SUPPLEMENTAL INFORMATION

Supplement to: Patel RM, Knezevic A, Shenvi N, et al. Association of Red Blood Cell Transfusion, Anemia and Necrotizing Enterocolitis in Very Low Birth Weight Infants.

Abbreviations: NEC, necrotizing enterocolitis; RBC, red blood cell; VLBW, very low birth weight.

eTable 1. Baseline characteristics by NEC and RBC exposure

Baseline characteristics	RBC exposed N=319	RBC unexposed N=279	NEC N=44	No NEC N=554
Gestational age in weeks, mean (SD)	26.4 (2.1)	29.5 (2.2)	26.6 (2.3)	28.0 (2.6)
Birth weight in grams, mean (SD)	864 (240)	1189 (193)	820 (250)	1031 (269)
Male gender	163 (51.1%)	139 (49.8%)	20 (45.5%)	282 (50.9%)
Race				
Black	199 (62.4%)	147 (52.7%)	31 (70.5%)	315 (56.9%)
White	96 (30.1%)	104 (37.3%)	11 (25%)	189 (34.1%)
Asian	9 (2.8%)	16 (5.7%)	2 (4.5%)	23 (4.2%)
More than one race	12 (3.8%)	11 (3.9%)	0	23 (4.2%)
Other[a]	3 (0.9%)	1 (0.4%)	0	4 (0.7%)
Hispanic ethnicity	27 (8.5%)	23 (8.2%)	1 (2.3%)	49 (8.8%)
Singleton birth	231 (72.4%)	180 (64.5%)	34 (77.3%)	377 (68.1%)
Small for gestational age[b]	62 (19.4%)	76 (27.2%)	14 (31.8%)	124 (22.4%)
Born outside of study hospital	5 (1.6%)	2 (0.7%)	0	7 (1.3%)
5 minute Apgar score, median (IQR)[c]	7 (6-9)	9 (8-9)	7.5 (6-8.5)	8 (7-9)
SNAP score, mean (SD)[d]	12.4 (4.6)	8.1 (5.3)	11.5 (5.1)	10.3 (5.4)
Rupture of membranes >18 hours	80 (25.1%)	44 (15.8%)	5 (11.4%)	119 (21.5%)
Chorioamnionitis (clinical or histological)	73 (22.9%)	15 (5.4%)	9 (20.5%)	79 (14.3%)
Caesarean delivery	236 (74.0%)	227 (81.4%)	33 (75.0%)	430 (77.6%)
Receipt of ≥1 dose of antenatal steroids	261 (81.8%)	239 (86.0%)	33 (75.0%)	467 (84.5%)
Hemoglobin on day of birth in g/dL, mean (SD)	14.0 (2.4)	16.4 (2.2)	14.5 (2.2)	15.2 (2.7)
Highest level of respiratory support on day of birth				
Mechanical ventilation	200 (62.7%)	63 (22.6%)	23 (52.3%)	240 (43.3%)
CPAP	92 (28.8%)	177 (63.4%)	16 (36.4%)	253 (45.7%)
None of the above	27 (8.5%)	39 (14.0%)	5 (11.3%)	61 (11.0%)

Abbreviations: NEC, necrotizing enterocolitis; RBC, red blood cell; SD, standard deviation; IQR, interquartile range; SNAP, score for neonatal acute physiology; CPAP, continuous positive airway pressure.

Unless otherwise noted, variables reported no. (%).

[a]American Indian, Alaska Native, Native Hawaiian or Other Pacific Islander, or other unidentified race.

[b]Birth weight <10th percentile for gestational age using intrauterine growth curves by Olsen et al.[1]

[c]5 minute APGAR score is missing for two infants born outside of study hospital.

[d]Scores measured on day of birth and range from 0 to 42, with higher scores indicating greater illness severity.

eTable 2. Clinical characteristics by NEC and RBC exposure

Clinical characteristics	RBC exposed N=319	RBC unexposed N=279	NEC N=44	No NEC N=554
Death	30 (9.4%)	2 (0.7%)	13 (30.0%)	19 (3.4%)
Receipt of surfactant therapy in 1st week of life	220 (69.0%)	78 (28.0%)	30 (68.2%)	268 (48.4%)
Mechanical ventilation in 1st week of life	230 (72.1%)	74 (26.5%)	26 (59.0%)	278 (50.2%)
Patent ductus arteriosus	118 (37.0%)	36 (12.9%)	17 (38.6%)	137 (24.7%)
Intraventricular hemorrhage (≥grade II)	64 (20.0%)	16 (5.7%)	9 (20.5%)	71 (12.8%)
Positive blood culture	58 (18.2%)	4 (1.4%)	12 (27.3%)	50 (9.0%)
Receipt of any antibiotics in 1st 10 days of life	312 (97.8%)	239 (85.7%)	43 (97.7%)	508 (91.7%)
Duration of antibiotic therapy in 1st 10 days of life in days, median (IQR)	6 (3-8)	3 (2-4)	6.5 (3.5-8)	3 (3-7)
Ever fed breast milk	270 (84.6%)	250 (89.6%)	43 (97.7%)	477 (86.1%)
Age at first feed in days, median (IQR)	3 (2-5)	2 (1-2)	3 (2-5)	2 (2-4)
Maximum storage age of transfused RBC in days, median (IQR)[a]	12 (9-17)	-	12 (8-15)	12 (9-17)
Maximum storage age after irradiation of RBC in days, median (IQR)[a,b]	3 (0-7)	-	4 (0-9)	3 (0-7)

Abbreviations: NEC, necrotizing enterocolitis; RBC, red blood cell; IQR, interquartile range.

Unless otherwise noted, variables reported as no. (%) and characteristics assessed throughout the entire study period from birth to 90 days, hospital discharge, transfer to a non-study affiliated hospital or death.

[a]For 319 RBC transfused infants.

