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# Thrombocytopenia in the First 24 Hours After Birth and Incidence of Patent Ductus Arteriosus

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## KEY WORDS

ductal closure, patent ductus arteriosus, preterm infant, platelets, very low birth weight

## ABBREVIATIONS

COX—cyclooxygenase

DA—ductus arteriosus

DOL—days of (postnatal) life

ELBW—extremely low birth weight

NS—not significant

OR—odds ratio

PDA—patent ductus arteriosus

ROC—receiver operating characteristic

VLBW—very low birth weight

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Dr Sallmon and Dr Hansmann wrote the manuscript; and all authors made substantial contributions to conception and design, acquisition of data, analysis, and interpretation of data. Thus, all persons designated as authors qualify for authorship, and all those who qualify are listed. Each author has participated sufficiently in the work to take public responsibility for appropriate portions of the content. All authors commented on and/or revised the manuscript, and approved the final version.

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**WHAT'S KNOWN ON THIS SUBJECT:** To date, 4 small to moderate sized studies have revealed conflicting results on the clinically important question whether thrombocytopenia contributes to persistent patent ductus arteriosus (PDA) in very immature, preterm infants.



**WHAT THIS STUDY ADDS:** Thrombocytopenia in the first 24 hours after birth was not associated with the incidence of PDA at postnatal day of life 4 to 5 in a large cohort of preterm infants with <1500 g birth weight. Platelet dysfunction, rather than platelet number, might play a role in ductus arteriosus patency.

## abstract

**BACKGROUND:** Experimental studies suggest that platelet-triggered ductal sealing is critically involved in definite ductus arteriosus closure. Whether thrombocytopenia contributes to persistently patent ductus arteriosus (PDA) in humans is controversial. This was a retrospective study of 1350 very low birth weight (VLBW; <1500 g) infants, including 592 extremely low birth weight (ELBW; <1000 g) infants.

**METHODS:** All infants who had a platelet count in the first 24 hours after birth and an echocardiogram performed on day of life 4 to 5 were included. The incidence of thrombocytopenia was analyzed in infants with and without PDA, and in those who did or did not undergo PDA intervention. The impact of thrombocytopenia, gestational age, birth weight, gender, and sepsis on PDA was determined by receiver operating characteristic curve, odds ratio, and regression analyses.

**RESULTS:** Platelet numbers within the first 24 hours after birth did not differ between VLBW/ELBW infants with and without spontaneous ductal closure. Platelet numbers were not associated with subsequent PDA treatment. Low platelet counts were not related to failure of pharmacologic PDA treatment and the need for subsequent surgical ligation. Lower gestational age or birth weight, male gender, and sepsis were linked to the presence of PDA in VLBW infants on day of life 4 to 5.

**CONCLUSIONS:** Thrombocytopenia in the first 24 hours after birth was not associated with PDA in this largest VLBW/ELBW infant cohort studied to date. Impaired platelet function, due to immaturity and critical illness, rather than platelet number, might play a role in ductus arteriosus patency. *Pediatrics* 2012;130:e623–e630

A patent ductus arteriosus (PDA) affects ~60% of preterm infants born after <28 weeks of gestation.<sup>1</sup> Physiologic ductus arteriosus (DA) closure occurs in the first 3 days after birth.<sup>1–3</sup> The persistence of a PDA beyond the first postnatal days of life, however, is frequently associated with multiple severe complications, predominantly in the most immature infants. Depending on the degree of left-to-right ductal shunting, an exaggerated postnatal increase in pulmonary blood flow can cause left ventricular volume overload, pulmonary edema, and impairment of lung compliance, and may be associated with the development or progression of pulmonary vascular and chronic parenchymal lung disease.<sup>4</sup> In addition, cardiopulmonary deterioration with decreased systemic perfusion increases the risk of neonatal death.<sup>5</sup> Recently, the threshold for PDA intervention has been raised, given the high spontaneous DA closure rate and low comorbidity with small ductal shunt or mild symptoms.<sup>6</sup> The exact indications for PDA interventions are controversial.<sup>7</sup> Therefore, understanding the risk factors, disease mechanisms, and modifiers that underlie DA closure is of pivotal clinical importance for tailored intensive care of preterm infants.

