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Original Article

Frequent Use of Fresh Frozen Plasma Is a Risk Factor for Venous Thrombosis in Extremely Low Birth Weight Infants: A Matched Case-control Study

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Percutaneously inserted central catheters (PICCs) are often used in neonatal medicine. Venous thrombosis (VT) is one of the complications associated with PICC use. According to some reports, fresh frozen plasma (FFP) may be a risk factor for VT. The purpose of this study was to determine whether FFP use is associated with VT in extremely low birth weight infants (ELBWIs). We performed a matched case-control study on risk factors for VT in ELBWIs born over a period of 5 years in the neonatal intensive care unit of a tertiary hospital. Controls were infants from the unit matched for gestational age and birth weight. We performed univariate analyses and created receiver operating characteristic (ROC) curves for the cut-off values of continuous parameters such as FFP. We also conducted multivariate conditional logistic regression analysis and calculated adjusted odds ratios and their 95% confidence intervals. Thirteen VT cases and 34 matched controls were examined. Using an ROC curve, FFP by day 5 > 50 mL/kg was selected as the cut-off value. In multivariate conditional logistic regression analysis, FFP by day 5 > 50 mL/kg exhibited an adjusted odds ratio of 5.88 (95% confidence interval: 1.12–41.81, $p = 0.036$). FFP by day 5 > 50 mL/kg may be a risk factor for VT in ELBWIs.

Key words: extremely low birth weight infants, fresh frozen plasma, venous thrombosis

Percutaneously inserted central catheters (PICCs) are often used in neonatal medicine. Venous thrombosis (VT) is a complication associated with PICC use. In a Canadian multi-center study, the incidence of VT in babies admitted to the neonatal intensive care unit (NICU) was 0.24% [1]. In Holland, the incidence of VT in neonates has been reported to be 0.15% [2]. A nationwide postal questionnaire survey of PICC complications in Japanese neonates uncovered only 10 symptomatic thrombosis cases in 5 years [3]. According to an unpublished hospital report, the incidence of VT in babies admitted to the NICU of Osaka Medical Center and the Research Institute for Maternal and Child Health (OMC) in the last 5 years was 1.2%. The incidence of VT in extremely low birth weight infants (ELBWIs) was 6.1%, which is high in comparison to previously reported rates.

Risk factors for VT include indwelling catheters

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(*e.g.*, PICCs), hypercoagulation, polycythemia, hypoperfusion, infection and maternal disease [1, 2, 4–6]. Fresh frozen plasma (FFP) contains coagulation factors and platelet-derived microparticles that participate in clot formation [7]. Excessive FFP may cause an increase in the concentration of these components. Patiroglu *et al.* reported middle cerebral artery thrombosis after FFP and recombinant activated factor VII infusion in a patient with hypofibrinogenemia [8]. Puetz *et al.* reported that 7% of patients treated for coagulopathy or bleeding with FFP infusion develop thrombosis [9]. These 2 reports suggest that FFP may be associated with VT. Although FFP is often used as a volume expander in treating ELBWIs, some guidelines advise against the use of FFP for this purpose [10]. Accordingly, the aim of the present study was to determine whether frequent FFP use is a risk factor for VT in ELBWIs.

Materials and Methods

We performed a matched case-control study on risk factors for VT in ELBWIs born over a period of 5 years (from April 1, 2004 to March 31, 2009) at the NICU of OMC. Group A (n = 13), the case group, consisted of ELBWIs with VT diagnosed by ultrasound or autopsy. Ultrasound was routinely performed at least once a week up to day 28 post birth. An additional test for VT was performed when thrombocytopenia occurred without infection. The searching sites for ultrasound were the superior vena cava (SVC), the inferior vena cava (IVC) and the heart. Group B (n = 34), the control group, consisted of NICU infants matched for gestational age and birth weight. For each case subject, a maximum of three ELBWI controls were selected whose gestational age (every 2 weeks) and birth weight (every 100 g) were matched.

