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Fresh frozen plasma transfusion – a risk factor for pulmonary hemorrhage in extremely low birth weight infants?

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Abstract

Aim: To evaluate risk factors for pulmonary hemorrhage (PH) in extremely low birth weight infants (ELBW) taking into consideration coagulation screens, platelet counts, transfusion of fresh frozen plasma (FFP), and platelet concentrates prior to PH.

Patients and methods: A retrospective case-control study consisting of 20 ELBW infants with PH and 40 matched controls. Coagulation screens, platelet counts at birth and at onset of PH, and transfusion frequencies prior to PH were compared to case-controls at birth and 24–96 h after birth.

Results: While the initial platelet counts, fibrinogen concentrations, and international normalized ratios were similar in PH infants and controls, the activated partial prothrombin time was prolonged ($P = 0.05$). Compared to 28% of case controls ($P < 0.05$), 55% of infants with later PH received FFP prior to PH. Platelet counts were significantly lower at onset of PH (median 81/nL; range: 37–236/nL) compared to controls (166/nL; 27–460/nL; $P < 0.005$). Multivariate analysis indicated a lack of antenatal steroids, supplemental oxygen, and transfusion of FFP as independent risk factors for PH.

Conclusion: Prolonged activated partial thromboplastin time (aPTT) might be associated with PH. PH does not primarily depend upon severe thrombocytopenia. A developmental mismatch in hemostasis by transfusion of adult donor plasma should be considered a risk factor for PH.

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Keywords: Bleeding; coagulation disorder; extremely low birth weight; fresh frozen plasma; platelet transfusion; thrombocytopenia.

Introduction

In extremely low birth weight infants (ELBW; <1000 g), pulmonary hemorrhage (PH) is a rare but life-threatening event associated with mortality rates as high as 50%–68% [1–3]. The risk of PH is inversely related to gestational age. Numerous findings are associated with PH, such as intrauterine growth restriction, lung hypoplasia, sepsis, coagulopathy, thrombocytopenia, surfactant application, intraventricular hemorrhage (IVH), and patent ductus arteriosus (PDA) [1, 2]. While previous studies indicate that antenatal glucocorticoids may be protective against PH [4], putative adverse effects of transfusions of adult red blood cells, platelets, or plasma are a matter of discussion. In particular, the routine performance of coagulation screens at admission causes an increased use of fresh frozen plasma (FFP), although their clinical benefit has to be confirmed in randomized clinical trials [3]. Thus, we reassessed risk factors for PH with specific emphasis on routine coagulation screens, including platelet counts and transfusion of blood products.

Patients and methods

This retrospective case-control study included 20 consecutive ELBW infants with PH admitted to our neonatal intensive care unit (NICU) between 2002 and 2012. PH was defined by the presence of frank tracheal blood requiring prompt intervention (e.g. red blood cell transfusions, increased respiratory support) and by multi-lobular opacity on chest X-rays [2, 5]. The cohort was compared with gestational age and weight-matched controls (n = 40).

We considered two different time points of blood sampling in infants with PH and case-controls. Immediately after birth, ELBW infants had routine blood tests, including full blood cell count, interleukin-6 (IL-6), and coagulation screens. The specimens were taken via direct venipuncture or through a non-heparinized umbilical line. At the second time point, a full blood cell count was performed at least once 24–96 h after birth, according to standard procedure. In

patients with PH, another examination of full blood count was initiated as soon as PH was recognized.

