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Enteral Iron Supplementation, Red Blood Cell Transfusion and Risk of Bronchopulmonary Dysplasia in Very Low Birth Weight Infants

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Abstract

BACKGROUND: Enteral iron supplementation and red blood cell (RBC) transfusions are routinely administered to very low birth weight infants (VLBW), although the potential risks of these exposures have not been adequately quantified. This study evaluated the association between the cumulative dose of enteral iron supplementation, total volume of RBC transfused and risk of bronchopulmonary dysplasia (BPD) in VLBW infants.

STUDY DESIGN AND METHODS: Retrospective, multicenter observational cohort study in Atlanta, GA. Cumulative supplemental enteral iron exposure and total volume of RBC transfused were measured until the age at assessment of BPD. Multivariable generalized linear models used to control for confounding and the reliability of the factors assessed in 1000 bootstrap models.

RESULTS: A total of 598 VLBW infants were studied. In multivariable analysis, a greater cumulative dose of supplemental enteral iron exposure was associated with an increased risk of BPD (adjusted relative risk per 50mg increase: 1.07, 95% CI 1.02–1.11; P=0.002). Similarly, a greater volume of RBC transfused was associated with a higher risk of BPD (adjusted relative risk

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per 20 ml increase: 1.05, 95% CI 1.02–1.07; $P < 0.001$). Both factors were reliably associated with BPD (>50%). Volume of RBC transfused was similar to gestational age in reliability as a risk factor for BPD (present in 100% of models) and was more reliable than mechanical ventilation at 1 week of age.

CONCLUSION: The cumulative dose of supplemental enteral iron exposure and total volume of RBC transfusion are both independently associated with an increased risk of BPD in VLBW infants.

Keywords

blood transfusion; neonate; preterm; chronic lung disease; epidemiology; outcome

INTRODUCTION

Iron is an essential micronutrient for infant brain development and erythropoiesis¹. The American Academy of Pediatrics currently recommends that all preterm infants should receive at least 2 mg/kg per day of enteral iron for the first year of life,² although data from one randomized trial does not support supplementation of more than 2 mg/kg per day for very low birth weight (VLBW) infants³. Over half of VLBW infants will receive additional iron from red blood cell (RBC) transfusions⁴. Although short-term exposure to high-doses of iron have not been associated with oxidative stress,⁵ the cumulative chronic exposure to both enteral iron supplementation and RBC transfusions may potentially place infants at risk of iron overload⁶. Several reports have shown an association between RBC transfusion and a higher risk of BPD^{7,8}, although it is unclear whether the risk is due to the transfusion itself or the likelihood that infants who have worse respiratory disease receive more RBC transfusion. The mechanistic role of iron and oxidative stress in the development of BPD has been postulated,⁹ although no clear relationship has been identified¹⁰. Importantly, none of these studies have evaluated the contribution of iron from enteral supplementation on the risk of BPD.

In this study, we examined the association between cumulative supplemental enteral iron exposure and the risk of BPD in VLBW infants. As a secondary aim, we evaluated the independent association between the cumulative volume of transfused RBCs and the risk of BPD.

METHODS

Study population

We conducted a secondary, retrospective analysis using prospectively collected data from a multicenter observational birth-cohort study investigating the transfusion-transmission of cytomegalovirus in preterm infants (TT-CMV study)^{11,12}. We included VLBW infants born at three neonatal intensive care units (NICUs) in Atlanta, Georgia with coverage provided by separate groups (two with coverage by an academic division and 1 with coverage by a private practice group). All enrolled VLBW infants were followed from birth to 90 days, hospital discharge, transfer to a non-study affiliated hospital, or death (whichever was sooner). Our inclusion criteria were: 1) birth weight > 1500 grams and 2) postnatal age < 5

days. Our exclusion criteria included: 1) infant not expected to survive beyond 7 days of life based on the assessment by the treating neonatologist; 2) severe congenital abnormality; 3) transfusion before enrollment; or 4) maternal refusal to participate. This study was approved by the Institutional Review Board and/or Research Oversight Committees at all participating centers. This study was reported according to the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) statement¹³.