We retrospectively collected perinatal data for the first 5 days of life, using the following parameters general characteristics (sex, gestational age, birth weight, height, head circumference and Apgar score), PICC factors (PICC tip in right atrium and FFP or red cell concentrates in mannitol-adenine-phosphate (MAP) solution through the PICC), hypercoagulation factors (coagulation test at birth and total amount of platelet concentrate and FFP by day 5), polycythemia factors (hemoglobin (Hb) at birth and on day 3 and total amount of MAP by day 5), hypoperfusion factors (enteral feeding and urine output on day 5, rather than supra vena cava or IVC flow), infection factors (immunoglobulin M (IgM) at birth, C-reactive protein (CRP) at birth and on day 3, Blanc classification of placenta and sepsis by day 5), maternal disease factors (pregnancy-induced hypertension, diabetes mellitus, systemic lupus erythematosus, and antiphospholipid syndrome), as well as complete blood count (CBC), pH, pCO_2, base excess (BE) at birth, and total amount of calcium on day 5. Some factors either lacked data or data were outside the range of measurement.

Data are expressed as mean and standard deviation (SD) for continuous variables and frequency and percentage (%) for binary or categorical variables. The matched case-control study was analyzed using conditional logistic regression. We first performed univariate analyses of the variables in conditional logistic regression. We then created a receiver operating characteristic (ROC) curve for cut-off values of continuous parameters such as FFP, and calculated sensitivity, specificity, positive likelihood ratio, and area under curve (AUC). A multivariable model was constructed using selection criteria with a significance level of < 0.2 in univariate analysis, given the small size of the study group. Variables with missing data were eliminated from the model. We performed multivariate conditional logistic regression analysis and calculated adjusted odds ratios (ORs) and their 95% confidence intervals (95% CIs). Apgar scores at 1 and 5 min were similar, so only the 5-min score was used. Two cases with fibrinogen levels of < 50 mg/dL were assigned values of 0. Fibrinogen values were divided by 100 because the indication for FFP is a fibrinogen value of less than 100 mg/dL in some guidelines [10]. The fibrinogen degradation products (FDP) factor was eliminated due to the large number of missing data. A new category was created for gestational age and birth weight because the 2 variables were related and data were sparse. In the appropriate for gestational age (AGA) group, birth weight was > 400 g at 22 weeks, > 500 g at 23 weeks, > 600 g at 24 weeks, > 700 g at 25 weeks, > 800 g at 26 weeks, and > 900 g at 27 weeks. The small for gestational age (SGA) group comprised the remaining cases. BE was divided by 5; when asphyxia was assessed, the cut-off value for BE was -12 [11]. In the present study,

mean BE values were −7.5 and −9.3 in Groups A and B, respectively. The difference between the mean and −12 was approximately 5. Sodium bicarbonate was used when BE was < −5. SGA infants suffer coagulation abnormality and require significant amounts of FFP [12]. Thus, sensitivity analysis was performed after eliminating cases of severe SGA (Wt < −2 SD).

Statistical analysis was performed using JMP 7.0.1 (SAS Institute, Inc., Cary, NC, USA). This study was approved by the OMC ethics committee.

Results

Groups A and B had 13 and 34 cases, respectively. Gestational ages were 24.8 (1.4) weeks and 24.6 (1.0) weeks, and birth weights were 644 (103) g and 657 (97) g in Groups A and B, respectively. FFP by day 5 values were 55 (34) mL/kg and 52 (58) mL/kg in Groups A and B, respectively, and minimum, 25%tile, median, 75%tile and maximum FFP by day 5 were 0, 26, 53, 78, 118 and 0, 11.5, 40.5, 80.6, 305. An FFP by day 5 value of < 51 mL/kg corresponded to sensitivity, specificity, positive likelihood ratio and AUC values of 0.69, 0.65 (1−specificity = 0.35), 0.34 and 0.59, respectively. An FFP value of 50 mL/kg was chosen as the cut-off value. Nine group A cases (69%) and 12 group B cases (35%) had an FFP by day 5 value of > 50 mL/kg (OR: 4.13; 95% CI: 1.05–16.26; $p = 0.052$).