[b]Unavailable for 2 infants, each transfused with RBCs once with storage age after irradiation unknown.

eTable 3. NEC diagnosis, surgical intervention and Bell's staging

		Number of episodes	Number of infants	Number of infants who underwent surgical intervention
Clinical NEC diagnosis		53	48	18
Bell's stage	I	5	4	0
	II	28	24	1[a]
	III	20	20	17

Abbreviation: NEC, necrotizing enterocolitis.

During prospective data collection, 48 infants with a clinical diagnosis of NEC were identified (53 total episodes of NEC: 4 infants had 2 episodes and 1 infant had 3 episodes). Of the 48 infants, 18 were confirmed to have surgical intervention for NEC. All 53 NEC episodes were adjudicated and staged according to Bell's staging criteria. 5 events (in 4 infants) did not meet the criteria for NEC Bell's Stage II (confirmed NEC) or greater. 44 infants who were confirmed to have NEC Bell's Stage II or greater were included in the analysis. Of the 18 surgical NEC cases, 17 were determined to be Bell's stage III.

[a]1 infant who underwent surgery had a negative exploratory laparotomy and was determined to be Bell's stage II.

eTable 4. Cumulative incidence of NEC and mortality by baseline and clinical characteristics (N=598)

			NEC No. events/No. at risk, cumulative incidence (95% CI)			Mortality No. events/No. at risk, cumulative incidence (95% CI)	
		N	4 weeks	8 weeks	P	8 weeks	P
Birth cohort		598	22/542, 3.7% (2.4, 5.7)	42/329, 7.7% (5.7, 10.3)	-	19/403, 3.3% (2.0, 5.0)	-
NEC risk factors		**N**	**4 weeks**	**8 weeks**	**P**	**8 weeks**	**P**
Gestational age[a]	< 28wk	275	15/248, 5.4% (3.4, 8.5)	29/224, 10.7% (7.5, 15.3)	0.008	16/232, 5.8% (3.5, 9.6)	0.004
	≥ 28wk	323	7/294, 2.3% (1.3, 4.0)	13/178, 4.6% (2.7, 7.8)		3/286, 1.0% (0.3, 3.2)	
Birth weight	<1000g	283	17/251, 5.8% (3.7, 9.2)	32/210, 11.5% (8.3, 16.0)	<0.001	19/225, 6.7% (4.2, 10.7)	-
	1000-1500g	315	5/293, 1.8% (0.9, 3.4)	10/184, 3.6% (2.0, 6.5)		0[b]	
Race	Black	346	14/312, 4.5% (2.9, 7.1)	30/210, 9.3% (6.4, 13.7)	0.15	11/227, 3.3% (1.8, 5.9)	0.85
	White	200	8/183, 2.8% (1.3, 5.7)	11/114, 5.8% (3.1, 10.6)		7/178, 3.6% (1.7, 7.7)	
	Other	52	0/52, 1.9% (0.5, 7.7)	1/40, 3.9% (0.9, 17.4)		1/50, 2.0% (0.3, 14.0)	
5 minute Apgar score[c]	< 5	56	3/51, 5.9% (2.9, 12.2)	7/44, 12.1% (5.7, 25.6)	0.19	4/40, 7.2% (2.9, 17.4)	0.09
	≥ 5	540	19/489, 3.5% (2.3, 5.3)	35/289, 7.2% (5.3, 9.9)		15/479, 2.9% (1.8, 4.7)	
Mortality risk factors		**N**	**4 weeks**	**8 weeks**	**P**	**8 weeks**	**P**
Gender	Male	302	10/268, 3.4% (2.0, 5.6)	23/157, 6.9% (4.5, 10.5)	0.50	10/261, 3.1% (1.6, 5.8)	0.76
	Female	296	12/274, 4.1% (2.5, 6.7)	19/211, 8.4% (5.7, 12.4)		9/209, 3.5% (1.9, 6.6)	
SNAP score[d]	< 11	288	8/262, 3.4% (2.0, 5.8)	17/160, 6.9% (4.6, 10.4)	0.54	3/171, 1.1% (0.3, 3.5)	0.01
	≥ 11	310	14/280, 4.0% (2.3, 7.1)	25/203, 8.3% (5.5, 12.4)		16/275, 5.2% (3.1, 8.7)	
Multiple birth	No	411	19/364, 4.2% (2.8, 6.4)	33/219, 8.7% (6.2, 12.2)	0.17	15/350, 3.8% (2.3, 6.3)	0.32
	Yes	187	3/178, 2.6% (1.3, 5.3)	9/120, 5.4% (3.0, 9.7)		4/136, 2.2% (0.8, 5.9)	
Receipt of ≥1 dose of antenatal steroids	No	97	4/84, 5.8% (3.0, 11.1)	11/62, 11.8% (7.2, 19.4)	0.10	4/86, 4.3% (1.7, 11.1)	0.55
	Yes	501	18/458, 3.3% (2.1, 5.3)	31/278, 6.9% (5.0, 9.5)		15/336, 3.1% (1.7, 5.5)	

Abbreviations: NEC, necrotizing enterocolitis; CI, confidence interval; SNAP, score for neonatal acute physiology.

Competing risks: 44 infants with NEC and 32 deaths total; 13 infants with NEC died. 44 events used to estimate cumulative incidence for NEC and 19 deaths used to estimate cumulative incidence for mortality. Estimates obtained from the cumulative incidence function and updated as additional events occur in each risk factor group. Therefore, the cumulative incidence may not match the simple fraction of number of events/number at risk at any given time point.

[a]Categorized by above or below median.

[b]All observed deaths occurred in the <1000g group.

[c]5 minute Apgar score is missing for two infants born outside of study hospital.