Definitions

All study data were collected in standardized case report forms (CRFs) using DataFax (DF/Net Research, Inc., Seattle, WA). The dose (in mg) and duration (in days and number of doses) of enteral iron supplementation was systematically recorded. Changes in absolute doses of iron and body weight were updated at scheduled assessments each week. The cumulative dose was determined by multiplying the absolute iron dose by the total number of doses with updating as the dose of iron changed up through the age of assessment of BPD (e.g. 36 weeks postmenstrual age). Iron supplementation was given as ferrous sulfate or multivitamin with iron with initiation and dosing adjustments at the discretion of the treating team. No infants received parenteral iron dextran or iron sucrose. Each RBC transfusion, including the volume transfused, was recorded. All RBC transfusions administered were leukoreduced, citrate phosphate dextrose adenine (CPDA-1) units from cytomegalovirus-seronegative donors. The primary outcome was any severity of BPD, defined according to the consensus definition by the National Institutes of Health¹⁴. Additional analyses were limited to only moderate-to-severe BPD. Study exposures were measured up until the postnatal age at assessment of BPD. Baseline illness severity was ascertained using the score for neonatal acute physiology (SNAP)¹⁵. Race and ethnicity were determined by maternal self-report using options defined by federally funded study guidelines.

Statistical Analysis

The sample size was fixed based on enrollment into the primary study. Any missing data was quantified by reporting of denominators or in footnotes. Relative risks were calculated to measure the degree of association between individual risk factors and BPD by fitting a modified Poisson regression model using a robust error variance¹⁶. We used SAS GENMOD Procedure (SAS Institute, Version 9.4, Cary, North Carolina) to implement the regression model as previously described¹⁶.

Two-stage modeling was used to control for confounding. In the first stage, longitudinal weight measurements were modeled using a random coefficients model to estimate infant-specific weight gain¹⁷, which allowed us to account for doses of iron exposure specific to an infant's weight over time. This was used to avoid bias from higher iron doses given to infants with greater birth weight and because the study did not record weight each study day. Weight gain was estimated using a mixed effects model specifying that weight follows a linear regression over time (between days 7–90), with random slope and intercept for each infant. The infant-specific coefficient deviations (random effects) were added to the overall coefficient (fixed effects) to obtain an infant-specific weight gain, which was then included in multivariable modified Poisson models in the second stage of modeling. Control for confounding was performed based on available knowledge of factors thought to be

associated with the risk of BPD or respiratory illness severity that were measured within the 1st week of life¹⁸. We included adjustment for total volume of RBCs transfused (also a secondary exposure of interest), gestational age, small for gestational age, receipt of any antenatal steroids, illness severity (SNAP), positive airway pressure on the day of birth (including both continuous positive airway pressure and mechanical ventilation), surfactant treatment on the day of birth, caffeine therapy on the day of birth, mechanical ventilation at 1 week of age, days until birth weight regained, weight gain, ever fed breast milk and clinical center. We used the same model to evaluate the relationship between the rate of iron exposure and BPD by replacing the variable for cumulative supplemental iron exposure with median rate of iron exposure (cumulative iron dose, in mg, divided by time-at risk, measured from birth up to the age of assessment of BPD, in days). Next, we evaluated the dose exposure-outcome relationships by replacing the continuous exposure variable in the above model with parameters for quartiles of cumulative enteral iron exposure. We also assessed a model that only included variables reliably associated with BPD that appeared in at least 50% of 1000 bootstrapped models. Each model fit was evaluated using a modified Akaike Information criterion goodness-of-fit statistic¹⁹. In addition, we evaluated for heterogeneity in the relationship between enteral iron exposure and BPD among infants fed any breast milk compared to no breast milk using interaction terms in a multivariable model and also evaluated the outcome of moderate-to-severe BPD. To avoid overfitting, the latter two models were reduced and included adjustment for volume of RBC transfusion, weight gain (with patient-specific intercept used as a measure of birth weight), days until birth weight regained, receipt of antenatal steroids, illness severity (SNAP), positive airway pressure on the day of birth, surfactant treatment on the day of birth, caffeine therapy on the day of birth, ever fed breast milk and center. A two-sided P value of <0.05 indicated statistical significance.