Blood cell counts were determined using the automated analyzer XE-2100™ (Sysmex Corporation, Kobe, Japan). Plasma IL-6 concentrations were measured by means of an automated immunoassay (COBAS 6000 or COBAS 8000, Roche Diagnostics, Mannheim, Germany). Coagulation screens, including activated partial thromboplastin time (aPTT), international normalized ratio (INR), and fibrinogen concentration, were performed on the COBAS t 411 coagulation analyzer (Roche Diagnostics, Mannheim, Germany). Thrombocytopenia was defined by a platelet count <150/nL. Fibrinogen, INR, and aPTT were interpreted considering age-dependent reference values [6, 7]. Abnormal values were defined as those outside of two standard deviations of the mean (aPTT > 64 s, INR > 2.7, fibrinogen < 0.71 g/L). Early-onset infection was diagnosed by increased IL-6 concentration (>100 ng/L) [8]. Septicemia was defined by positive blood culture within 48 h. Echocardiographic examination for PDA included assessment of ductal shunt direction by color Doppler and measurement of the minimal internal ductal diameter in B-mode on the 4th or the 5th day of life. The left-atrium-to-aortic-root ratio was determined using the M-mode in the parasternal long axis view. Ibuprofen treatment was only initiated in hemodynamically significant PDA with left-to-right shunt, according to the following conditions: (1) respiratory setback with supplementary oxygen > 30% and/or invasive ventilation, (2) left atrium to aortic root ratio ≥ 1.4 or a PDA diameter ≥ 2.5 mm. Ibuprofen (Pedea) was given with three doses of 10, 5, and 5 mg/kg per dose at 24-h intervals. Cerebral ultrasound examinations were performed on the 1st, 3rd, and 7th day after birth. In patients with PH, cerebral ultrasound examination was additionally performed after diagnosis of PH. The grade of intraventricular hemorrhage (IVH) was defined according to Papile et al. [9]. Indomethacin treatment for IVH prevention was initiated immediately after birth if ELBW infants were at high risk for IVH. According to our internal standard operating procedure, we calculated a risk score based on previously published data [10, 11], including completion of prenatal steroids for lung maturation [12], gestational age, sex [13], multiple birth [14], and IL-6 concentration at birth [15]. Indomethacin was used at a dose of 0.1 mg/kg intravenously for 3 consecutive days if the patients were classified as high risk for IVH. Other bleeding events were defined as bloody staining of oral, nasal, or gastric secretion, puncture site oozing, or macrohematuria.

Platelet transfusions were administered according to previously published recommendations [16]. Briefly, platelets were transfused under the following conditions: (1) ELBW infants with acute major bleeding and a platelet count below 100/nL. (2) Non-bleeding ELBW infants, if the platelet count fell below 50/nL in the first week of life. (3) ELBW infants with concurrent coagulopathy or signs of minor bleeding and a platelet count of 50–99/nL in the first week. Only single donor (apheresis-derived), leukoreduced, and irradiated platelet concentrates were transfused. The transfusion volume was 10 mL/kg body weight, administered over 1–2 h, preferentially via a peripheral venous line.

FFP was transfused in infants with abnormal coagulation screens. Plasma packages were produced from whole blood, pooled from various donors, and stored at −30°C until use. Normally, 10–20 mL/kg FFP was transfused over approximately 4 h.

For this study, we recorded the number and time points of FFP and platelet concentrate transfusions in the first week after birth. In patients with PH, the transfusion of blood products was only recorded, when given prior to the onset of PH (minimum clearance 24 h). We analyzed the frequency (none/one/more than one) of FFP/platelet concentrates transfused.

For statistical analysis of platelet counts and coagulation screens, the initial blood examination at birth (first time point) was considered. Platelet counts were analyzed at onset of PH and compared to the second routine blood examination performed within 24–96 h postnatally in case-controls (second time point). The difference of the individual platelet counts between the first and second time point were compared using the Wilcoxon matched-pairs signed-rank test. To assess the relation between risk factors and PH, multivariate logistic regression was used [yielding adjusted odds ratio (OR) with 95% confidence interval (CI)]. Variables significantly associated with PH in the univariate model (FFP transfusion, fraction of inspired oxygen, antenatal steroids) were included in the multivariate model. As published data also suggests an association between sex and PH in ELBW infants, this variable was also included in the multivariate model. Statistics were performed with Stata® (Stata Statistical Software: release 13. STATA Cooperation, College Station, TX, USA), using the χ^2-test or the Mann-Whitney *U-test* as appropriate.