After birth, an increase in oxygen tension results in initial ductal constriction. Falling levels of prostaglandin E<sub>2</sub> promote functional DA closure (medial smooth muscle cell contraction that causes DA wall thickening, luminal obliteration, and shortening). The subsequent definite DA closure is characterized by vascular fibrosis due to an infolding of the endothelium, neointima formation, subintimal disruption, and vasa vasorum ingrowth. Although several molecular pathways (eg, nitric oxide, vascular endothelial growth factor, the cyclooxygenase [COX]/prostaglandin system<sup>8–11</sup>) are known to be involved in permanent DA sealing, the complex

cellular and molecular mechanisms contributing to DA closure remain poorly understood.<sup>12</sup>

Intriguingly, Echtler et al<sup>13</sup> reported that platelet-triggered ductal sealing (with subsequent vascular remodeling) seems to be an important mechanism for definite DA closure. In their mouse models, platelets were recruited to the endothelium of the DA soon after birth, and induced platelet dysfunction or defective platelet formation resulted in persistent PDA. Addressing the clinical implications, Echtler et al conducted a small retrospective study in 123 infants born at 24 to 30 weeks' gestation in a German tertiary center, and concluded that mild thrombocytopenia (platelet counts 101 000–140 000/ $\mu$ L) on the first day of life (DOL) was a risk factor for failure of DA closure.<sup>13</sup>

A subsequent small retrospective single-center study presented at the 2011 Pediatric Academic Societies' meeting evaluated the association between platelet counts on DOL 1 to 3 and DA closure in 148 extremely low birth weight (ELBW; <1000 g) infants in the United States. Both spontaneous and indomethacin-induced DA closure were found to be less frequent in ELBW infants with platelet counts <150 000/ $\mu$ L (K. Dwarakanath, N.R. Dereddy, D. Chabra, C. Schabacker, J. Calo, L.A. Parton, unpublished data, 2011). However, the latter study has confounders in the thrombocytopenic group, and 2 retrospective studies from Japan (142 born at 24–30 weeks' gestation)<sup>14</sup> and the United States (497 born at <28 weeks' gestation who received prophylactic indomethacin)<sup>15</sup> did not find an association between thrombocytopenia and incidence of PDA.

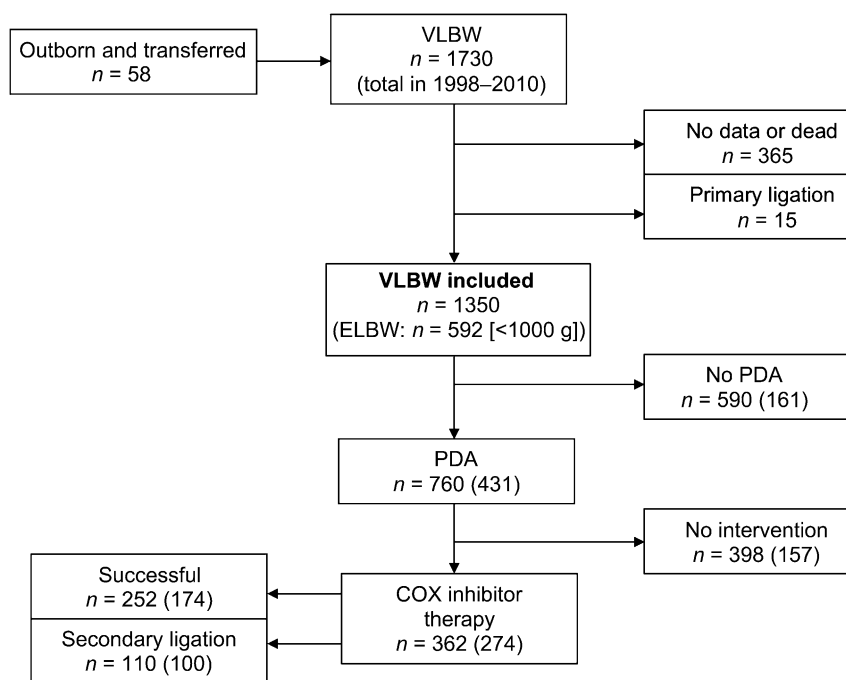
Because of the aforementioned controversy, we conducted a large retrospective study in a cohort from 2 German tertiary centers including 1350 very low birth weight infant (VLBW) (<1500 g) infants, of whom 592 had ELBW. We

demonstrate that thrombocytopenia in the first 24 hours after birth is not associated with higher incidence of PDA in either VLBW or ELBW infants. In addition, we found that sepsis, male gender, and lower gestational age or birth weight is associated with higher PDA incidence.