RESULTS

We evaluated a total of 598 VLBW infants. The median birth weight and gestational age of the cohort was 1024 grams (interquartile range [IQR] 790–1233) and 28 weeks (IQR 26–30), respectively (Table 1). Two infants received erythropoiesis-stimulating agents. A total of 495 (82.8%) infants received enteral iron supplementation. The cumulative supplemental enteral iron dose was 69 mg (18–186) overall and 101 mg (IQR 40–221) among those receiving supplementation. The median duration of iron supplementation was 29 days (IQR 17–41). The median rate of enteral iron supplementation was 1.2 mg/day (IQR 0.4–3.6) overall and 2.0 mg/day (IQR 0.8–4.0) among those infants receiving supplementation. The majority of infants were fed breast milk (87.0%). Baseline characteristics among only infants receiving enteral iron supplementation were similar to the full cohort of infants (Supplementary Table 1).

BPD of any severity developed in 240 infants (40.1%) and moderate-to-severe BPD occurred in 136 infants (22.7%). The median dose of supplemental iron exposure among infants with BPD was 70 mg (IQR 24–194), compared to 59 mg (IQR 11–172) among those without BPD (Table 2). Infants with BPD received more RBC transfusion (median 56ml vs. 0 ml), had lower daily weight gain (21 g/d vs 27 g/d), received more surfactant (63% vs. 27%) and had higher SNAP scores on the day of birth (12 vs. 9). The incidence of any

severity of BPD among RBC transfused infants was 64% compared to 11% among infants not RBC transfused (Supplementary Table 2).

In multivariable analysis, a greater cumulative dose of enteral iron exposure was associated with a higher risk of BPD (adjusted relative risk [RR] per 50 mg increase: 1.07, 95% CI 1.02–1.11; $P=0.002$) (Table 3). Similarly, a greater total volume of RBC transfusion was independently associated with a higher risk of BPD (adjusted RR per 20 ml increase in RBCs transfused: 1.05, 95% CI 1.02–1.07; $P<0.001$). Both of these exposures were reliably associated with BPD (present in 50% of 1000 bootstrap models). In regression modelling, infants with BPD had slower weight gain compared to those without BPD (21.4 ± 0.4 g/d vs. 27.3 ± 0.4 g/d; $P<0.0001$) and lower weight at 30 days of age (1260 ± 21 g vs 1726 ± 17 g; $P<0.0001$). The association between enteral iron exposure and BPD was similar when only including covariates that were reliably associated with BPD (Table 4). When evaluating the outcome of moderate-to-severe BPD, we found similar point estimates of the association with enteral iron exposure as prior analyses, although the association was not statistically significant (adjusted RR per 50 mg increase 1.05; 95% CI 0.97–1.14). In this analysis, RBC transfusion was associated with moderate-to-severe BPD (adjusted RR per 20 ml increase 1.14; 95% CI 1.10–1.19). Infants in the highest quartile of iron exposure had a 1.8 times (95% CI 1.34–2.47) greater risk of BPD compared to infants in the lowest quartile (Figure 1). Findings were consistent, although diminished in magnitude, when limiting the analysis to only infants receiving enteral iron supplementation (Supplementary Figure 1). When evaluating the rate, instead of dose, of iron exposure in the primary model, with adjustment for other confounders, to account for potential differences in the time at risk iron supplementation, we found no significant association between each 1 mg/day increase in iron and risk of BPD (adjusted RR 1.04; 95% CI 0.99–1.10). In subgroup analyses by feeding type, no difference in the relationship between cumulative enteral iron exposure and BPD was detected among those infants fed any breastmilk (RR per 50 mg increase 1.05; 95% CI 1.01–1.09) and those infants not fed breast milk (1.27; 95% CI 1.13–1.42; interaction $P=0.06$).

