associations were found for gestational age, birth weight, and mature platelet counts on days 3 and 7, which all showed an area under the curve > 0.5, indicating a negative correlation between these variables and the incidence of a haemodynamically significant patent ductus arteriosus. Immature platelet fraction (percentage) values on days 3 and 7 of life exhibited an area under the curve < 0.5 and, thus, a positive
correlation with haemodynamically significant patent ductus arteriosus (Table 2; Fig 2).

**Logistic regression analysis**

In addition, logistic regression analysis was performed to investigate whether mature platelet counts and immature platelet fraction values on postnatal day 7 are independent risk factors for haemodynamically significant patent ductus arteriosus. While gestational age, birth weight, and mature platelet counts on day 7 were identified as independent risk factors for haemodynamically significant patent ductus arteriosus, immature platelet fraction (percentage) on postnatal day 7 was not found to be an independent predictor of haemodynamically significant patent ductus arteriosus (p = 0.585; Table 3).

**Discussion**

The role of platelets in ductal closure has first been demonstrated in murine models. Observational studies on the clinical significance of this finding in preterm infants have yielded conflicting results. However, two recent meta-analyses indicate a moderate correlation with haemodynamically significant patent ductus arteriosus. While gestational age, birth weight, and mature platelet counts on day 7 were identified as independent risk factors for haemodynamically significant patent ductus arteriosus, immature platelet fraction (percentage) on postnatal day 7 was not found to be an independent predictor of haemodynamically significant patent ductus arteriosus (p = 0.585; Table 3).

In contrast, immature platelet fraction values, which can be increased in response to thrombocytopenia, if megakaryopoiesis and thrombopoiesis are intact. A recent study found an association between immature platelet fraction (percentage) and bleeding risk in children with immune thrombocytopenic purpura, independently from platelet counts. The latter results support previous findings on immature platelets having distinct functional properties which affect their haemostatic potential.

Based on these observations, we hypothesised that, due to their distinct functional properties, mature and immature platelets are independently associated with incidence of haemodynamically significant ductus arteriosus in preterm infants. Here, for the first time, we show that lower absolute counts of mature platelets on days 3 and 7, but not on day 1 after birth, are associated with ductal patency.

In addition, we found higher immature platelet fraction (percentage) values in infants with haemodynamically significant patent ductus arteriosus as compared to those without. However, logistic regression analysis revealed that only mature platelet counts on postnatal day 7, in contrast to immature platelet fraction values, were independently associated with haemodynamically significant patent ductus arteriosus in our cohort. Our results suggest that lower counts of mature platelet contribute to a higher incidence of haemodynamically significant patent ductus arteriosus. In contrast, immature platelet fraction values, which can be regarded as a physiological response to thrombocytopenia in preterm infants, do not independently affect ductal closure. Of note, we found similar results indicating a role for lower mature platelet counts in failed spontaneous ductal closure when comparing all infants with patent ductus arteriosus (not only haemodynamically significant ones) to those without a patent ductus arteriosus (data not shown).

Our study is limited by its relatively low sample size. Thus, we were unable to assess a potential association between low mature platelet counts and treatment failure as only 24 infants underwent pharmacological treatment for patent ductus arteriosus in our cohort. In addition, the current study did not address a potential
Figure 2. Receiver operating characteristic (ROC) curve analyses. ROC curve analysis for mature platelet counts and immature platelet fraction (percentage) on day 7 of life, birth weight, and gestational age comparing infants with and without haemodynamically significant patent ductus arteriosus (hsPDA). All ROC curves presented in this figure show an unequal division resulting in an area under the curve (AUC) of $>0.5$ or $<0.5$ (for confidence intervals, see Table 2). An AUC $>0.5$ (gestational age, birth weight, and mature platelet day 7) indicates a negative and an AUC $<0.5$ (IPF day 7) a positive correlation between the variable and hsPDA incidence. IPF = immature platelet fraction (percentage); MPC = mature platelet count.

Table 2. ROC curve analyses in VLBW and ELBW infants

|                | AUC      | 95% CI       |
|----------------|----------|--------------|
| MPC day 1      | 0.625    | 0.481–0.769  |
| MPC day 3      | 0.664    | 0.531–0.796  |
| MPC day 7      | 0.854    | 0.762–0.946  |
| IPF (%) number 1| 0.429    | 0.277–0.581  |
| IPF (%) number d3 | 0.338    | 0.203–0.473  |
| IPF (%) number day 7 | 0.202    | 0.093–0.310  |
| IPC (#) number 1 | 0.574    | 0.423–0.726  |
| IPC (#) number d3 | 0.463    | 0.308–0.617  |
| IPC (#) number day 7 | 0.606    | 0.459–0.753  |
| Birth weight   | 0.833    | 0.308–0.933  |
| Gestational age| 0.935    | 0.459–0.996  |

Statistical significance is indicated by bold values.
AUC = area under the curve; CI = confidence interval; d = day of life; ELBW = extremely low birth weight; IPC(#) = immature platelet count; IPF (%) = immature platelet fraction (percentage); MPC = mature platelet count; ROC = receiver operating characteristic; VLBW = very low birth weight.

Table 3. Logistic regression analysis for prediction of hemodynamically significant patent ductus arteriosus

|                        | Regression coefficient (B) | Standard error | Wald  | Df | Significance level (p-values) | Exponent (B) |
|------------------------|---------------------------|----------------|-------|----|--------------------------------|---------------|
| Birth weight           | 0.007                     | 0.003          | 4.276 | 1  | 0.041                          | 1.007         |
| Gestational age        | –1.789                    | 0.554          | 10.44 | 1  | 0.001                          | 0.167         |
| IPF day 7              | –0.037                    | 0.147          | 0.62  | 1  | 0.803                          | 0.964         |
| MPC day 7              | –0.018                    | 0.008          | 5.38  | 1  | 0.020                          | 0.982         |

66/69 (95.7%) of cases with a complete dataset were included in this analysis. Statistical significance is indicated by bold values.
Df = degree of freedom; IPF = immature platelet fraction (percentage); MPC = mature platelet count; Wald = Wald chi-square test.
association between inflammation, as seen in septic preterm neonates, mature and immature platelets, and ductus arteriosus. Furthermore, ductal closure is a process that is characterised by a complex interplay between molecular and morphological changes within the vessel wall, which, in addition to platelets, is triggered by vascular adhesion and migration of mononuclear blood cells to the muscular layer of the vessel wall. Assessing those complex molecular and cellular processes requires a highly sophisticated experimental design and is beyond the scope of this paper. Thus, further research is required to elucidate the complex interplay between immature and mature platelets on ductal closure and to assess their specific functions and association with bleeding risk and thromboembolic complications in preterm infants.25,26

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Conflicts of Interest. Malte Cremer served as an advisory board member for Sysmex Deutschland GmbH, Hamburg, Germany.

Ethical Standards. The study was carried out in accordance with national and international recommendations, including the Declaration of Helsinki, and has been approved by the local ethics committee (EA1/229/08). Written informed parental consent was obtained.

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