## METHODS

### Patients

This retrospective cohort study was conducted at the NICUs in the Department of Neonatology, Campus Virchow, Charité University Medical Center, Berlin, and the Division of Neonatology, University Hospital Essen, both in Germany. All VLBW infants who were admitted between 1998–2008 and 2005–2010, respectively, were included if they had received a complete blood cell count within the first 24 hours after birth and undergone an echocardiogram on DOL 4 to 5 (Fig 1). None of the infants had received PDA-specific pharmacotherapy (indomethacin or ibuprofen) before echocardiography on DOL 4 to 5. The ELBW population is a subset of the total VLBW cohort; both groups were analyzed separately for better comparison with other populations. Infants were stratified in 3 groups according to their platelet number within the first 24 hours after birth and the incidence of PDA (platelet count cutoff 150 000, 100 000, and 50 000/ $\mu$ L). Furthermore, we analyzed whether the frequency of pharmacologic or surgical PDA treatment was associated with severity of thrombocytopenia. Sepsis data were collected from the German mandatory neonatal care quality and outcome assessment. Only infants with sepsis-onset  $\leq$ DOL 5 were considered as “harboring an infection.” Parental consent was given after patient admission to the NICUs, and data were collected through chart review. The study was approved by the ethics committees of the 2 study centers.

**FIGURE 1**

Study design. The flowchart illustrates the numbers of VLBW and ELBW infants who were included in this retrospective cohort study. As shown (numbers given in parentheses), the ELBW population is a subset of the total VLBW infant cohort.

### PDA Diagnosis/Algorithm for PDA Intervention

Infants were evaluated for a hemodynamically significant PDA on DOL 4 to 5 and when clinically indicated. Echocardiographic studies included the assessment of ductal shunt direction by using color Doppler (high upper parasternal short axis) and measurement of the minimal internal ductal diameter in B-mode (average of 3–5 measurements). The left atrium to aortic root ratio was determined by using M-mode in the parasternal long axis views. Doppler measurements of the resistance index in the anterior cerebral artery was conducted at the same time.

Imaging was performed by 3 experienced echocardiographers using HDI 3500 equipment (ATL Philips Medical Systems, Hamburg, Germany; ACUSON Sequoia 512, Siemens, Erlangen, Germany) with a curved array transducer (7.5–10 MHz).

In both study centers, COX inhibitor treatment was only initiated in VLBW/

ELBW infants with hemodynamically significant PDA (hsPDA). A PDA with left-to-right shunt was considered hemodynamically significant under the following conditions: (1) respiratory setback with supplemental inspiratory oxygen >30% and/or mechanical invasive or non-invasive ventilation; (2) left atrium to aortic root ratio  $\geq 1.4$  by using M-mode; and/or (3) ductal diameter  $\geq 2.5$  mm; and/or (4) a decreased end diastolic flow in the anterior cerebral artery with a resistance index  $\geq 0.85$  in the cerebral ultrasound indicating significant ductal steal.

Although the center in Essen primarily used indomethacin for PDA intervention, in Berlin indomethacin was replaced by ibuprofen in April 2001 as an alternative COX inhibitor with potentially fewer undesirable short-term adverse effects.<sup>16,17</sup> Due to the uncertain impact of ibuprofen on bilirubin/albumin binding capacity, the Berlin study center returned to indomethacin as the first-choice drug in January 2005.<sup>5,18</sup>

Successful response to COX inhibitor treatment was defined as absence of ductal shunt flow 24 to 48 hours after the end of pharmacotherapy; all other cases were defined as COX inhibitor treatment failure. Ligation was performed as a rescue therapy in ventilated infants after failure of pharmacotherapy. Infants with successful pharmacologic closure of the PDA underwent routine echocardiography before discharge from the hospital.

### COX Inhibitor Therapy

Infants received indomethacin, starting with 3 intravenous doses of 0.2 mg/kg per dose in 12-hour intervals followed by daily maintenance doses of 0.1 to 0.2 mg/kg for a maximum of 6 days. Ibuprofen (Pedea) was used with 3 doses of 10, 5, and 5 mg/kg per dose at 24-hour intervals to treat PDA according to the Safety Survey Report (Patient Use of Ibuprofen Orphan Europe/PEDEA Report IBU/SURVEY, April 2003).

### Statistical Analysis

Values are expressed as absolute number of patients and percentage unless stated otherwise. Demographic characteristics are given as median and range. Comparisons between groups were made by using Kruskal-Wallis or Mann-Whitney *U* tests as appropriate for continuously scaled data and by using  $\chi^2$  tests for categorical data. *P* values < .05 were considered statistically significant. Receiver operating characteristic (ROC) curves, odds ratios (ORs), and logistic multivariate regression analyses were conducted by using SPSS version 19 software (SPSS Inc, Chicago, IL).