DISCUSSION

In this observational study, we demonstrate that a greater cumulative dose of supplemental enteral iron exposure is associated with a higher risk of BPD in VLBW infants. To our knowledge, this is the first study reporting this finding, which is hypothesis generating. Potential biological explanations underlying the observed relationship between enteral iron exposure and BPD were not assessed in this epidemiologic study. In neonatal murine studies, enteral iron intake has been associated with increases in oxidative stress, although the relevance of this murine model to preterm infants may be limited²⁰. In two prior studies in preterm infants, no association between short-term high dose enteral iron supplementation and oxidative stress markers in preterm infants was found. In a study of 16 infants, short term enteral iron supplementation beginning at a postmenstrual age of 30 weeks in doses ranging from 3 to 12 mg/kg/day with treatment for 2 to 3 weeks had no effect on markers of oxidative injury such as urine and blood isoprostanes²¹. In another study, 21 healthy VLBW infants were given high-doses (18 mg/daily) of iron for 1 week and no changes in urine 8-isoprostane or plasma total hydroperoxides, both markers of oxidant injury, were observed

within 1 week of treatment⁵. Both of these studies suggest that short-term exposure to high doses of enteral iron are unlikely to mediate oxidant injury in preterm infants. However, neither study evaluated the effects of long-term cumulative supplemental exposure in which the risk of iron overload may be increased⁶. As this was an epidemiologic study, we did not obtain serum markers of iron status or measures of oxidative injury, which prevented us from determining if these factors have any role in mediating the relationship between cumulative supplemental enteral iron exposure and BPD or whether any enrolled patient exhibited any laboratory findings to support iron overload.

We assessed for heterogeneity in the relationship between enteral iron exposure and BPD depending on the type of feeding. Two recent studies have demonstrated formula to be a risk factor for BPD, while breast milk to be protective in a dose-dependent manner^{22,23}. We found potential differences in the relationship between enteral iron exposure and BPD depending on if an infant received any breast milk feeding. However, these subgroup analyses should be viewed cautiously as they were exploratory.

We found an association between a greater total volume of RBCs transfused and higher risk of BPD. This finding has been previously reported in prior observational studies, including a prospective observational study in the UK²⁴ and more recent single-center observational studies from China^{7,25}. A study from Korea found similar associations between volume and number of RBC transfusions and BPD in a univariable analysis, but no association with ferritin levels or average iron supplementation during hospitalization and BPD²⁶. By contrast, a recent prospective study from Japan found hyperferritinemia (ferritin 500ng/mL) to be associated with a higher risk of BPD²⁷. However, ferritin is an acute phase reactant and, as BPD has an associated inflammatory component, the relationship may reflect inflammation that influences the risk of BPD instead of iron status. RBC transfusion has been shown to increase non-transferrin bound iron in preterm infants, which exists partly in the ferrous form and may increase the generation of reactive oxygen species²⁸. In addition, in the setting of hyperoxia, iron in the redox-active state may accumulate in the lung without raising serum ferritin in mice²⁹. However, the relationship between free iron and oxidative injury measured by lipid peroxidation is not clear¹⁰ and the relationship between oxidative stress and BPD may depend on antioxidant capacity^{30,31}. Furthermore, a systematic-review and meta-analysis of randomized trials comparing liberal vs. restrictive RBC transfusion approaches have not demonstrated a higher risk of BPD among preterm infants that receive more liberal RBC transfusions compared to more restrictive RBC transfusions (relative risk 0.99; 95% CI 0.92–1.06)³². Therefore, the association between RBC transfusion and BPD may be confounded by indication for RBC transfusion, with the decision to transfuse RBCs dependent on the level of respiratory support an infant is receiving.

