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Relationship between circulating platelet counts and ductus arteriosus patency following indomethacin treatment

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Abstract

Objective—To determine if low platelet counts are related to the incidence of patent ductus arteriosus (PDA) after indomethacin treatment in preterm human infants.

Study design—Multivariable logistic regression modeling was used for a cohort of 497 infants, who received indomethacin (within 15 hours of birth).

Results—Platelet counts were not related to the incidence of permanent closure following indomethacin constriction. There was a relationship between platelet counts and the initial degree of constriction; however, this relationship appeared to be primarily influenced by the high end of the platelet distribution curve. PDA incidence was similar in infants with platelet counts $<50 \times 10^9/L$ and those with platelet counts above this range. Only when platelet counts were consistently $>230 \times 10^9/L$ was there a decrease in PDA incidence.

Conclusion—In contrast to the evidence in mice, low circulating platelet counts do not affect permanent ductus closure (or ductus reopening) in human preterm infants.

Keywords

thrombocytopenia; NSAID; premature newborn; baboon

In full term newborn infants[C1], closure of the ductus arteriosus is initiated by smooth muscle constriction. This is followed by permanent anatomic closure as the neointimal layer

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of the ductus expands to form protrusions, or mounds, that fill and occlude the residual lumen (1,2). In contrast, preterm infants frequently fail to constrict their ductus or undergo anatomic remodeling after birth. Nonsteroidal anti-inflammatory drugs (NSAIDs), such as indomethacin, effectively constrict and narrow the ductus lumen in preterm infants, but frequently fail to cause permanent anatomic closure (3). Neointimal mound formation is less well developed in premature infants (especially those born before 28 weeks of gestation) than in full term infants and often fails to occlude the residual lumen (1,4-6).

The murine ductus arteriosus, just like the preterm human ductus, fails to develop extensive neointimal mounds after birth. Recently, Echtler et al reported that mice require platelet aggregation and thrombus formation (within the ductus lumen) before permanent closure can occur (7). Following indomethacin treatment, mice with normal circulating platelet counts, constrict and permanently close their ductus, even though indomethacin is known to alter platelet function (8). NSAIDs, like indomethacin, inhibit platelet activation and increase bleeding times (9); however, recent studies suggest that, in vivo, NSAIDs may have a pro- rather than an anti-thrombotic effect (7,10-12). In contrast to mice with normal circulating platelet counts, mice with low circulating platelet counts constrict their ductus following indomethacin treatment, but fail to produce permanent ductus closure (7). The findings in mice are consistent with recent reports of an association between circulating platelet counts in preterm infants and both spontaneous (7) and indomethacin-induced ductus closure (13).

Therefore, we examined a large cohort of preterm infants who were managed with a defined PDA-care oriented protocol that used prophylactic indomethacin treatment (starting within 15 hours of birth) to close the ductus arteriosus. We used multivariable logistic regression modeling to determine if there was an independent relationship between low platelet counts during the course of indomethacin treatment and persistence of ductus patency following indomethacin treatment.

Methods

This project was approved by the Institutional Review Board of the University of California San Francisco. Between January 1995 and December 2009, all infants <28^{0/7} weeks gestation, admitted within 15 hours of birth to the William H. Tooley Nursery at University of California San Francisco, were treated according to the following PDA-care oriented protocol. A full description of this approach has been published elsewhere (14). Infants received either a short 3-dose course of prophylactic indomethacin (0.2, 0.1, 0.1 mg/kg, administered at 24 hr intervals) or an extended 6-dose course (0.2, 0.1, 0.1, 0.1, 0.1, 0.1 mg/kg, at 24 hr intervals) starting within 15 hr of birth. A Doppler examination was performed on the third day after birth, just before the 3rd indomethacin dose. If the ductus was closed on echocardiogram and there was no evidence of ductus patency on the Doppler examination, infants received the third dose (prophylactic indomethacin was stopped after the third dose). If there was any evidence of ductus patency on the exam, infants received the extended 6-dose course. A repeat echocardiogram/Doppler examination was performed on the seventh day after birth (24 hours after the last indomethacin dose) to determine the ductus' response to prophylactic indomethacin. During the first week after birth platelet counts were measured with an LH750 Beckman Coulter automated platelet counter prior to each prophylactic indomethacin dose and at least every other day thereafter. Platelet counts <100 × 10⁹/L were routinely repeated for accuracy and all platelet counts <50 × 10⁹/L were checked manually. Indomethacin dosing was not interrupted for low platelet counts unless the platelet count fell to <50 × 10⁹/L. If the platelet count fell to <50 × 10⁹/L, infants were transfused with platelet concentrates and the indomethacin dosing continued. Six of the 497 infants received a platelet transfusion as part of the prophylactic indomethacin protocol.