## RESULTS

### Incidence of PDA

Of 1730 VLBW infants who were born in the respective period, 1350 VLBW infants (including 592 ELBW infants) met the inclusion criteria; 365 infants

**TABLE 1** Demographic Characteristics and Incidence of Thrombocytopenia and Sepsis in all VLBW Infants (Total VLBW Cohort)

Characteristic	VLBW	Without PDA	PDA	PDA Pharm	PDA Secondary Ligation	PDA Without Therapy
All VLBW	1350 (100)	590 (43.7)	760 (56.3)	252 (18.7)	110 (8.1)	398 (29.5)
Female	693 (51.3)	341 (57.8)	352 (46.3)	113 (44.8)	52 (47.3)	187 (47.0)
Male	657 (48.7)	249 (42.2)	408 (53.7)	139 (55.2)	58 (52.7)	211 (53.0)
BW, g	1060 (270–1495)	1230 (356–1495)	945 (270–1495)	870 (270–1495)	728 (360–1440)	1080 (382–1495)
GA, wk	28 2/7 (22 0/7–37 3/7)	29 6/7 (22 0/7–37 3/7)	27 2/7 (22 6/7–35 1/7)	26 4/7 (23 0/7–32 0/7)	25 0/7 (23 0/7–30 6/7)	28 2/7 (22 6/7–35 1/7)
PLT >150/nL	1128 (83.6)	496 (84.1)	632 (83.2)	204 (81.0)	95 (86.4)	333 (83.7)
PLT <150/nL	222 (16.4)	94 (15.9)	128 (16.8)	48 (19.0)	15 (13.6)	65 (16.3)
PLT <100/nL	46 (3.4)	14 (2.4)	32 (4.2)	11 (4.4)	3 (2.7)	18 (4.5)
PLT <50/nL	16 (1.2)	6 (1.0)	10 (1.3)	4 (1.6)	1 (0.9)	5 (1.3)
Sepsis	54 (4)	12 (2.0)	42 (5.5)	25 (9.9)	6 (5.5)	11 (2.8)

Demographic characteristics and incidence of thrombocytopenia in the total cohort of infants with VLBW (birth weight [BW] <1500 g). In the top row, data in parentheses represent percentages referring to the total number of 1350, included VLBW preterm infants (horizontal comparison within the first row). For all other data shown, percentages in parentheses refer to the total number in the upper first row of the same column (vertical comparison within the same column). Data are presented as *n* (%) or median, range. GA, gestational age; Pharm, pharmacologic therapy; PLT, platelet count.

were excluded because they either died early postnatally or data (complete blood cell count, echocardiography, or demographic data) were missing; 15 infants who underwent primary PDA ligation were also excluded (Fig 1). Demographic characteristics are shown in Table 1 (total VLBW cohort) and Table 2 (ELBW subset). In the VLBW infant cohort, 657 (48.7%) of 1350 were male; of these 657 male infants, 408 (62.1%) had a PDA, 139 (21.2%) received pharmacologic PDA treatment, and 58 (8.8%) underwent secondary surgical ligation. The according relative proportions for female VLBW infants (693 of 1350 [51.3%]) were as follows: 352 (50.8%) of 693 had a PDA, 113 (16.3%) received pharmacologic PDA treatment, and 52 (7.5%) underwent secondary surgical ligation. The frequency of PDA was significantly higher in male versus female patients in both the total VLBW

cohort and the ELBW subset (Tables 1 and 2).

As expected, given the degree of immaturity, the rate of PDA on DOL 4–5 was higher in the ELBW subset than in the total VLBW cohort (72.8% vs 56.3%, respectively). In addition, 63.6% (274 of 431) of all ELBW infants with PDA received treatment (pharmacologic COX inhibition with or without secondary surgical ligation) versus only 47.6% (362 of 760) of all VLBW infants. Failure of pharmacologic treatment (ie, secondary ligation) was observed in 36.5% ELBW (100 of 274) and 30.4% VLBW (110 of 362) infants.

### Thrombocytopenia and PDA

Platelet counts did not differ between VLBW infants with and those without PDA. Platelet counts <150 000/ $\mu$ L were found in 16.8% versus 15.9% of VLBW infants, and in 19.3% versus 21.1% of

ELBW infants, with and without PDA, respectively (Tables 1 and 2). Among the 222 VLBW infants with thrombocytopenia (platelet count <150 000/ $\mu$ L) in the first 24 hours' postnatally, 128 had a PDA (57.7%) whereas 632 (56.0%) of the 1128 nonthrombocytopenic infants had a PDA (not significant [NS]). A near-even distribution was also found in ELBW infants: PDA was evident in 91 (74.6%) of 122 and 340 (72.3%) of 470 of the thrombocytopenic and nonthrombocytopenic infants, respectively (NS). Lower gestational age and birth weight had significant effects on the prevalence of PDA, the need for therapy, and the rate of pharmacologic treatment failure in both VLBW and ELBW infants (Table 3). Because thrombocytopenia could affect the need for PDA treatment or influence the success rate of treatment (pharmacologic COX inhibition versus secondary ligation), we investigated platelet number