[d]Scores measured on day of birth and range from 0 to 42, with higher scores indicating greater illness severity.

eTable 5. Characteristics of RBC transfusion practices by study centers

RBC transfusion rates	Center		
	A	B	C
Patients receiving ≥ 1 RBC transfusion	76/113 (67.3%)	105/147 (71.4%)	138/338 (40.8%)
Transfusion rate (per 30 infant days)[a]	1.58	1.66	0.70
95% CI	1.41 - 1.75	1.51 - 1.82	0.63 - 0.76
Hemoglobin values measured within 24 hours before a RBC transfusion			
No. of Hb measurements[b]	286	435	450
Central tendency of Hb (g/dL)			
Median	9.9	10.0	10.3
75th percentile	10.9	10.8	11.8
25th percentile	8.9	9.0	9.1
Lowest quartile of Hb (g/dL)			
20th percentile	8.4	8.6	8.9
10th percentile	7.9	8.1	8.2
1st percentile	6.5	6.6	6.9
Minimum	5.6	5.9	5.7

Abbreviations: RBC, red blood cell; CI, confidence interval; Hb, hemoglobin.

[a]RBC transfusion rates per 30 infant days and 95% CIs were estimated using methods based on the Poisson distribution (Poisson regression implemented with SAS Proc Genmod, version 9.4).Transfusion rates reported for a total of 319 (53.3%) of 598 enrolled infants who received at least 1 RBC transfusion. The overall transfusion rate for the full cohort including all centers was 1.20 (95% CI 1.14-1.27) per 30 infant days.

[b]For Hb values recorded within 24 hours before a RBC transfusion.

eTable 6. Reduced Cox models to account for potential collinearity between RBC transfusion and severe anemia (N=598)

Reduced multivariable model without RBC transfusion	**β**	**SE**	**CSHR for NEC (95%CI)**	**P (Reliability[d])**
Birth weight *(per 100g increase)*	-0.00285	0.000732	0.75 (0.65-0.87)	<.0001 (98%)
RBC transfusion in a given week[a]			*Parameter removed*	
Severe anemia *(Hb ≤ 8g/dL)* in a given *week*[a,b]	1.30714	0.49949	3.70 (1.39-9.84)	0.009 (63%)
Breast fed in first 10 days *(per 1 day increase)*	0.09908	0.04599	1.10 (1.01-1.21)	0.03 (39%)
SNAP score on day of birth *(per 1 unit increase)*	-0.00631	0.03389	0.99 (0.93-1.06)	0.85 (8%)
Days of antibiotic treatment in 1st 10 days *(per 1 day increase)*	0.02138	0.05283	1.02 (0.92-1.13)	0.69 (5%)
Reduced multivariable model without severe anemia	**β**	**SE**	**CSHR for NEC (95%CI)**	**P (Reliability[d])**
Birth weight *(per 100g increase)*	-0.0032	0.000795	0.73 (0.62-0.85)	<.0001 (98%)
RBC transfusion in a given week[a,c]	-0.43028	0.4722	0.65 (0.26-1.64)	0.36 (24%)
Severe anemia *(Hb ≤ 8g/dL)* in a given week[a]			*Parameter removed*	
Breast fed in first 10 days *(per 1 day increase)*	0.08807	0.04614	1.00 (1.00-1.20)	0.06 (32%)
SNAP score on day of birth *(per 1 unit increase)*	-0.00222	0.0359	1.00 (0.93-1.07)	0.95 (9%)
Days of antibiotic treatment in 1st 10 days *(per 1 day increase)*	0.02538	0.05596	1.03 (0.92-1.15)	0.65 (7%)

Abbreviations: RBC, red blood cell; β, estimated regression coefficient; SE, standard error; CSHR, cause-specific hazard ratio; NEC, Necrotizing enterocolitis; CI, confidence interval; Hb, hemoglobin; SNAP, score for neonatal acute physiology.

Model includes adjustment for center (not shown).

[a]Time dependent covariate. Infants may switch from one risk group to the other (e.g. not severely anemic to severely anemic) in a given week. If no Hb measurement was performed in a given week, then the previous week's Hb value was used to determine if an infant had severe anemia (last observation carried forward).

[b]CSHR based on 4,565 hemoglobin measurements over 12 weeks (post-NEC hemoglobin data excluded for NEC patients).

[c]CSHR based on 1,430 RBC transfusions given over 12 weeks (post-NEC transfusion data excluded for NEC patients).

[d]Percentage of time risk factor appears in 1000 bootstrap models. Risk factors that appear in ≥50% of the models are reliable.

eTable 7. Risk factors for NEC and mortality using bivariable Cox models in subset of transfused infants (N=319)