The strengths of our study include the systematic measurement of iron exposure from enteral supplementation and RBC transfusion and the systematic assessment of BPD. In addition, we were able to measure and adjust for multiple potential confounders, including baseline illness severity and respiratory support at 1 week of age, a major determinant of BPD. We also assessed the interaction between the type of enteral feeding and the risk of BPD. Finally, we were able to account for changes in weight gain over time and, therefore, could

control for a weight-based dosing of iron given dose changes of iron often do not occur on a daily basis with changes in weight and the effective weight-based dose of iron could change as an infant gains weight.

Our study has several limitations. We could not measure all sources of iron exposure, such as enteral iron from routine feeding due to the challenges of daily measurement of all enteral feeding amounts. However, we evaluated the interaction between feeding type and the relationship between iron exposure and BPD. Furthermore, iron supplementation was not standardized. While this allowed us to assess the exposure-outcome relationship across variable approaches to enteral supplementation, the external validity of our findings to centers with differing supplementation practices or routine use of erythropoiesis stimulating agents may be limited. We acknowledge the limitations of any particular definition of BPD and the possibility that our study definition may have led to a higher incidence of BPD than if we had used alternative definitions³³, evidenced by the results from our analysis evaluating moderate-to-severe BPD. However, we used a validated definition of BPD¹⁴ that has been shown to identify infants with higher risk of adverse pulmonary outcomes in early childhood³⁴ and the fewer events of moderate-to-severe BPD could have limited statistical estimation. In addition, we did not evaluate a composite outcome of death or BPD because overall mortality was low in this cohort and infants who died may not have been eligible for enteral iron supplementation, potentially leading to a biased assessment of the outcome of death or BPD. It is possible, but unlikely, that our results were biased by reverse causation, as most infants initiated enteral iron supplementation in the first month of life, before BPD could be diagnosed and we did not include any iron exposure after 36 weeks PMA. Finally, we could not account for all potential confounding variables, including early oxygen exposure, and, therefore, our findings are intended to be hypothesis generating.