**TABLE 2** Demographic Characteristics and Incidence of Thrombocytopenia and Sepsis in the Subset of ELBW Infants

Characteristic	ELBW	Without PDA	PDA	PDA Pharm	PDA Secondary Ligation	PDA Without Therapy
All ELBW	592 (100)	161 (27.2)	431 (72.8)	174 (29.4)	100 (16.9)	157 (26.5)
Female	304 (51.4)	99 (61.5)	205 (47.6)	113 (64.9)	49 (49.0)	80 (51.0)
Male	288 (48.6)	62 (38.5)	226 (52.4)	61 (35.1)	51 (51.0)	77 (49.0)
BW, g	785 (270–998)	837 (356–995)	770 (270–998)	773 (270–995)	705 (360–990)	825 (382–998)
GA, wk	26 2/7 (22 0/7–33 0/7)	27 1/7 (22 0/7–32 0/7)	26 0/7 (22 6/7–33 0/7)	26 0/7 (23 0/7–30 3/7)	24 6/7 (23 0/7–30 2/7)	26 6/7 (22 6/7–33 0/7)
PLT >150/nL	470 (79.4)	130 (80.7)	340 (78.9)	136 (78.2)	86 (86.0)	118 (75.2)
PLT <150/nL	122 (20.6)	31 (19.3)	91 (21.1)	38 (21.8)	14 (14.0)	39 (24.8)
PLT <100/nL	26 (4.4)	5 (3.1)	21 (4.9)	9 (5.2)	3 (3.0)	9 (5.7)
PLT <50/nL	7 (1.2)	1 (0.6)	6 (1.4)	3 (1.7)	1 (1.0)	2 (1.2)
Sepsis	38 (6.4)	7 (4.3)	31 (7.2)	22 (12.6)	6 (6.0)	3 (1.9)

Demographic characteristics and thrombocytopenia in the subset of infants with ELBW (birth weight [BW] <1000 g). In the top row, data in parentheses represent percentages referring to the total *N* of 592 included ELBW preterm infants (horizontal comparison within the first row). For all other data shown, percentages in parentheses refer to the total number in the upper first row of the same column (vertical comparison within the same column). Data are presented as *n* (%) or median, range. GA, gestational age; Pharm, pharmacologic therapy; PLT, platelet count.



**TABLE 3** ROC Curve Analysis in the Total VLBW Infant Cohort and the ELBW Subset

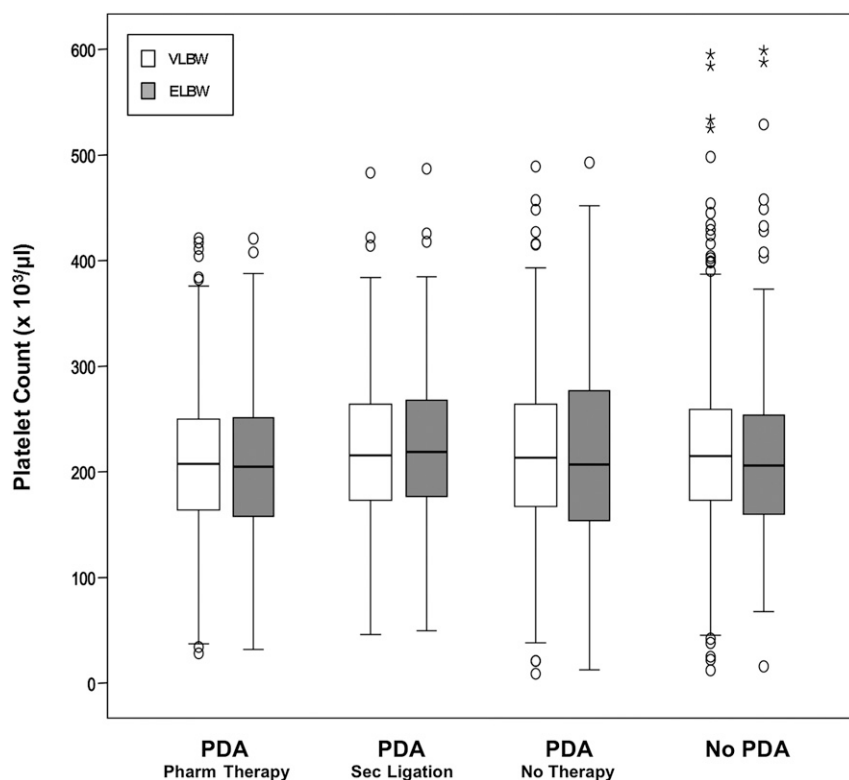
Variable	VLBW	ELBW
Platelet number		
PDA versus no PDA	0.483 (0.452–0.514)	0.499 (0.447–0.551)
PDA (therapy) versus PDA (no therapy)	0.487 (0.445–0.528)	0.494 (0.434–0.553)
PDA (pharma) versus PDA (secondary ligation)	0.541 (0.475–0.606)	0.564 (0.497–0.640)
Birth weight		
PDA versus no PDA	0.709 <sup>a</sup> (0.681–0.737)	0.597 <sup>a</sup> (0.545–0.690)
PDA (therapy) versus PDA (no therapy)	0.736 <sup>a</sup> (0.700–0.771)	0.635 <sup>a</sup> (0.579–0.690)
PDA (pharma) versus PDA (secondary ligation)	0.701 <sup>a</sup> (0.646–0.756)	0.639 <sup>a</sup> (0.575–0.704)
Gestational age		
PDA versus no PDA	0.752 <sup>a</sup> (0.655–0.710)	0.661 <sup>a</sup> (0.611–0.711)
PDA (therapy) versus PDA (no therapy)	0.771 <sup>a</sup> (0.738–0.804)	0.729 <sup>a</sup> (0.680–0.778)
PDA (pharma) versus PDA (secondary ligation)	0.757 <sup>a</sup> (0.703–0.810)	0.724 <sup>a</sup> (0.663–0.785)