			NEC			Mortality		
NEC risk factors	**n**	**N**	**CSHR**	**95% CI**	**P**	**CSHR**	**95% CI**	**P**
Gestational age *(per 1 week increase)*		319	0.87	0.73, 1.04	0.12	0.67	0.50, 0.89	0.006
Birth weight *(per 100g increase)*		319	0.74	0.62, 0.88	<0.001	0.57	0.44, 0.74	<0.001
Race *(White relative to Black)*	96	319	0.68	0.31, 1.52	0.35	1.33	0.52, 3.42	0.77
Race *(Other relative to Black)*	24	319	0.31	0.04, 2.17		0.73	0.09, 5.61	
5 minute Apgar *(per 1 point decrease)*		318	1.04	0.87, 1.24	0.66	1.17	0.97, 1.42	0.10
SNAP score *(per 1 point increase)*		319	0.97	0.90, 1.06	0.51	1.18	1.08, 1.29	<0.001
Hb at birth *(per 1 g/dL decrease)*		319	1.01	0.90, 1.14	0.84	1.13	0.89, 1.44	0.31
Ever with severe anemia *(Hb ≤ 8 g/dL)*	103	319	0.66	0.30, 1.44	0.29	1.20	0.48, 3.02	0.69
Severe anemia[a]		319	1.37	0.62, 3.05	0.44	5.54	2.02, 15.2	<0.001
Severe anemia in a given wk[b]		319	3.31	1.25, 8.78	0.02	3.99	1.09, 14.5	0.04
Nadir Hb measured in a given wk[b] *(per 1 g/dL decrease)*		319	1.44	1.14, 1.82	0.002	1.48	1.04, 2.10	0.03
Received RBC transfusion in a given wk[b]		319	1.03	0.67, 1.57	0.90	1.75	1.45, 2.11	<0.001
No. RBC transfusion(s) in a given wk[b]		319	1.18	0.52, 2.67	0.69	3.36	1.05, 10.7	0.04
Cumulative RBC transfusions over 12 weeks *(per 1 transfusion)*[b]		319	1.07	0.99, 1.15	0.07	1.32	1.18, 1.47	<0.001
Days of breast milk feeding in 1st 10 days *(per 1d increase)*		319	1.06	0.97, 1.16	0.18	0.86	0.76, 0.98	0.02
Days of antibiotic treatment in 1st 10 days *(per 1d increase)*		319	1.07	0.96, 1.19	0.23	1.08	0.93, 1.26	0.33
Age started enteral feeding *(per 1d increase)*		319	0.99	0.94, 1.05	0.69	0.86	0.71, 1.05	0.14
Maximum storage of transfused RBC in a given week (p*er 1d increase)*		319	0.99	0.94, 1.04	0.66	1.07	1.02, 1.12	0.003
Maximum storage after irradiation of RBC in a given week *(per 1d increase)*		319	1.10	0.98, 1.23	0.11	1.05	0.94, 1.19	0.39
			NEC			**Mortality**		
Additional mortality risk factors			**CSHR**	**95% CI**	**P**	**CSHR**	**95% CI**	**P**
Male gender	163	319	0.61	0.30, 1.22	0.16	0.85	0.35, 2.07	0.72
Multiple birth	88	319	0.68	0.30, 1.56	0.36	0.68	0.23, 2.03	0.49
Receipt of ≥1 dose of antenatal steroids	261	319	0.57	0.26, 1.21	0.14	0.82	0.27, 2.46	0.72
Receipt of surfactant therapy in first wk	220	319	1.37	0.62, 3.03	0.43	1.27	0.46, 3.50	0.64
Mechanical ventilation in first wk	230	319	0.76	0.37, 1.57	0.46	3.37	0.79, 14.4	0.10

Abbreviations: NEC, Necrotizing enterocolitis; CSHR, cause-specific hazard ratio; CI, confidence interval; SNAP, score for neonatal acute physiology; Hb, hemoglobin; RBC, red blood cell; wk, week.

Competing risks: 33 infants with NEC and 30 deaths total; 11 infants with NEC died. 33 events used to estimate CSHR for NEC and 19 deaths used to estimate CSHR for mortality.

Cumulative incidence of NEC at 8 weeks (95% CI) for subgroup of transfused infants: 10.4% (6.8, 13.8).

[a]Time dependent covariate defined as a dichotomous variable that can change at most once from unexposed to exposed over 12 weeks of follow-up (i.e. once exposed, always exposed).

[b]Time dependent covariate. Infants may switch from one risk group to the other (e.g. not severely anemic to severely anemic) in a given week. If no Hb measured in a given week, then the previous week's Hb value used (last observation carried forward).CSHR based on 4,565 Hb measurements over 12 weeks (post-NEC data excluded for NEC patients).

eTable 8. Risk factors for NEC and mortality using multivariable Cox models in subset of transfused infants (N=319)

	NEC			Mortality		
Risk factors	**CSHR**	**95% CI**	**P (Reliability[b])**	**CSHR**	**95% CI**	**P (Reliability[b])**
Birth weight *(per 100g increase)*	0.68	0.56, 0.83	<0.001 (97%)	0.67	0.51, 0.88	0.004 (96%)
Received RBC transfusion in a given week[a]	0.52	0.19, 1.40	0.19 (31%)	1.14	0.26, 5.08	0.87 (12%)
Severe anemia *(Hb ≤ 8 g/dL)* in a given week[a]	6.32	1.94, 20.6	0.002 (71%)	1.74	0.43, 7.08	0.44 (25%)
Days of breast milk feeding in 1st 10 days *(per 1 day increase)*	1.12	1.01, 1.24	0.04 (42%)	0.87	0.78, 0.98	0.02 (41%)
SNAP score on day of birth *(per 1 point increase)*	0.97	0.90, 1.05	0.45 (19%)	1.12	1.02, 1.23	0.02 (59%)
Days of antibiotic treatment in 1st 10 days *(per 1 day increase)*	1.04	0.93, 1.18	0.49 (5%)	0.99	0.82, 1.19	0.90 (10%)

Abbreviations: NEC, Necrotizing enterocolitis; CSHR, cause-specific hazard ratio; CI, confidence interval; RBC, red blood cell; Hb, hemoglobin; SNAP, score for neonatal acute physiology.

Competing risks: 33 infants with NEC and 30 deaths total; 11 infants with NEC died. 33 events used to estimate CSHR for NEC and 19 deaths used to estimate CSHR for mortality. Model includes adjustment for center (not shown).

Included in the cohort of transfused infants are 286 infants without NEC who received RBC transfusion and 33 infants with NEC who received RBC transfusion prior to NEC diagnosis.

[a]Time dependent covariate. Infants may switch from one risk group to the other (e.g. not severely anemic to severely anemic) in a given week. Post-NEC RBC transfusion and Hb observations were excluded for NEC patients.