Perinatal factors, laboratory data at birth and laboratory and clinical data on days 3 and 5 are shown in Tables 1–3, respectively, for VT patients and controls. In VT cases, the mean (SD) detection day was 28 (19), and the earliest was day 6. The detection sites were 1 SVC, 11 IVC and 1 pulmonary artery. The methods of detection were 6 thrombocytopenia, 6 routine ultrasound and 1 autopsy. The autopsy case had a sudden SpO_2 decrease and thrombus in the pulmonary artery, which was the cause of death.

The number of cases (blank/outside the measurement range) for each variable were as follows: birth height (2/0), birth head circumference (2/0), prothrombin time (PT) (1/2), activated partial thromboplastin time (APTT) (0/5), fibrinogen (0/2), FDP (10/0), antithrombin III (AT-III) (10/1), IgM (1/23), CRP at birth (0/29) and CRP on day 3 (0/13). The following factors exhibited p-values of < 0.2: sex, Apgar scores at 1 and 5 min, fibrinogen, FDP, FFP by day 5 > 50 mL/kg, day 3 Hb and BE.

Multivariate conditional logistic regression analysis (Table 4) yielded the following adjusted ORs (95% CI): FFP by day 5 > 50 mL/kg, 5.88 (1.12–41.81); and BE/5, 3.95 (1.41–14.39).

SGA/AGA cases in groups A and B were 5/8 and 10/24, respectively. For sensitivity analysis, we eliminated 6 infants with severe SGA (2 VT cases and 4 controls). The mean (SD) of FFP by day 5 (mL/kg) was 54 (26) in Group A and 45 (39) in Group B (mean difference, 9.33 (95%CI −16.23–34.89); p

Table 1 Perinatal factors

	Group A ($n = 13$)	Group B ($n = 34$)	OR or MD (95% CI)	p-value
Female	9 (69%)	13 (38%)	3.63 (0.93–14.24)	0.101
Gestational age (weeks)	24.8 (1.4)	24.6 (1.0)	0.21 (−0.50–0.92)	0.561
Birth weight (g)	644 (103)	657 (97)	−12.79 (−77.74–52.15)	0.693
Birth height (cm)*	31.3 (1.3)	30.5 (2.1)	0.82 (−0.47–2.11)	0.204
Birth head circumference (cm)*	22.2 (1.2)	21.9 (1.0)	0.37 (−0.36–1.10)	0.311
Apgar score at 1 min	4.5 (1.3)	3.7 (1.8)	0.76 (−0.36–1.87)	0.180
Apgar score at 5 min	6.8 (1.5)	5.8 (2.2)	0.98 (−0.35–2.30)	0.146
PIH	1 (7.7%)	0 (0%)	1 (–)	—
DM	0 (0%)	1 (2.9%)	−1 (–)	—
SLE	0 (0%)	0 (0%)	0 (–)	—
APS	0 (0%)	0 (0%)	0 (–)	—
Placental infection, Blanc 3	5 (38%)	18 (53%)	0.56 (0.15–2.05)	0.374

Data are expressed as mean (standard deviation) or case number (percentage).
OR, odds ratio; MD, mean difference; CI, confidence interval; PIH, pregnancy-induced hypertension; DM, diabetes mellitus; SLE, systemic lupus erythematosus; APS, antiphospholipid syndrome.
*Cases with (no data/outside the measurement range): birth height (2/0), birth head circumference (2/0).