The ELBW population is a subset of the total VLBW cohort. Data are presented as area under the curve (95% confidence interval). pharma, pharmacologic therapy.

<sup>a</sup> Statistically significant.

distributions in infants with PDA who subsequently were pharmacologically treated, ligated after treatment failure, and those who did not receive any specific treatment. As shown in Fig 2, platelet

counts did not differ between groups of VLBW and ELBW infants. Thus, platelet numbers were not associated with the frequency of PDA on DOL 4 to 5 or a specific therapeutic intervention.

**FIGURE 2**

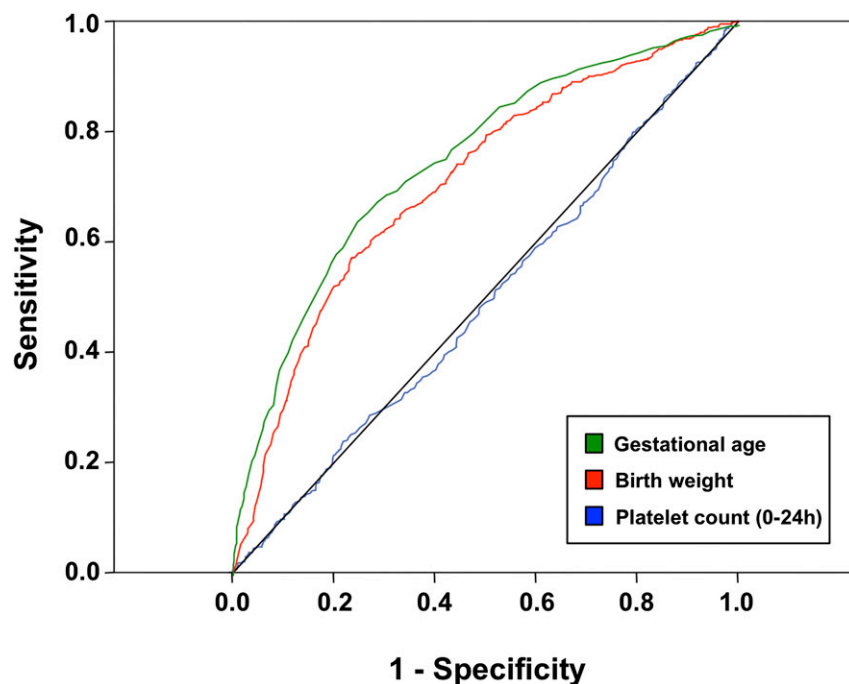
Boxplot: platelet count distribution. Boxplots show platelet count distributions within the first 24 hours of life with median and the 25th and 75th percentiles among 4 different groups of VLBW (white) and ELBW (gray) infants: PDA with pharmacologic (pharm) therapy, PDA with secondary (sec) ligation, PDA without therapy, and no PDA. There were no significant differences between the 4 groups in VLBW and ELBW infants, respectively. Of note, the ELBW population is a subset of the total VLBW infant cohort.