[b]Percentage of time risk factor appears in 1000 bootstrap models. Risk factors that appear in ≥50% of the models are reliable.

eTable 9. Multivariable Cox models with anemia specified as a continuous variable (N=319)

	NEC			Mortality		
Risk factors	**CSHR**	**95% CI**	**P (Reliability[c])**	**CSHR**	**95% CI**	**P (Reliability[c])**
Birth weight *(per 100g increase)*	0.67	0.55, 0.82	<.0001 (98%)	0.67	0.52, 0.88	0.003 (94%)
Received RBC transfusion in a given week[a]	0.47	0.18, 1.27	0.14 (45%)	0.86	0.16, 4.48	0.89 (14%)
Nadir Hb in a given week[b] *(per 1 g/dL decrease)*	**1.65**	**1.23, 2.12**	**<0.001 (92%)**	**1.29**	**0.87, 1.92**	**0.20 (45%)**
Days of breast milk feeding in 1st 10 days *(per 1 day increase)*	1.13	1.01, 1.25	0.03 (47%)	0.87	0.78, 0.98	0.02 (39%)
SNAP score on day of birth *(per 1 point increase)*	0.97	0.90, 1.05	0.47 (18%)	1.12	1.02, 1.22	0.02 (58%)
Days of antibiotic treatment in 1st 10 days *(per 1 day increase)*	1.05	0.92, 1.19	0.45 (8%)	1.00	0.84 ,1.19	0.96 (8%)

Abbreviations: NEC, Necrotizing enterocolitis; CSHR, cause-specific hazard ratio; CI, confidence interval; RBC, red blood cell; Hb, hemoglobin; SNAP, Score for neonatal acute physiology.

Competing risks: 33 infants with NEC and 31 deaths total; 12 infants with NEC died. 19 deaths used to estimate CSHR for mortality. Model includes adjustment for center (not shown).

Included in the cohort of transfused infants are 286 infants without NEC who received RBC transfusion and 33 infants with NEC who received RBC transfusion prior to NEC diagnosis.

[a]Time dependent covariate. Infants may switch from one risk group to the other (e.g. receipt of ≥1 RBC transfusion to no RBC transfusion) in a given week. Post-NEC RBC transfusion observations were excluded for NEC patients.

[b]Time dependent covariate, using the lowest measured Hb in a given week. If no Hb measurement was done in a given week, the previous week's Hb value was used (last observation carried forward). Post-NEC Hb data was excluded for NEC patients.

[c]Percentage of time risk factor appears in 1000 bootstrap models. Risk factors that appear in ≥50% of the models are reliable.

eTable 10. Longitudinal change in hemoglobin by NEC and RBC transfusion status

Scheduled assessment age (age window for recorded Hb values)	No. Hb measurements	No. infants with ≥1 Hb measured (as a % of infants on study)	No. infants on study[a]	NEC Mean Hb in g/dL (95% CI)	Non-NEC with RBC transfusion Mean Hb (95% CI)	Non-NEC without RBC transfusion Mean Hb (95% CI)	NEC vs non-NEC with RBC transfusion Difference in mean Hb (95% CI)	P
1 d (0-2)	811	593 (99%)	598	13.9 (13.4, 14.4)	13.4 (13.2, 13.6)	16.1 (15.9, 16.4)	0.52 (-0.02, 1.05)	0.06
4 d (3-5)	509	390 (65%)	598	11.9 (11.3, 12.5)	12.0 (11.8, 12.2)	14.7 (14.3, 15.0)	-0.11 (-0.71, 0.49)	0.72
7 d (6-9)	526	394 (66%)	595	11.6 (11.1, 12.2)	11.6 (11.4, 11.8)	13.2 (12.9, 13.6)	0.02 (-0.55, 0.59)	0.95
14 d (10-17)	687	458 (78%)	589	11.2 (10.7, 11.7)	11.1 (10.9, 11.2)	12.2 (11.9, 12.5)	0.11 (-0.42, 0.64)	0.68
21 d (18-24)	560	421 (74%)	570	10.9 (10.2, 11.5)	10.8 (10.6, 11.0)	10.9 (10.6, 11.2)	0.03 (-0.66, 0.72)	0.93
28 d (25-34)	528	412 (75%)	550	10.4 (9.7, 11.1)	10.5 (10.3, 10.8)	10.1 (9.8, 10.4)	-0.15 (-0.91, 0.61)	0.69
40 d (35-48)	476	370 (73%)	506	9.6 (8.5, 10.7)	10.1 (9.9, 10.3)	9.8 (9.5, 10.1)	-0.52 (-1.62, 0.59)	0.36
60 d (49-90)	468	293 (74%)	394	8.7 (7.2, 10.2)	10.2 (10.0, 10.4)	10.7 (10.3, 11.0)	-1.50 (-3.05, 0.04)	0.06

Number of hemoglobin measurements per infant by end of time on study

End of time on study[b]	No. of infants in each period	Median Hb measurements	Lower quartile of Hb measurements	Upper quartile of Hb measurements
Overall (birth to 90 d)	598	7.0	5.0	9.0
≤28 d	60	4.0	3.0	6.0
28-55 d	208	5.0	4.0	7.0
56-90 d	330	9.0	7.0	11.0

Abbreviations: NEC, Necrotizing enterocolitis; RBC, red blood cell; Hb, hemoglobin; CI, confidence interval.

All Hb measures are in g/dL and age at measurement were recorded at 8 scheduled assessments, with age windows becoming larger over time to account for less frequent measurements as infants neared discharge to home. Model-based Hb means were adjusted for birth weight. Analysis of repeated Hb measures was done using a mixed linear model (4,565 Hb measures from 598 VLBW infants), taking into account the age at which each Hb measure was obtained (see eMethods for additional details). Hb measurements taken post-NEC diagnosis date are excluded for the NEC group.

Group sample sizes: 44 infants with NEC (302 Hb measurements), 286 infants with no NEC and RBC transfusion (2,875 Hb measurements) and 268 infants with no NEC and no RBC transfusion (1,388 Hb measurements).

[a]Excludes patients who have been diagnosed with NEC before given study period, as their hemoglobin measurements are not included in this analysis.