Results

PH was diagnosed in 20 out of 761 (2.6%) ELBW infants admitted within the 11-year observation period. PH occurred, in median, on day (d) 3 (range: d2–d5). PH occurred most frequently within the first 72 h after birth (70%). The demographic and clinical data are summarized in Table 1. The overall short-term outcome in the PH group was poor, including a significantly higher incidence (55%) of severe IVH and a mortality rate of 35% within the first 7 days of life. In infants with PH, steroids were given significantly less often as compared to case-controls (P = 0.013). The frequency of intubation and surfactant application in the delivery room did not differ between infants with PH and case-controls. At admission to the NICU, the clinical risk index for babies (CRIB) score and the incidence of early-onset infection were similar in both groups. The median concentration of IL-6 did not differ between infants with PH (149 pg/mL, range 1–90,000) and controls (223 pg/mL, range 5–66,600; P = 0.269). Infants with later PH had a significantly higher concentration of supplemental oxygen at admission to NICU. The type of mechanical ventilation (conventional or high frequency oscillation) and rate of respiratory support (continuous positive airway pressure, CPAP) treatment at onset of PH were similar compared to case-controls. The frequency of indomethacin treatment for the prevention of IVH did not differ significantly between the groups. PH was associated with a significantly higher incidence of IVH, both considering any grade of IVH (P = 0.003) or only severe IVH (stage ≥ III°, P = 0.002). In 10 out of 11 patients with PH, severe IVH was first diagnosed after the occurrence of PH.

Table 1: Demographic and clinical characteristics of both groups of patients.

	Pulmonary hemorrhage (n = 20)	Case-controls (n = 40)	P-value
Females	5 (25%)	15 (37.5%)	0.33
Singletons	10 (50%)	27 (67.5%)	0.26
Birth weight (g)	713 (488–990)	737 (438–1024)	0.86
Small for gestational age	8 (40%)	16 (40%)	0.78
Gestational age (weeks + days)	26 + 2 (24 + 1–30 + 0)	26 + 0 (24 + 0–31 + 5)	0.52
Antenatal steroids	14 (70%)	38 (95%)	0.013
Intubation in delivery room	17 (85%)	38 (95%)	0.17
Surfactant replacement therapy	19 (95%)	37 (92.5%)	1.0
Fraction of inspired oxygen (%)[a]	58 (21–73)	40 (21–74)	0.003
CRIB score	7 (1–17)	6.5 (2–11)	0.63
Early-onset infection	11 (55%)	27 (67.5%)	0.90
Positive blood culture at birth	4 (20%)	2 (5%)	0.09
Platelet count (/nL) at birth	170 (61–446)	183 (45–443)	0.51
Platelet count (/nL) at onset of PH or within first 96 h (controls)	81 (37–236)	166 (27–460)	0.002
Conventional mechanical ventilation	15 (75%)	25 (62.5%)	0.67
High-frequency oscillatory ventilation	1 (5%)	2 (5%)	
CPAP respiratory support	4 (20%)	12 (30%)	
Early indomethacin (< 12 h of life) for IVH prevention	1 (5%)	8 (20%)	0.12
PDA	18 (90%)	33 (83%)	0.58
Ibuprofen treatment for hemodynamically significant PDA	2 (10%)	10 (25%)	0.17
IVH (all grades)	12 (60%)	8 (20%)	0.003
Severe IVH (grade ≥ III)	11 (55%)	6 (15%)	0.002
Minor bleeding[b]	3 (15%)	0	0.033
Death (≤ d7)	7 (35%)	4 (10%)	0.03
Death (> d7)	1 (5%)	5 (12.5%)	0.36
Overall death during hospital stage	8 (40%)	9 (22.5%)	0.16

Data are presented as median (range) or number (%). [a]Maximum fraction of inspired oxygen during the first 24 h after birth to achieve an oxygen saturation level > 85%. [b]Minor bleeding was defined as one or more of the following signs: blood staining of oral, nasal, or gastric secretion, puncture site oozing, or macrohematuria. CRIB, Clinical risk index for babies; CPAP, continuous positive airway pressure; IVH, intraventricular hemorrhage; PDA, patent ductus arteriosus; d, day of life.

In case-controls, IVH was diagnosed, in median, on day 3 (range d1–d3) in six infants. Other bleedings occurred in three infants with PH (two infants with gastrointestinal bleeding, one infant with macrohematuria), but not in controls. Among them, gastrointestinal bleeding was diagnosed contemporaneously to PH; macrohematuria occurred in one patient 7 days after the appearance of PH. Overall, the incidence of other bleeding events was significantly higher in PH patients (P = 0.033). Early mortality (≤ d7) was higher after PH compared to case-controls (P = 0.03), while the overall in-hospital mortality rate was not different.