Table 2 Laboratory data at birth

	Group A ($n=13$)	Group B ($n=34$)	MD (95% CI)	p-value
WBC ($10^3/\mu L$)	20.1 (21.1)	23.1 (18.9)	−3.01 (−15.84–9.82)	0.639
Hb (g/dL)	13.6 (2.6)	14.0 (2.3)	−0.43 (−1.98–1.12)	0.577
Platelet ($10^3/\mu L$)	211 (60)	221 (84)	−10.45 (−61.90–41.00)	0.684
pH	7.227 (0.109)	7.183 (0.112)	0.044 (−0.029–0.117)	0.230
pCO_2 (mmHg)	51.3 (11.7)	59.3 (35.1)	−7.94 (−28.07–12.19)	0.431
Base Excess (mmol/L)	−7.5 (4.1)	−9.3 (4.1)	1.77 (−0.92–4.46)	0.192
PT (%)*	42 (7.6)	45 (15.1)	−3.05 (−12.30–6.20)	0.509
APTT (s)*	102 (24)	92 (25)	10.68 (−7.12–28.50)	0.233
Fibrinogen (mg/dL)*	130 (93)	194 (155)	−64.38 (−160.83–32.08)	0.185
FDP (μg/mL)*	22.7 (44.5)	7.7 (7.3)	15.02 (−3.26–33.30)	0.104
AT-III (%)*	22.3 (4.2)	24.7 (13.3)	−2.46 (−10.51–5.60)	0.539
CRP (mg/dL)*	0.35 (0.35)	0.34 (0.39)	0.015 (−0.61–0.64)	0.960
IgM (mg/dL)*	7.7 (4.8)	15.2 (23.6)	−7.51 (−29.96–14.95)	0.495

Data are expressed as mean (standard deviation).
MD, mean difference; CI, confidence interval; WBC, white blood cell; PT, prothrombin time; APTT, activated partial thromboplastin time; FDP, fibrinogen degradation products; AT-III, antithrombin III; CRP, C-reactive protein.
*Cases with (no data/outside the measurement range): PT (1/2), APTT (0/5), fibrinogen (0/2), FDP (10/0), AT-III (10/1), CRP (0/29), IgM (1/23).

Table 3 Laboratory and clinical data on days 3 and 5

	Group A ($n=13$)	Group B ($n=34$)	OR or MD (95% CI)	p-value
Hb on day 3 (g/dL)	12.6 (1.3)	11.4 (1.5)	1.19 (0.24–2.13)	0.015
CRP on day 3 (mg/dL)*	0.21 (0.17)	0.44 (0.69)	−0.22 (−0.76–0.31)	0.405
Enteral feeding on day 5 (mL/kg/day)	10.5 (3.5)	15.3 (13.3)	−4.81 (−13.00–3.40)	0.244
Urine output on day 5 (mL/kg/h)	3.7 (1.5)	4.1 (1.2)	−0.39 (−1.23–0.45)	0.352
FFP by day 5 (mL/kg)	55 (34)	52 (58)	3.05 (−31.49–37.59)	0.860
FFP by day 5 > 50 mL/kg	9 (69%)	12 (35%)	4.13 (1.05–16.26)	0.052
MAP by day 5 (mL/kg)	22 (13)	20 (15)	1.92 (−7.50–11.35)	0.682
FFP or MAP through PICC by day 5	7 (54%)	15 (44%)	1.48 (0.41–5.33)	0.550
Platelet concentrate by day 5 (mL/kg)	0 (0)	3.3 (10)	−3.32 (−8.93–2.29)	0.240
PICC tip in right atrium on day 5	2 (15%)	4 (12%)	1.36 (0.22–8.52)	1.000
Calcium by day 5 (mg/kg)	5.2 (1.4)	5.2 (1.5)	−0.0079 (−0.98–0.97)	0.987
Sepsis by day 5	0 (0%)	0 (0%)	0 (–)	—

Data are expressed as mean (standard deviation) or case number (percentage).
OR, odds ratio; MD, mean difference; CI, confidence interval; CRP, C-reactive protein; FFP, fresh frozen plasma; MAP, mannitol-adenine-phosphate; PICC, percutaneously inserted central catheter.
*Cases with (no data/outside the measurement range): CRP on day 3 (0/13).

= 0.465). From the ROC curve, the AUC was the biggest when FFP was 51. We set 50 mL/kg as the cut-off value. OR was 5.33 (95% CI 1.16–24.60; $p=0.036$) in FFP by day 5 > 50 mL/kg.