[b]Time until death, onset of NEC, hospital discharge or transfer to a non-study affiliated hospital.

eTable 11. Modeling propensity for RBC transfusion with risk factors potentially associated with RBC exposure

Bivariable logistic regression analysis (N=598)

Baseline factors	RBC exposed (N=319)	RBC unexposed (N=279)	β (SE)	Odds ratio (95% CI); P
Mechanical ventilation on the day of birth	200 (63%)	63 (23%)	1.751 (0.184)	5.76 (4.02, 8.27); <0.001
Receipt of ≥1 dose of antenatal steroids	261 (82%)	240 (86%)	-0.313 (0.226)	0.73 (0.47, 1.14); 0.17
Birth weight *(per 100g increase)*			-0.0061 (0.00050)	0.54 (0.49, 0.60); <0.001
Gestational age *(per 1 week increase)*			-0.6836 (0.0566)	0.51 (0.45, 0.56); <0.001
SNAP score on day of birth *(per 1 point increase)*			0.169 (0.019)	1.19 (1.14, 1.23); <0.001
5 minute Apgar score[a] *(per 1 point decrease)*			-0.400 (0.056)	1.49 (1.33, 1.67); <0.001
Hemoglobin at birth *(per 1 g/dL decrease)*			-0.4659 (0.0456)	1.59 (1.46, 1.74); <0.001

Multivariable logistic regression analysis[b] (N=596)

Baseline factors	β (SE)	Odds ratio (95% CI); P
Mechanical ventilation on the day of birth	0.2709 (0.2959)	1.31 (0.73, 2.34); 0.36
Receipt of ≥1 dose of antenatal steroids	0.0458 (0.3581)	1.05 (0.51, 2.11); 0.90
Birth weight *(per 100g increase)*	-0.00447 (0.0007)	0.64 (0.56, 0.74); <0.001
Gestational age *(per 1 week increase)*	-0.3311 (0.0866)	0.72 (0.61, 0.85); <0.001
SNAP score on day of birth *(per 1 point increase)*	0.0875 (0.0279)	1.09 (1.03, 1.15); 0.002
5 minute Apgar score[a] *(per 1 point decrease)*	0.0352 (0.0795)	0.97 (0.83, 1.13); 0.66
Hemoglobin at birth *(per 1 g/dL decrease)*	-0.3553 (0.0611)	0.70 (0.62, 0.79); <0.001

Abbreviations: RBC, red blood cell; β, estimated regression coefficient; SE, standard error; CI, confidence interval; SNAP, score for neonatal acute physiology.

Model includes adjustment for center. Bivariable odds ratio for RBC exposure for highest vs. lowest transfusing center: 3.32 (95% CI 2.36-4.67); P<0.0001. Multivariable odds ratio for RBC exposure for highest vs. lowest transfusing center: 5.32 (95% CI 3.01-9.39); P<0.001.

[a]5 minute Apgar score was missing for two infants born outside of study hospital, and these two subjects were excluded from the multivariable logistic regression analysis.

[b]The multivariable logistic regression model with the 7 factors above plus center was used to estimate the probability of RBC exposure. This probability, the propensity score, was used in Models 4 and 5 in Table 3 for the analysis of study outcomes. The model demonstrated good fit (Hosmer-Lemeshow goodness of fit statistic P=0.25). See eMethods for additional details.

eTable 12. Assessment of covariate balance after propensity stratification (N=596)

Baseline covariate	RBC unadjusted[a] Test Statistic	RBC unadjusted P Value	RBC adjusted[b] Test Statistic	RBC adjusted P Value	RBC by PS adjusted P Value
Mechanical ventilation on the day of birth	100.8	<0.001	0.2	0.67	0.32
Receipt of ≥1 dose of antenatal steroids	2.0	0.16	0.4	0.53	0.57
Birth weight in grams	261.6	<0.001	3.6	0.06	0.01
Gestational age in weeks	258.9	<0.001	1.6	0.21	0.96
SNAP score on day of birth	101.9	<0.001	0.6	0.43	0.09
5 min Apgar score	62.1	<0.001	0.2	0.65	0.22
Hemoglobin at birth (g/dL)	146.8	<0.001	0.1	0.79	0.80
Center	49.9	<0.001	0.2	0.67	0.32

Abbreviations: RBC, red blood cell; PS, propensity score; SNAP, score for neonatal acute physiology.

Two-way models were used to assess covariate imbalance after propensity stratification[2]. Test statistics and P values for models without propensity adjustment (unadjusted) and with propensity adjustment (adjusted) are reported. SAS Proc Genmod was used for the two-way models. The model was run separately for each possible confounder. All adjusted models contain RBC exposure, propensity score and the interaction between RBC exposure and propensity score. The summary listing displays the test statistics and p-values for both the RBC exposure effect and RBC exposure by propensity strata interaction. For comparison, the unadjusted RBC exposure effect was also included. For each covariate, there is reduction in imbalance produced by the propensity scoring (smaller test statistics and larger p-values). There is some residual imbalance for birth weight, where the RBC exposure effect varies by propensity quintile. Therefore, modeling of outcomes using PS as a continuous covariate or use of inverse probability of treatment weighting was favored over evaluation of outcomes across PS quintiles.

[a]Testing for covariate balance without PS.

[b]All models contain RBC exposure, PS quintile and the interaction between RBC exposure and PS quintile.

eTable 13. Standardized differences before and after propensity score adjustment and within each propensity score quintile (N=596).