The incidence of thrombocytopenia and median platelet count at birth did not differ between both groups (platelet count 170/nL vs. 183/nL, Figure 1). In infants with PH, platelet counts decreased at onset of PH and were significantly lower (platelet count 81/nL vs. 166/nL, P = 0.002) than in case-controls at a comparable time

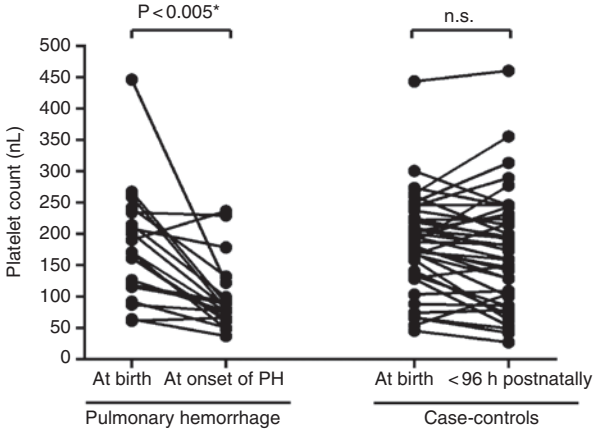

Figure 1: Individual platelets counts of each patient suffering from pulmonary hemorrhage (PH) (n = 20) and matched case-controls (n = 40) at birth and at onset of PH or within 96 h postnatally, respectively. *Results obtained with the Wilcoxon matched-pairs signed-rank test.

point (24–96 h postnatally). In contrast, platelet counts in case-controls did not significantly change during the observation period (P = 0.405). Only 10% of the infants displayed platelet counts < 50/nL at the onset of PH, while in the case-control group, 7.5% presented platelet counts < 50/nL (Figure 1).

Due to the small sample volume, aPTT measurement could not be performed in one infant with PH. aPTT measurement was missing in nine, and fibrinogen and INR in 11 case-controls, respectively. In infants with PH, the median aPTT was significantly longer (75 s) compared to case-controls (62 s, P = 0.033). Thirteen infants with PH (68%) had prolonged aPTT compared to 12 case-controls (41%, P = 0.061). The median concentration of fibrinogen did not differ between both groups (PH: median 131 mg/dL, range: 30–378; controls: 178 mg/dL, 55–469; P = 0.107). Five of twenty infants (25%) with PH had an abnormal fibrinogen concentration compared to two case-controls (6%, P = 0.073). The INR did not differ significantly between the groups (PH: median INR 2.05, range: 0.92–4.3; controls: 1.6, 0.48–5.4; P = 0.559).

There was no difference in the administration rate of platelet concentrates in infants with later PH vs. case-controls: Five out of 20 infants (25%) were transfused prior to the onset of PH compared to four out of 40 case-controls (10%, P = 0.13). In four patients with PH, platelet concentrates were transfused when PH occurred. In contrast, infants with later hemorrhage received FFP prior to the onset of PH significantly more often (minimum clearance 24 h) compared to controls. In patients with later hemorrhage, FFP was transfused in median 1.5 days (range 1–3 days) before PH occurred. Eleven out 20 infants (55%) received FFP prior to the onset of PH; among them, two patients received FFP twice. Eleven out of 40 case-controls (27.5%) were transfused with FFP once in the first 5 days (P = 0.033) (Table 2). We performed uni- and multivariate logistic regressions to study the associations between different risk factors and PH. FFP transfusion remained an independent risk factor for PH [OR 12.9 (95% CI 2.07–76.94); P = 0.006], even if adjusted to the application of antenatal steroids, the fraction of inspired oxygen, and sex (Table 3).

Discussion

A priori, this retrospective single-center case-control study, emphasizing the comparison of coagulation factors and platelet counts, was unlikely to identify a single major risk factor for PH, but we aimed to add our data to the ongoing discussion on preventive strategies [1, 2, 17]. Based on our data and other studies, the following avenues for further clinical recommendations and research might become more obvious.

Table 2: Blood product transfusions in infants with pulmonary hemorrhage and case-controls.