In conclusion, higher amounts of cumulative supplemental enteral iron exposure may potentially be associated with an increased risk of BPD in VLBW infants. Therefore, additional studies are needed to compare the risks and benefits of routine enteral iron supplementation, including the optimal dose and timing of initiation, given iron is an essential micronutrient for neurodevelopment and hematopoiesis. Until such studies are conducted, we believe it is reasonable to follow the recommendation of the American Academy of Pediatrics and provide 2 mg/kg/day of supplemental iron to human milk fed infants starting by 1 month of age², but suggest caution with higher doses of iron or the routine supplementation of iron to formula-fed preterm infants. In addition, the strength of association between volume of RBC transfusion and risk of BPD in our study supports the need for further studies into the potential role of RBC transfusion in lung injury in preterm infants. Ongoing randomized trials comparing liberal and restrictive RBC transfusion thresholds will provide additional data on the effect of RBC transfusion on the risk of BPD in extremely preterm infants. Until these trial data are available, we recommend transfusing RBCs within the approaches, including specific hemoglobin threshold triggers that have been studied in randomized trials to date³⁵.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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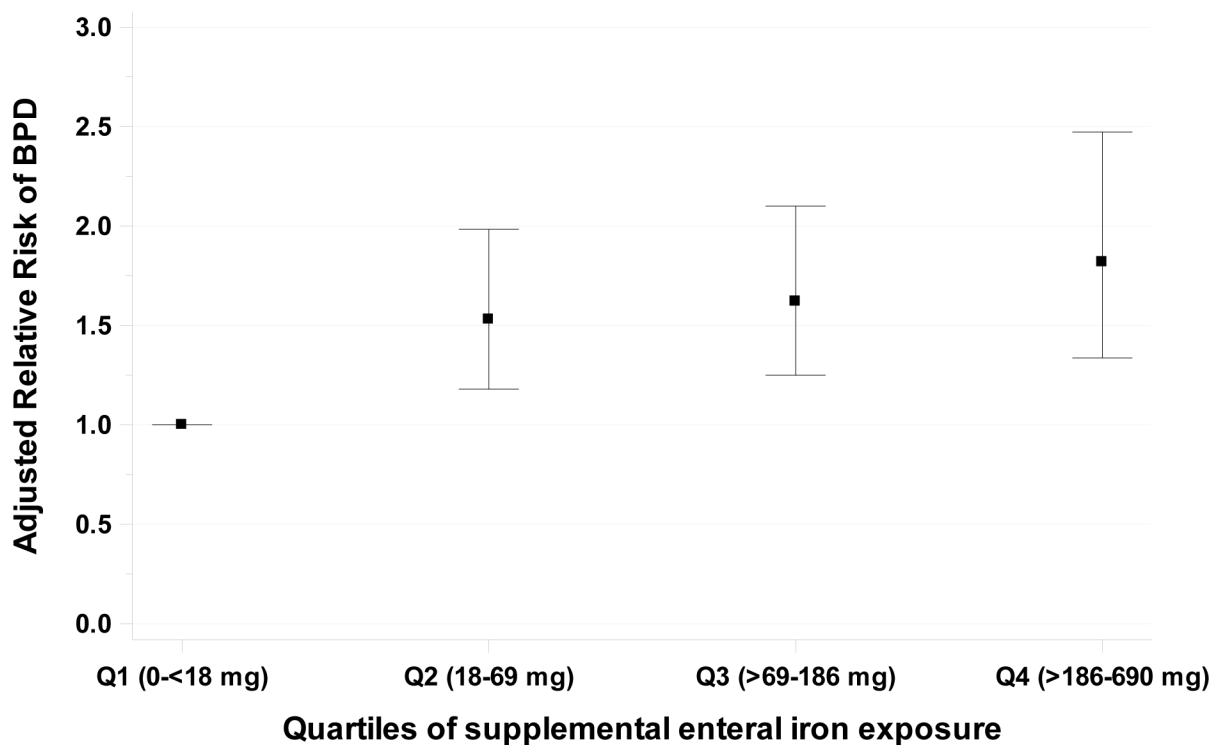
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Enteral iron quartile (mg of enteral iron exposure)	$\beta \pm SE$	Adjusted relative risk of BPD (95% CI)	P
Q1 (0-<18 mg)		Reference	
Q2 (18-69 mg)	0.4252 \pm 0.1325	1.53 (1.18-1.98)	0.0013
Q3 (>69-186 mg)	0.4824 \pm 0.1324	1.62 (1.25-2.10)	0.0003
Q4 (>186-690 mg)	0.5978 \pm 0.1569	1.82 (1.34-2.47)	0.0001

Figure 1. Association between enteral iron supplementation and BPD by quartiles of iron exposure.

Relative risk estimates derived from multivariable model that includes 592 infants, with the 1st (lowest) quartile as reference. Error bars reflect 95% confidence interval.

Table 1.

Characteristics of the study cohort

Patient characteristics	All infants (N=598)
Birth weight, median g (IQR)	1024 (790-1233)
Gestational age, median wk (IQR)	28 (26-30)
Small for gestational age	138/598 (23.1%)
Male gender	302/598 (50.5%)
Race	
Black	346/598 (57.9%)
White	200/598 (33.4%)
Other ^a	52/598 (8.7%)
Antenatal steroids given	501/598 (83.8%)
SNAP, median (IQR)	11 (6-14)
Positive airway pressure on DOB	532/598 (89.0%)
Surfactant on DOB	248/598 (41.5%)
Caffeine on DOB	313/598 (52.3%)
Days until birth weight regained, median d (IQR)	7 (5-10)
Ever fed breast milk	520/598 (87.0%)
Receipt of an erythropoiesis stimulating agent	2 (0.3%)
Total enteral iron exposure, median mg (IQR) ^{b,c}	69 (18-186)
Rate of enteral iron supplementation, median mg/day (IQR) ^{b,c}	1.2 (0.4, 3.6)
Receipt of any enteral iron supplementation	495/598 (82.8%)
Total enteral iron exposure among infants receiving supplementation, median mg (IQR) ^{c,d}	101 (40-221)
Duration of supplementation, median d (IQR) ^{c,d}	29 (17-41)
Death during hospitalization	32/598 (5.4%)