Following the prophylactic indomethacin treatment, all infants were examined daily for the appearance of clinical symptoms indicative of a PDA (systolic murmur, widened pulse pressure, hyperdynamic precordium). If any of these occurred a repeat echocardiogram/ Doppler examination was performed within 24 hours. If the repeat examination found new left-to-right flow through the ductus (and the previous Doppler examination showed that the ductus was closed) the infant was considered to have reopened the PDA and to have failed to achieve permanent ductus closure after indomethacin treatment.

A single neonatologist prospectively evaluated and recorded all of the antenatal and neonatal risk factors during the hospitalization. Gestational age was determined by the date of last menstrual period and early ultrasounds (before 24 weeks gestation). If there were discrepancies, the early ultrasound dating was used. Small for gestational age was defined as birthweight less than the tenth-percentile for gestational age using the growth curves from Alexander et al (15). Intracranial hemorrhage (ICH) was classified using the four-level grading system (16). All infants were examined with serial bedside cranial ultrasounds initiated within the first 3 days after birth. These were repeated weekly or biweekly for the first 4 weeks. Infants were excluded from the analysis if they died before the echocardiographic evaluations.

Statistical Models

Our intent was to determine if there was a relationship between the lowest platelet count (platelet nadir) during the course of indomethacin treatment and the incidence of persistent ductus patency following treatment. We studied three aspects of persistent ductus patency: (1) ductus patency just prior to the 3rd dose of prophylactic indomethacin (on day 3); (2) ductus patency after the last dose of prophylactic indomethacin (on day 7); and (3) ductus re-opening following initial indomethacin-induced ductus closure. The first two aspects reflect the ability of the ductus to constrict after prophylactic indomethacin treatment. The third aspect reflects the ability of the ductus to undergo anatomic remodeling and permanent closure after indomethacin-induced ductus constriction.

We first examined the distribution of potential antenatal and neonatal risk factors among the groups with an open and closed ductus. Continuous variables were compared using the non-parametric Mann-Whitney rank sum test, and p-values for categorical and binary variables were based on the Chi-square test.

The predictor of primary interest, lowest platelet count (platelet nadir), was characterized in analyses in a variety of ways: as a continuous variable, as a binary variable (to explore a potential plateau effect), and as a categorical variable (in order to account for possible non-linearity), and examined using logistic regression models in order to determine how best to represent it in multivariable models. Univariate logistic regression models were used to obtain odds ratios for the potential predictors of ductus closure.

Adjusted multivariable logistic regression models were built for each of the three aspects of persistent ductus patency to determine if the effects of the platelet nadir were independent of other antenatal/neonatal risk factors. Multivariable logistic regression models were constructed as follows: platelet nadir (variously characterized) were adjusted for variables shown in univariate analysis to be strongly associated with ductus closure ($p < 0.05$), then forward stepwise regression was utilized on the remainder of the variables to obtain a final model. Models including different characterizations of platelet nadir were compared to determine which best fit the data. This process was repeated for each of the three aspects of persistent ductus patency to determine if the effects of the platelet nadir were independent of other antenatal/neonatal risk factors. All analyses were performed using SAS version 9.1.

The final multivariate models included the following variables: 1) a platelet variable (either absolute platelet nadir or distribution of platelet nadirs), plus 2) gestation, plus 3) antenatal betamethasone, plus 4) preeclampsia, plus 5) Respiratory Severity Score (Mean Airway Pressure $\times$ FiO₂) at 24 hours after birth, plus 6) each of the following perinatal/neonatal risk factors: maternal diabetes, birthweight, small for gestational age, male sex, respiratory distress syndrome, surfactant treatment, intracranial hemorrhage (grades 3 and 4), chorioamnionitis/early neonatal septicemia, and fluid intake.

Results

Table I lists the distribution of antenatal and neonatal risk factors among the groups with an open and closed ductus following prophylactic indomethacin treatment. Despite the administration of prophylactic indomethacin within 15 hr of birth, 34% of the infants failed to close their patent ductus after the 2nd prophylactic dose, and 19% continued to have a PDA after the last dose of prophylactic indomethacin. Among those who closed their ductus initially with prophylactic indomethacin, 14% failed to develop permanent anatomic closure of their lumen after indomethacin treatment and reopened their PDA at a later time (Table I).