Baseline covariate	STDIFF, unadjusted	STDIFF, adjusted (averaging across strata)	STDIFF, 1st quintile	STDIFF, 2nd quintile	STDIFF, 3rd quintile	STDIFF, 4th quintile	STDIFF, 5th quintile[a]
Mechanical ventilation on the day of birth	0.89	-0.06	-0.35	0.08	0.21	-0.09	
Receipt of ≥1 dose of antenatal steroids	-0.11	-0.11	-0.10	-0.01	-0.18	0.32	
Birth weight in grams	-1.49	-0.33	0.09	0.14	0.06	-0.95	
Gestational age in weeks	-1.47	-0.23	-0.12	-0.14	-0.15	-0.36	
SNAP score on day of birth	0.86	0.17	-0.33	0.61	-0.04	-0.16	
5 min Apgar score	-0.67	-0.10	-0.46	0.09	-0.30	0.33	
Hemoglobin at birth (g/dL)	-1.06	-0.05	-0.18	-0.14	-0.29	0.15	
Center	0.60	-0.05	0.63	-0.02	0.09	-0.30	

Abbreviations: STDIFF, standardized difference; SNAP, score for neonatal acute physiology; RBC, red blood cell;

This table provides standardized differences for the unadjusted sample (without propensity scoring), averaged across propensity scores (adjusted) and within each propensity score stratum. Most of the unadjusted standardized differences were greater than 0.25 while all standardized differences averaged over the strata were <0.25, which is considered to indicate balance of the potential confounder between RBC exposure groups[3]. Others have proposed a threshold of 0.10 to indicate a negligible difference in the mean or prevalence of a covariate between exposure groups[4]. Within-propensity score strata demonstrated several standardized differences greater than 0.25, reflecting the difficulty in producing and assessing balance with relatively small sample sizes within each stratum (particularly the 1st and 4th quintiles). Therefore, modeling of outcomes using the propensity score as a continuous covariate and use of inverse probability of treatment weighting was favored over evaluation of outcomes across propensity score quintiles.

[a]Limited data in the RBC unexposed group prevented an estimate of the standardized difference for the 5th quintile.

eTable 14. Summary of weighted standardized differences for baseline covariates in propensity score regression analysis (N=596)

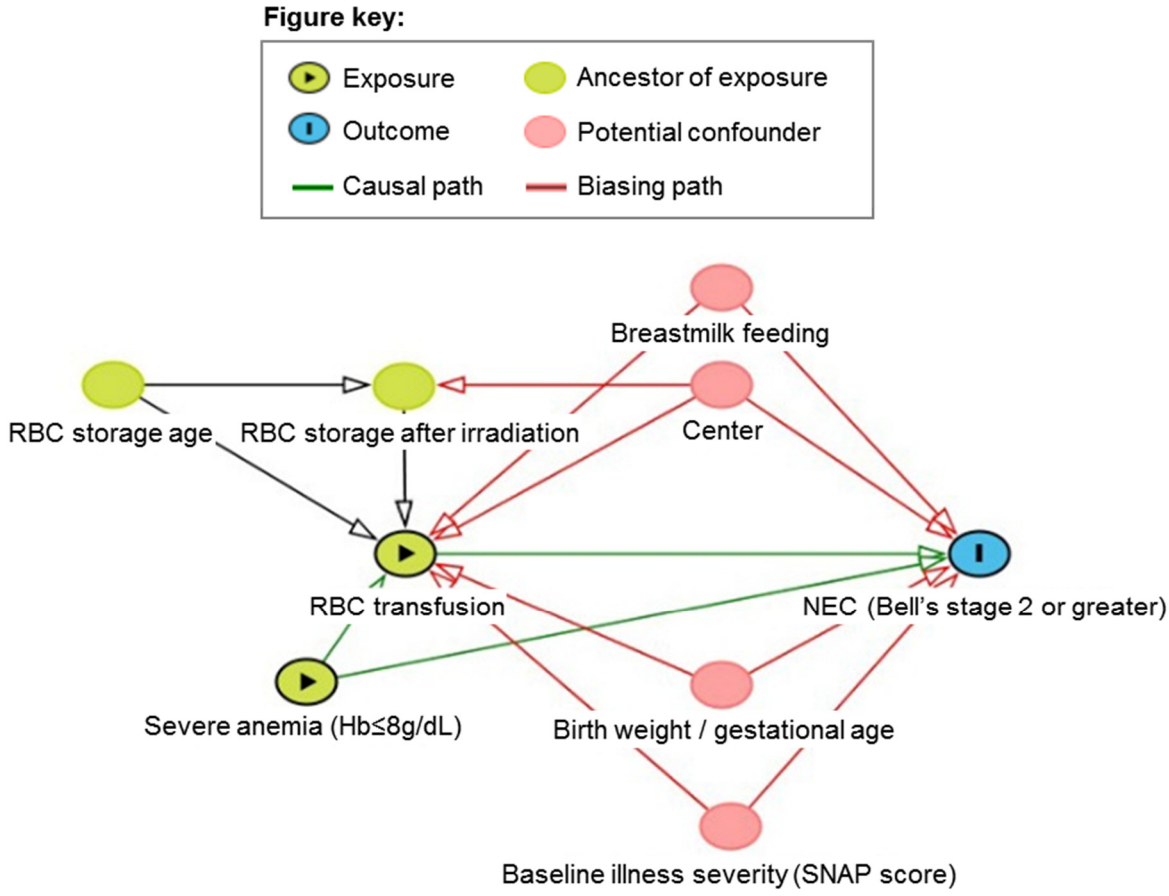
Baseline covariate	Sample size	Mean	Standard deviation	Minimum	Maximum
Mechanical ventilation on the day of birth	596	0.04	0.01	0.00	0.05
Receipt of ≥1 dose of antenatal steroids	596	0.08	0.03	0.00	0.11
Birth weight in grams	596	0.51	0.22	0.00	0.73
Gestational age in weeks	596	0.19	0.08	0.00	0.28
SNAP score on day of birth	596	0.30	0.13	0.00	0.43
5 min Apgar score	596	0.08	0.03	0.00	0.12
Hemoglobin at birth (g/dL)	596	0.03	0.02	0.00	0.05
Center	596	0.21	0.08	0.00	0.30

Abbreviations: RBC, red blood cell; SNAP, score for neonatal acute physiology; IPTW, inverse probability of treatment weighting.