Values are n (%), unless indicated otherwise. Abbreviations: IQR, interquartile range; DOB, day of birth; BPD, bronchopulmonary dysplasia.

^aIndicates Asian, American Indian, Alaska Native, Native Hawaiian or other Pacific Islander, more than one race or other unidentified race.

^bAmong all 598 infants, including those who did not receive iron supplementation.

^cEvaluated from birth until postnatal age of BPD assessment.

^dAmong 495 infants receiving enteral iron supplementation.

Table 2.

Univariable Analysis of Risk factors for BPD (n=598)

Factor	BPD (n=240)	No BPD (n=358)	Relative Risk (95% CI)	Relative risk per
Supplemental enteral iron, median mg (IQR)	70 (24-194)	59 (11-172)	1.02 (0.98, 1.06)	50 mg increase
Age at first enteral iron dose, median d (IQR) ^a	24 (15, 42)	14 (11, 25)	1.16 (1.12, 1.19)	Per 7 day increase
Volume of RBC transfused, median ml (IQR)	56 (24-105)	0 (0-22)	1.14 (1.11, 1.18)	20 ml increase
Gestational age at birth, median weeks (IQR)	26 (25, 27)	29 (28, 30)	0.73 (0.71, 0.76)	1 week increase
Small for gestational age, n (%)	44 (18%)	94 (26%)	0.74 (0.57, 0.98)	yes vs. no
Birth weight, median g (IQR)	800 (682-979)	1158 (983-1325)	0.78 (0.75, 0.81)	100 g increase
Received 1 dose of antenatal steroids, n (%)	200 (83%)	301 (84%)	0.98 (0.75, 1.27)	yes vs. no
Score for Neonatal Acute Physiology, median (IQR)	12 (10-15)	9 (4-13)	1.07 (1.05, 1.08)	1 unit increase
Positive airway pressure on DOB, n (%)	222 (93%)	310 (87%)	1.53 (1.02, 2.30)	yes vs. no
Surfactant on DOB, n (%)	151 (63%)	97 (27%)	2.39 (1.95, 2.94)	yes vs. no
Caffeine on DOB, n (%)	136 (57%)	177 (49%)	1.19 (0.98, 1.45)	yes vs. no
Mechanical ventilation at 1 week of age, n (%)	194 (81%)	120 (34%)	3.81 (2.89, 5.04)	yes vs. no
Days until birth weight regained, median (IQR) ^c	8 (5-11)	7 (5-10)	1.15 (1.05, 1.25)	Per 7 day increase
Mean weight gain, g/day (SE) ^b	21.4 (0.4)	27.3 (0.4)	0.90 (0.88, 0.91)	1 g/d increase
Ever fed breast milk, n (%)	215 (90%)	309 (86%)	1.21 (0.87, 1.70)	yes vs. no

Abbreviations: BPD, bronchopulmonary dysplasia; IQR, interquartile range; DOB, day of birth; SE, standard error.

^a Among the 495 infants receiving enteral iron supplementation.

^b Results from mixed-effects linear model, with random intercept and slope for each infant.

^c N=594. Four infants died before birth weight regained.

Table 3.