We were particularly interested in the relationship between low platelet counts during the course of indomethacin treatment and the incidence of persistent ductus patency following treatment. The associations presented in Table II represent the adjusted odds-ratios of the effects of low platelet counts on the incidence of persistent ductus patency when antenatal and neonatal variables are entered into the model.

Although the platelet nadir was significantly associated with the incidence of ductus patency on days 3 and 7 (Table [C2] II), we were struck by the observation that the average platelet nadir in the open ductus group (Table I) was still greater than the number that is usually considered to be the lower limit of normal for full term newborn infants ($150 \times 10^9/L$) (17).

Due to non-linearity of the platelet nadir effect, we determined that the best representation of platelet nadir was achieved by categorizing the platelet nadirs into quintiles (with the lowest quintile as the reference group). We examined the effects of each quintile on the incidence of ductus patency to determine if there was a specific range of platelet counts that had the greatest effect on ductus patency (Table II). To our surprise, we found no difference in the incidence of PDA between infants whose platelet count nadirs were in the lowest quintile compared with those whose platelet count nadirs were in the three middle quintiles (Table II). This observation was the same even if we compared the incidence of ductus patency among infants whose platelet nadirs were $<50 \times 10^9/L$ with those whose platelet nadirs were $\geq 50 \times 10^9/L$ (at 3 days: PDA incidence in the platelet nadir group $<50 \times 10^9/L$ group = 25%, in the group $\geq 50 \times 10^9/L$ = 34%; at 7 days: PDA incidence in the platelet nadir group $<50 \times 10^9/L$ group = 16%, in the group $\geq 50 \times 10^9/L$ = 19%). It appears that the relationship between platelet count nadirs and the incidence of ductus patency at 3 and 7 days is more affected by the high end of the platelet count distribution curve than it is by the low end of the curve: infants whose platelet counts never fell below the highest quintile, had the lowest incidence of PDA (Table II).

Although there was a significant relationship between the infants' lowest platelet counts and the incidence of initial ductus closure (by constriction) at 3 and 7 days, we found no relationship (in either the unadjusted (Table I) or adjusted analyses (Table II) between low platelet counts and the ability of the ductus to remodel and remain permanently closed after its initial constriction.

Discussion

Our findings in preterm human infants contrast markedly with the results reported in mice. In mice, platelets and platelet thrombi appear to be essential for *permanent anatomic closure* of the ductus lumen (7). In contrast, we found that the incidence of permanent ductus closure is *not related* to the number of circulating platelets in preterm human infants (Table II).

Although the number of circulating platelets did not affect the incidence of permanent ductus closure, it did seem to be related to the degree of initial, indomethacin-induced, ductus constriction (Table II). Whether this relationship represents a true cause and effect relationship is still a matter of debate. Two smaller studies also found an association between the presence of a PDA and low platelet counts (7,13). However, in both studies, the average platelet counts, in the open ductus groups, were in the mild thrombocytopenia range ($100 - 150 \times 10^9/L$) (a range that has not been associated with prolongation of the bleeding time in preterm infants (18)). The importance of mild or even moderate thrombocytopenia in premature infants is still unclear because a randomized, controlled trial of platelet transfusions (to maintain the platelet count over $150 \times 10^9/L$) failed to alter the incidence of PDA in thrombocytopenic premature infants (19).

Several pieces of evidence lead us to believe that low circulating platelet numbers are *not* responsible for a persistent PDA in human infants. Full term infants with severe thrombocytopenia, secondary to Wiskott-Aldrich Syndrome or alloimmune thrombocytopenia, close their ductus normally and do not have a higher incidence of PDA (20,21). Adherent platelets and platelet thrombi have not been observed (in histological studies of both full-term human ductus and nonhuman primate ductus) during early ductus closure (4) and have been observed only infrequently during the first month after birth (2). Similarly, we were unable to detect the presence of adherent platelets during ductus closure in premature baboons (Table III; available at www.jpeds.com).