Discussion

Our clinical hypothesis was that frequent use of FFP may contribute to VT in ELBWIs. One of the risk factors for VT is hypercoagulation [5]. FFP contains several coagulation factors and platelet-derived microparticles [7], and some previous studies have shown that FFP is associated with VT [8, 9]. In the present study, the adjusted OR of FFP by day 5 > 50 mL/kg was high at 5.88. Thus, excessive FFP may be a risk factor for VT.

The mean (SD) values of FFP by day 5 in Groups A and B were 55 (34) mL/kg and 52 (58) mL/kg, respectively. The difference of mean seemed small, but variability was different and the difference may

Table 4 Multivariate conditional logistic regression analysis

	Adjusted OR	95% CI	*p*-value
SGA*	0.86	0.17–3.99	0.850
FFP by day 5 > 50 mL/kg	5.88	1.12–41.81	0.036
Fibrinogen (mg/dL)/100	0.61	0.28–1.12	0.117
Apgar score at 5 min	0.80	0.52–1.20	0.289
Base Excess (mmol/L)/5	3.95	1.41–14.39	0.008
Hb on day 3 (g/dL)	1.05	0.79–1.38	0.741
Female	3.89	0.83–22.11	0.085

CI, confidence interval; SGA, small for gestational age.
*In the appropriate for gestational age (AGA) group, birth weight was > 400 g at 22 weeks, > 500 g at 23 weeks, > 600 g at 24 weeks, > 700 g at 25 weeks, > 800 g at 26 weeks and > 900 g at 27 weeks. The remaining cases were included in the SGA group.

have affected the prognosis. Following sensitivity analysis, the mean (SD) values of FFP by day 5 in Groups A and B were 54 (26) mL/kg and 45 (39) mL/kg, respectively. Thus, the difference in means was slightly higher after sensitivity analysis. A previous report suggests that the recommended FFP volume is 10 mL/kg/dose [10]. Based on this, 50 mL/kg by day 5 corresponds to one dose per day, up to day 5. Accordingly, a high adjusted OR indicates that 50 mL/kg by day 5 may be the critical dose.

Patients in Group A showed signs or symptoms of coagulation perturbations or hemodynamic compromise. Furthermore, asphyxia has been reported to cause coagulation abnormalities [13]. However, there were no differences in pH or pCO_2 at birth in the VT and control patients in the present study. Apgar scores and BE were better in Group A, and enteral feeding and urine output on day 5 were lower in these subjects.

Fibrinogen at birth was lower and APTT and FDP were high in Group A, while PT and AT-III were similar in both groups, possibly indicating that Group A patients were already in a hypercoagulative state at birth and that fibrinogen had been consumed. As a result, a high volume of FFP may have been required. Coagulation abnormalities at birth have been reported in SGA infants [12]. While there were more SGA infants in Group A than in Group B, the adjusted OR of SGA was 0.86 (95% CI: 0.17–3.99). Furthermore, infection can cause increases in fibrinogen [14]. In fact, there were more placental infections in Group B. While the observed fibrinogen increase may have been caused by infection, this contradicts the previous observation that infection is a risk factor for VT [2].

Group A had more females than Group B (adjusted OR: 3.89; 95% CI: 0.83–22.11); however, childhood arterial ischemic stroke and cerebral sinovenous thrombosis occur more often in males than in females [15, 16]. Some causes include increased testosterone, estrogen and prostacyclin. Accordingly, gender cannot be considered a risk factor.

There are a number of limitations worth noting. First, the study included only a small number of VT and control cases because data were collected from a single hospital. Second, a coagulation test on day 5 would have been useful to determine coagulation status. Finally, although ultrasound tests were performed routinely, silent thrombi may have been present in Group B.

In conclusion, the results of the present matched case-control study suggest that FFP by day 5 > 50 mL/kg may be a risk factor for VT in ELBWIs.

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