	Pulmonary hemorrhage (n = 20)	Case-controls (n = 40)	P-value
Platelet transfusion prior to onset of PH, or within the first 96 h (controls)	5 (25%)	4 (10%)	0.13
No FFP transfusion prior to onset of PH, or within the first 96 h (controls)	9 (45%)	29 (72.5%)	
1 FFP transfusion prior to onset of PH, or within the first 96 h (controls)	9 (45%)	14 (27.5%)	
≥ 2 FFP transfusions prior to onset of PH, or within the first 96 h (controls)	2 (10%)	0	0.033[a]

Data are presented as median (range) or number (%). [a]Comparison of all three categories. PH, Pulmonary hemorrhage; FFP, fresh frozen plasma.

Table 3: Association between different risk factors and pulmonary hemorrhage.

	Univariate association		Multivariate association[a]	
	OR (95% CI)	P-value	OR (95% CI)	P-value
Female gender	0.55 (0.16–1.84)	0.336	0.44 (0.09–2.21)	0.324
Antenatal steroids	0.12 (0.02–0.68)	0.016	0.11 (0.01–0.86)	0.035
Fraction of inspired oxygen (%)	1.07 (1.02–1.12)	0.003	1.06 (1.01–1.12)	0.031
FFP transfusion prior to onset of PH, or within the first 96 h (controls)	13.5 (2.74–66.32)	0.001	12.9 (2.07–76.94)	0.006

Data are presented as OR (95% CI) for pulmonary hemorrhage for the binary exposures female gender, application of antenatal steroids, FFP transfusion, and for a 1% increase in fraction of inspired oxygen. [a]The adjusted logistic regression model accounts OR for all variables of the univariate model. CI, Confidence interval; FFP, fresh frozen plasma; OR, odds ratio.

Our study underlines the beneficial effects of antenatal steroids beyond fetal lung maturation. As in previous studies [1, 2, 4], we found a protective effect of antenatal steroids for PH prevention. Antenatal steroids might not have been given due to the necessity of rapid delivery as a result of maternal illness or fetal distress. Although not reflected by higher CRIB scores, the reduced incidence of PH in response to steroids may result, on the cellular level, from enhanced microvascular maturation and premature focal capillary fusion [18]. The lower rate of fetal lung maturation is also reflected by the higher concentration of supplemental oxygen on the day of admission in infants with later PH. However, at the time point of PH, the ventilation mode did not differ between infants with PH and case-controls. Therefore, we assume that mechanical ventilation associated with micro trauma of the lung alone does not seem to be a major risk factor for PH. Severe IVH was detected in 10 out 11 infants after PH occurred and is thought to be a consequence of clinical deterioration during PH. However, other bleedings were only detected in infants with PH. Although small for gestational age (SGA) has been described as a risk factor for PH, and SGA infants are prone to coagulopathy [19], we did not find a correlation between SGA and the occurrence of PH.

The follow-up analysis of the trial on indomethacin prophylaxis in preterms (TIPP) indicated that prophylactic indomethacin reduced the rate of early serious PH [1]. Rates of infants with PH who had received prophylactic indomethacin were only 25% of those in controls, but this difference was not significant. In another recent case-control analysis, infants who received indomethacin were less likely to die, particularly in the PH group, in which moderate-to-large PDA was attributed to PH [2]. In order to avoid side effects, such as decreased cerebral blood flow, prophylactic indomethacin might be administered according to a risk score for major PH or IVH.

Although COX inhibitors, such as indomethacin and ibuprofen, are known to impair platelet function [20], their use for the prevention and therapy of PDA did not increase the occurrence of IVH in very low birth weight infants with platelet counts > 100/nL [21]. However, Brunner et al. showed that ibuprofen treatment significantly increased the risk for IVH in infants with platelet counts of 50–99/nL [21]. In our study cohort, the rate of ibuprofen or indomethacin treatment did not differ in infants with PH and case-controls. Therefore, we assume that impaired platelet function due to COX inhibitor exposure does not play a significant, or plays only a minor, role in the pathophysiology of PH in non-thrombocytopenic infants.