In our study, the average “lowest circulating platelet count” in the open ductus group was greater than $150 \times 10^9/L$ (Table I). Rather than finding higher rates of PDA among infants with the lowest platelet counts, we found that the relationship between the incidence of PDA and platelet counts appeared to be primarily influenced by the high end of the platelet distribution curve (Table II). There was no difference in the incidence of PDA between infants whose platelet counts fell into the severe thrombocytopenia range ($<50 \times 10^9/L$) and those whose platelet counts were above this range. On the other hand, infants whose platelet counts never fell below the highest quintile ($>230 \times 10^9/L$) had the lowest incidence of PDA on days 3 and 7 (Table II).

We speculate that, rather than contributing to PDA closure, circulating platelet counts act as a marker of inherent PDA risk in preterm infants. We included variables in our statistical models that are known to affect both the rate of ductus closure and the number of circulating platelets; however, other factors, not included in the models, may account for the relationship between platelet counts and PDA frequency. Only 2% of healthy newborns have platelet counts below $150 \times 10^9/L$ during the first week of life (17). On the other hand, the incidence of platelet counts below $150 \times 10^9/L$ is much higher in sick, hypoxic, preterm infants (22,23). These sick, preterm infants are the same babies that are likely to have delayed ductus closure after birth (24). We speculate that rather than contributing to PDA closure, platelet counts that remain in the highest quintile are merely an independent, surrogate marker of neonatal well being and of the presence of developmental factors that promote ductus closure after birth (25).

In conclusion, we found that, in contrast to the evidence in mice, low circulating platelet counts do not seem to have an effect on permanent ductus closure (or ductus reopening) in human preterm infants.

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Abbreviations

PDA	patent ductus arteriosus
ICH	intracranial hemorrhage
MAP	mean airway pressure
FiO2	fractional concentration of inspired oxygen
RSS	Respiratory Severity Score, (MAP × FiO2) at 24 hours after birth
RDS	respiratory distress syndrome

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Table 1

Distribution of antenatal/neonatal risk factors among infants with either an open ductus or a closed ductus after prophylactic indomethacin.

Variable	Ductus status					
	At 3 days		At 7 days			After initial closure
	Open (n = 169)	Closed (n = 328)	Open (n = 94)	Closed (n = 403)	Re-open (n = 54)	Closed (n = 343)
Betamethasone (%)	65	80*	57	79*	67	82*
Preeclampsia (%)	11	17	4	17*	13	17*
Diabetes (%)	7	6	5	6	4	7
Chorio/sepsis (%) ¹	21	25	21	24	21	25
Gestation (wks)	25.6±1.1	25.9±1.1*	25.4±1.1	25.9±1.1*	25.4±1.0	26.0±1.0*
Birthweight (gm)	831±176	822±180	814±166	828±182	791±157	834±185
SGA (%) ²	4	9*	2	7*	6	9
Male (%)	49	54	50	53	57	52
RDS (%) ³	93	83*	96	84*	87	83
Surfactant (%)	98	92*	99	93*	93	93
RSS @ 24 hr (units) ⁴	2.3±1.9	2.0±1.4*	2.5±1.9	2.0±1.5*	2.5±2.0	1.9±1.4*
ICH (3 or 4) (%) ⁵	17	10*	16	11*	15	10
Fluid Intake (ml/kg/d) ⁶	146±28	137±28*	151±30	137±28*	140±28	137±28
Platelet nadir during first 3 days after birth ($\times 10^9/L$)	185±59	198±81	182±54	197±78	190±79	198±78
Platelet nadir during first 7 days after birth ($\times 10^9/L$)	-	-	154±64	177±76*	162±73	181±76

¹ Chorio/Sepsis: chorioamnionitis or positive blood culture within first 3 days after birth

² SGA: small for gestational age (< 10th percentile)

³ RSS @ 24 hr: Respiratory Severity Score (MAP $\times$ FiO₂) at 24 hours after birth

⁴ RDS: clinical course and X-ray consistent with Respiratory Distress Syndrome

⁵ ICH (3 or 4): intracranial hemorrhage (grades 3 or 4)

⁶ Average Daily Fluid intake during first 4 days after birth in ml/kg/day

* p<0.05, for either open versus closed or re-open versus closed

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Table 2

Relationship between lowest platelet count (platelet nadir) and the incidence of either persistent ductus arteriosus patency after indomethacin prophylaxis or the incidence of ductus re-opening after initial ductus closure.