### ROC Curve Analysis (Platelet Count, Gestational Age, and Birth Weight)

ROC curve analysis was performed to determine whether thrombocytopenia, birth weight, or gestational age show any association with PDA. We thus ruled out the possibility that low platelet counts of a certain threshold might be associated with PDA. Figure 3 shows ROC curve analyses for the associations between platelet counts, birth weight, and gestational age with PDA in VLBW infants, by comparing infants with and without PDA. Lower birth weight and lower gestational age were associated with a higher PDA incidence, although platelet number did not seem to have any effect on PDA incidence. A ROC analysis for the ELBW subset revealed similar results (data not shown). Comparisons between (1) infants with PDA who received treatment (pharmacologic treatment and secondary ligation) versus infants with PDA who did not receive treatment and (2) between infants with successful pharmacologic treatment versus infants who underwent secondary ligation, with respect to effects of thrombocytopenia, birth weight, or gestational age are shown in Table 3. Although gestational age and birth weight were inversely associated with PDA on DOL 4 to 5, platelet number showed no such association (Fig 3).

### Influence of Sepsis and Male Gender on PDA Frequency

Moreover, we calculated ORs for PDA and sepsis or male gender in VLBW and ELBW infants, respectively. There were significantly increased ORs (approximate relative risk) for PDA in VLBW infants for both variables (Table 4). In ELBW infants, only male gender was associated with an increased OR for PDA. Sepsis increased the OR for PDA that required specific treatment compared with PDA without the need for such intervention in both VLBW and ELBW infants. Finally,



**FIGURE 3**

ROC curve analyses. Representative ROC curves for platelet count, birth weight, and gestational age comparing VLBW infants with and without PDA. The platelet curve divides the plot into 2 almost equal parts (indicating no association with PDA). In contrast, the ROC curves for birth weight and gestational age show an unequal division resulting in an area under the curve  $>0.5$ , indicating a negative correlation of gestational age or birth weight with PDA incidence. Similar results were obtained in the ELBW population, which is a subset of the total VLBW infant cohort (data not shown).

regression analyses in VLBW infants confirmed that sepsis, male gender, and gestational age are independent predictors of PDA (data not shown).

## DISCUSSION

Because of controversial data on the relationship between platelet number and PDA frequency,<sup>13–15</sup> we performed a large retrospective study at 2 tertiary centers to investigate higher numbers of VLBW/ELBW infants, with balanced gender

ratio and similar ethnic background compared with the study published by Echtler et al.<sup>13</sup> We herein report platelet numbers, PDA incidence, and PDA intervention in the largest VLBW ( $n = 1350$ ; median gestational age: 28 2/7 weeks) and ELBW ( $n = 592$ ; median gestational age: 26 2/7 weeks) cohorts published to date. In our study, the estimated incidence of PDA on DOL 4 to 5 (56% of all VLBW infants), frequency of PDA pharmacotherapy (27%), and secondary PDA ligation (8%) was within the (wide)

range of other published reports on VLBW infants.<sup>5,13,19–21</sup>

We found that mild ( $<150\,000/\mu\text{L}$ ), moderate ( $<100\,000/\mu\text{L}$ ), or severe ( $<50\,000/\mu\text{L}$ ) thrombocytopenia in the first 24 hours after birth was not associated with a higher incidence of PDA in either VLBW or ELBW infants not prophylactically treated with either indomethacin or ibuprofen. However, sepsis and lower gestational age or birth weight were associated with a higher PDA incidence.

In contrast to the previously published small retrospective, single-center study by Echtler et al,<sup>13</sup> which demonstrated an association between moderate thrombocytopenia and frequency of PDA (123 infants born at 24–30 weeks' gestation), and preliminary data (K. Dwarakanath, N.R. Dereddy, D. Chabra, C. Schabacker, J. Calo, L.A. Parton, unpublished data, 2011), 2 very recent retrospective studies from Japan<sup>14</sup> and the United States<sup>15</sup> did not find such an association. However, the moderately sized US study by Shah et al<sup>15</sup> focused on the relation between platelet numbers and DA closure after prophylactic indomethacin treatment within the first 15 hours after birth (497 infants  $<28$  weeks' gestation), and the other 2 published studies<sup>13,14</sup> are hampered by a relatively small sample size, as well as different ethnic background and gender distribution<sup>14</sup> in the cohorts studied.<sup>22</sup> The yet unpublished report by Dwarakanath et al (2011) is confounded by lower average birth weight, higher rates of intrauterine growth retardation, and maternal preeclampsia in the thrombocytopenic infants.