Importantly, the implication of platelet and coagulation data on the individual risk for PH should be revisited for the following reasons: First, platelet counts and INR and fibrinogen concentration at birth did not differ between infants with PH and case-controls. The aPTT was significantly longer in infants with later PH, and there was a trend towards a higher proportion of abnormal aPTT and fibrinogen concentration. This finding might explain the higher rate of FFP usage in infants with PH. Second, platelet counts at onset of PH were lower than those at birth and also lower than the lowest values observed in controls during the first 96 h after birth. We assume that the decreased platelet count is a result of platelet consumption during acute hemorrhage. However, platelet counts < 50/nL, widely thought to define severe thrombocytopenia, was seen not only in 10% of infants with PH, but also in 7.5% of controls. This finding is in line with the outcome study on very low birth weight infants with platelet counts < 60/nL (PlaNet1), in which 9% of children developed major hemorrhage [5].

Finally, infants with PH received more transfusions of FFP than controls. FFP transfusion was an independent risk factor associated with PH. It is tempting to speculate that the receipt of FFP is merely a marker of critical illness, but this cannot be concluded from other parameters such as requirement of mechanical ventilation, CRIB score, or surfactant treatment, as well as diagnosis of early-onset infection (Table 1). Thus, treatment with blood products deserves more attention. In 1996, a randomized controlled multicenter trial including 776 infants born before 32 weeks of gestation compared early FFP transfusion vs. maintenance glucose infusion (controls) with the primary outcome measure survival without disability. This trial provided no evidence that FFP transfusions affect the risks of death, IVH, or other major disability [22, 23]. Notably, the transfused plasma derived from adult donors may negatively interfere with neonatal platelet function, and hyporeactivity of neonatal platelets may be required for balancing primary hemostasis. Significant effects on platelet aggregation have been detected after 'developmental mismatch *in vitro* transfusions': the addition of adult platelets to cord blood plasma of thrombocytopenic neonates resulted in shorter clotting times, which in turn increased the bleeding risk as a consequence of prothrombotic effects. In the opposite approach, the addition of adult donor plasma to platelets of preterm infants led to prolonged bleeding time parameters. In line with this, no evidence is given that in preterm neonates, prophylactic plasma transfusion based on coagulation screens improved clinical outcomes in terms of mortality and morbidity [6, 23–25]. However, routine screening of coagulation in neonates leads to increased use of plasma for transfusion in neonatal intensive care [3]. Indeed, our

analysis of blood coagulation screens did not indicate significant differences between ELBW infants with regard to fibrinogen and INR in infants with PH and age-matched controls. As indicated by prolonged aPTT in infants with later PH and occurrence of other bleeding symptoms, some patients might suffer from temporarily deranged coagulation, which contributes to the occurrence of PH.

Several, somewhat obscure, findings might be of particular interest for further research on bleeding disorders of very premature infants. Platelet counts dropped significantly more often after birth in patients with PH than in controls. The exact time point of the decrease in platelet count can only be estimated between birth and onset of PH. Platelet count might be decreased at onset of PH due to platelet consumption during clotting in the bleeding sites in the lung parenchyma. On the other hand, the platelets might be involved in the pathogenesis of PH. Besides their primary role in coagulation, activated platelets mediate pulmonary neutrophil tethering and activation [26]. In the developmental-stage-dependent balanced coagulation system, changes in platelet counts and function (eventually related to their granula content, e.g. platelet-released Dickkopf-1 [Dkk1] or chemokines such as CCL5/RANTES [27, 28]) could contribute to damage of the pulmonary endothelial barrier with subsequent bleeding. Such mechanisms for the response to local pulmonary inflammatory processes and other mechanisms for the augmented tension in the capillary wall (e.g. PDA, intrauterine growth restriction) deserve further experimental research in animal models for lung diseases in prematurity.

In summary, antenatal steroids are associated with a reduced risk for PH. We cannot conclude whether the abnormal coagulation or the transfusion of FFP is causative for PH. However, as transfusion of adult plasma was associated with later PH, clinical trials on laboratory based values as transfusion criteria are justified. *In vivo* experiments remain needed to understand suggested adverse effects on primary hemostasis by transfusing adult plasma to ELBW infants.

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Author's Statement

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Material and methods: Informed consent: Informed consent has been obtained from all individuals included in this study.

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