Platelet nadir (1 st 3 days after birth)		Incidence of PDA on day 3		
	Range of platelet nadirs ($\times 10^9/L$)	(%)	Adjusted Analysis: ¹	
			OR	(95% CI)
Absolute platelet nadir ²	20 - 521		0.73*	(0.55 – 0.98)
Distribution of platelet nadirs (quintiles) ³				
Lowest quintile	20 - 132	32	1.00	
2 nd quintile	133 - 170	36	1.36	(0.71 – 2.61)
3 rd quintile	171 - 207	39	1.31	(0.69 – 2.50)
4 th quintile	208 - 252	47	1.88	(0.98 – 3.60)
Highest quintile	253 - 521	16	0.43*	(0.20 – 0.91)

Platelet nadir (1 st 7 days after birth)		Incidence of PDA on day 7		
	Range of platelet nadirs ($\times 10^9/L$)	(%)	Adjusted Analysis:	
			OR	(95% CI)
Absolute platelet nadir ²	20 - 437		0.65*	(0.45 – 0.93)
Distribution of platelet nadirs (quintiles) ³				
Lowest quintile	20 – 111	24	1.00	
2 nd quintile	112 – 150	24	0.84	(0.41 – 1.74)
3 rd quintile	151 – 182	18	0.71	(0.33 – 1.51)
4 th quintile	183 – 230	21	0.82	(0.39 – 1.70)
Highest quintile	231 – 437	8	0.27*	(0.10 – 0.68)

Platelet nadir (1 st 7 days after birth)		Incidence of ductus reopening		
	Range of platelet nadirs ($\times 10^9/L$)	(%)	Adjusted Analysis:	
			OR	(95% CI)
Absolute platelet nadir ²	20 - 437		0.98	(0.94 – 1.03)
Distribution of platelet nadirs (quintiles) ³				
Lowest quintile	20 – 111	16	1.00	
2 nd quintile	112 – 150	16	1.18	(0.44 – 3.20)
3 rd quintile	151 – 182	15	1.18	(0.44 – 3.20)
4 th quintile	183 – 230	13	1.09	(0.38 – 3.10)
Highest quintile	231 - 437	9	0.74	(0.24 – 2.30)

Additional variables in the adjusted models that were significant and independent of the other variables in the model were:

Incidence of PDA on day 3: *gestation* (OR=0.84 (0.70-0.99)); *antenatal betamethasone* (OR=0.52 (0.34-0.81)); *preeclampsia* (OR=0.37 (0.14-0.99)); *respiratory distress syndrome* (OR=2.73 (1.37-5.40)); and *fluid intake* (OR=1.09 (1.00-1.19)).

Incidence of PDA on day 7: *gestation* (OR=0.73 (0.58-0.90)); *antenatal betamethasone* (OR=0.50 (0.30-0.83)); *preeclampsia* (OR=0.26 (0.09-0.76)); and *respiratory distress syndrome* (OR=3.28 (1.12-9.60))

Incidence of ductus reopening: *gestation* (OR=0.63 (0.48-0.84))

¹The adjusted multivariate regression models were constructed as described in *Methods (Statistical Models)*. The Odds Ratios for ductus patency presented in Table 2 are for models that included: a) a *platelet variable* (either absolute platelet nadir or distribution of platelet nadirs), plus b) *gestation*, plus c) *antenatal betamethasone*, plus d) *preeclampsia*, plus e) *Respiratory Severity Score* (Mean Airway Pressure $\times$ FiO₂ at 24 hours after birth, plus 6) *small for gestational age*. The Odds Ratios for the models that substituted each of the other perinatal or neonatal variables (maternal diabetes, birthweight, male gender, respiratory distress syndrome, surfactant treatment, intracranial hemorrhage (grades 3 and 4), chorioamnionitis/early neonatal septicemia, and fluid intake) for the variable *small for gestational age* were similar.

²OR for each $100 \times 10^9/L$ unit change in platelet count

³OR for each quintile compared with the lowest quintile group

*
p<0.05

Table 3
Detection of Platelet-specific and Monocyte-specific RNA transcripts in Preterm Fetal and Neonatal Baboon Ductus Arteriosus.

Gene	Preterm Fetus (140 d gestation)		Preterm Newborn (14 d-open ductus)		Preterm Newborn (14 d-closed ductus)	
	ΔCT (MDH – gene)		ΔCT (MDH – gene)		ΔCT (MDH – gene)	
	mean	SD	mean	SD	mean	SD
PF4	n.d.		n.d.		n.d.	
CD14	-4.711	0.584	-3.998*	0.620	-3.186**	0.461