Although the study by Fujioka et al<sup>14</sup> revealed results similar to ours, it has been particularly criticized for its small sample size ( $N = 142$ ) and an apparent unbalanced gender ratio in infants with low platelet counts ( $n = 16$ ; 84% males). In contrast, both our thrombocytopenic and nonthrombocytopenic VLBW population had a balanced gender ratio (thrombocytopenic males: 28 of 48

**TABLE 4** OR Analysis in the Total VLBW Infant Cohort and the ELBW Subset

Variable	VLBW	ELBW
Sepsis		
PDA versus no PDA	2.82 <sup>a</sup> (1.47–5.40)	2.07 (0.85–5.05)
PDA (therapy) versus PDA (no therapy)	3.30 <sup>a</sup> (1.63–6.66)	4.35 <sup>a</sup> (1.50–12.65)
PDA (pharma) versus PDA (secondary ligation)	1.91 (0.76–4.79)	2.27 (0.89–5.8)
Male gender		
PDA versus no PDA	1.64 <sup>a</sup> (1.32–2.04)	1.76 <sup>a</sup> (1.22–2.56)
PDA (therapy) versus PDA (no therapy)	1.07 (0.80–1.42)	1.24 (0.84–1.84)
PDA (pharma) versus PDA (secondary ligation)	1.12 (0.72–1.76)	1.24 (0.76–2.03)

The ELBW population is a subset of the total VLBW cohort. Data are presented as OR (95% confidence interval). pharma, pharmacologic therapy.

<sup>a</sup> Statistically significant.

[58.3%]; nonthrombocytopenic males: 626 of 1302 [48.1%]; NS). Notably, Echtlar et al<sup>13</sup> argue that, in contrast to their own study, Fujioka et al only included Asian infants and, therefore, ethnic differences might have contributed to the contradictory results.<sup>14</sup> The VLBW/ELBW infants in our study ( $N = 1350$ ) and those studied by Echtlar et al ( $N = 123$ ) were born in the same country at tertiary centers, had a predominately caucasian ethnic background, and a similar in utero environment. Thus, potential differences in NICU management or ethnicity cannot explain the discrepancy in the findings.

Shah et al<sup>15</sup> assessed serial platelet counts (DOL 1 to 7) in preterm infants born <28 weeks' gestation, all of whom received prophylactic indomethacin starting on DOL 1. Patency of DA was not related to platelet count at any time in the first week of life. Instead, high platelet numbers may have been associated with initial DA constriction/closure. Neither high nor low platelet numbers had an impact on final, permanent DA closure. However, the influence of platelet numbers on DA in the absence of indomethacin could not be determined in the study by Shah et al. We also did not find an association between platelet number in the first 24 hours after birth and PDA on DOL 4 to 5

in VLBW/ELBW preterm infants, regardless of whether the infants had subsequently received pharmacologic COX inhibition, secondary ligation, or no therapy (Fig 2). Moreover, when we used the exact same inclusion criteria as Echtlar et al<sup>15</sup> (24–30 weeks' gestation), we still did not find any association between platelet number and PDA ( $n = 947$ ; data not shown).

Our study did not reveal an association between thrombocytopenia and PDA incidence, but sepsis, lower gestational age, and lower birth weight were independent risk factors for DA patency. Interestingly, all the latter variables may affect platelet function.<sup>23,24</sup> Given the findings on platelet-triggered ductal sealing in mice,<sup>13</sup> we speculate that platelet dysfunction (due to immaturity<sup>25–27</sup> or critical illness<sup>23,24,28</sup>) rather than platelet number contributes to the pathogenesis of PDA.<sup>22</sup>

Although the platelet numbers reported in our study were determined in the first 24 hours after birth, and before any indomethacin or ibuprofen administration, we cannot rule out that a platelet nadir occurred on DOL 2 to 5, which may have influenced the efficiency of COX inhibition or the need for secondary surgical ligation in infants

with hemodynamically significant PDA, especially when other risk factors such as sepsis/inflammation were present. However, the data reported by Shah et al<sup>15</sup> argue against the assumption that a platelet nadir after the first 24 hours may have influenced PDA closure rate in our study because no association between platelet number at any time in the first week of life and DA patency or permanent closure was found.<sup>15</sup> Furthermore, correction of thrombocytopenia by transfusions or medications that alter hemostasis and platelet function such as ampicillin<sup>29</sup> are unaccounted for in our retrospective study. These open questions should be addressed by prospective studies that must include variables of platelet function to shed light on the current controversy about the causal role of platelets in DA closure.

## CONCLUSIONS

We show that mild, moderate, or severe thrombocytopenia in the first 24 hours after birth is not associated with a higher rate of PDA in a large cohort of VLBW and ELBW infants, the vast majority of whom were born at 26 to 28 weeks' gestation. Impaired platelet function, due to immaturity and critical illness, rather than platelet number, may contribute to DA patency.

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