Multivariable Analysis of Risk Factors Associated with BPD (n=592)

Risk Factor^a	Estimated β (SE)	Relative Risk of BPD (95% CI)	P	Reliability (%)^b
<i>Primary exposure</i>				
Enteral iron (per 50 mg increase) ^c	0.0013 (0.0004)	1.07 (1.02, 1.11)	0.002	69
<i>Secondary exposure</i>				
RBC transfusion (per 20 ml increase) ^c	0.0022 (0.0006)	1.05 (1.02, 1.07)	<0.001	100
<i>Other factors in multivariable model</i>				
Gestational age (per 1 week increase)	-0.1633 (0.0350)	0.85 (0.79, 0.91)	<0.001	100
Small for gestational age	0.2062 (0.1294)	1.23 (0.95, 1.58)	0.111	48
Antenatal steroids	0.0455 (0.1029)	1.05 (0.86, 1.28)	0.658	4
SNAP (per 1 point increase)	0.0109 (0.0088)	1.01 (0.99, 1.03)	0.215	6
Positive airway pressure on DOB	-0.1495 (0.1772)	0.86 (0.61, 1.22)	0.399	6
Surfactant on DOB	0.1970 (0.0963)	1.22 (1.01, 1.47)	0.041	54
Caffeine on DOB	0.0721 (0.1091)	1.07 (0.87, 1.33)	0.509	6
Mechanical ventilation at 1 week of age	0.5727 (0.1582)	1.77 (1.30, 2.42)	<0.001	90
Days until birth weight regained (per 7 day increase)	0.0053 (0.0058)	1.04 (0.96, 1.12)	0.358	30
Weight gain (g/day)	-0.0218 (0.0121)	0.98 (0.96, 1.00)	0.072	18
Ever fed breast milk	0.1763 (0.1449)	1.19 (0.90, 1.58)	0.224	14

Abbreviations: BPD, bronchopulmonary dysplasia; SE, standard error; CI, confidence interval. RBC, red blood cell; SNAP, Score for Neonatal Acute Physiology; DOB, day of birth.

^aModel fitted to outcome of BPD (n=240 events) and includes adjustment for center (not shown). Reference category is no, unless indicated otherwise. Four infants died before birth weight regained and 2 infants with missing weight gain data were excluded from the analysis.

^bPercentage of time risk factors appears in 1000 bootstrap models (factors > 50% are reliable).

^cCumulative exposure was evaluated from birth until postnatal age of BPD assessment.

Table 4.

Multivariable Analysis of Risk Factors for BPD with Reliability 50% (n=592)

Risk Factor ^a	Estimated β (SE)	Relative Risk of BPD (95% CI)	P	Reliability (%) ^b
<i>Primary exposure</i>				
Enteral iron (per 50 mg increase) ^c	0.0010 (0.0004)	1.05 (1.01, 1.09)	0.01	69
<i>Secondary exposure</i>				
RBC transfusion (per 20 ml increase) ^c	0.0025 (0.0006)	1.05 (1.03, 1.08)	<0.001	100
<i>Other factors in multivariable model</i>				
Gestational age (per 1 week increase)	-0.1853 (0.0288)	0.83 (0.79, 0.88)	<0.001	100
Surfactant on DOB (yes vs. no)	0.2396 (0.0951)	1.27 (1.05, 1.53)	0.01	54
Mechanical ventilation at 1 week of age (yes vs. no)	0.6262 (0.1551)	1.87 (1.38, 2.53)	<0.001	90

Abbreviations: BPD, bronchopulmonary dysplasia; SE, standard error; CI, confidence interval. RBC, red blood cell; DOB, day of birth.

^aModel fitted to outcome of BPD (n=240 events) and includes adjustment for center (not shown, P=0.0005). Four infants died before birth weight regained and 2 infants with missing weight gain data were excluded from the analysis.

^bPercentage of time risk factors appears in 1000 bootstrap models (factors 50% are reliable).

^cCumulative exposure was evaluated from birth until postnatal age of BPD assessment.