This table summarizes the weighted standardized differences for assessing covariate balance in the propensity score regression analysis. Two-way models were used to assess covariate imbalance after propensity score regression analysis[5]. All models contain RBC exposure, propensity score and the interaction between RBC exposure and propensity score. SAS Proc Genmod was used for the two-way models. The model was run separately for each possible confounder. Parameter estimates from each model were used to compute the standardized differences. A standardized difference of <0.25 is considered to indicate balance of the potential confounder between RBC exposure groups. Birth weight and SNAP score had standardized differences greater than the generally accepted <0.25 threshold, indicating further assessment of the propensity model may be warranted. Therefore, we pursued an IPTW approach.

Application of IPTW resulted in balance for both birth weight and SNAP score. For example, the weighted mean for birth weight was nearly identical for RBC exposed infants (mean = 1091g) compared to RBC unexposed infants (mean = 1116g). The standardized difference for birth weight (0.10) met the most conservative threshold of 0.10. The weighted mean for SNAP score was similar for RBC exposed infants (mean = 8.9) compared to RBC unexposed infants (mean = 9.6). In time-to-event analyses, applying propensity scores using inverse probability of treatment/exposure weights minimizes bias relative to other methods of applying propensity scores[6]. The results of this analysis are reported in Model 5 in Table 3 in the primary manuscript.

eFigure 1. Directed acyclic graph of causal relationship between exposures of interest, potential confounders and outcome

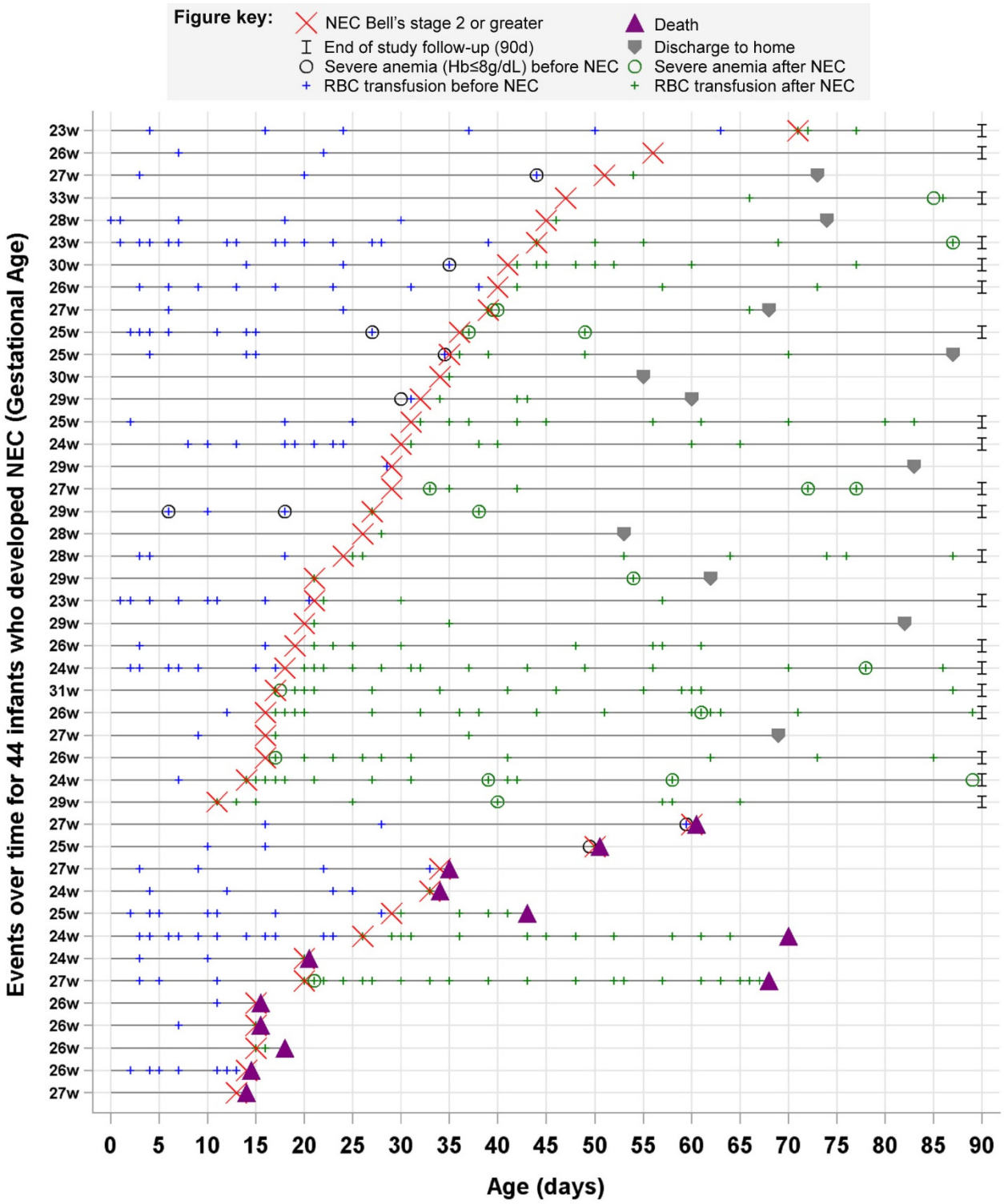


Causal diagram depicts hypothesized relationship between RBC transfusion (primary exposure of interest), severe anemia (secondary exposure of interest) and NEC (outcome of interest), with depiction of important potential confounders. Black lines connect ancestors of exposure to the primary exposure of interest. Figure created using DAGitty, available online at http://dagitty.net/dags.html.

Reference: Textor J, Hardt J, Knüppel S. DAGitty: A Graphical Tool for Analyzing Causal Diagrams. Epidemiology, 5(22):745, 2011.

Abbreviations: RBC, red blood cell; NEC, necrotizing enterocolitis; Hb, hemoglobin; SNAP, score for neonatal acute physiology.

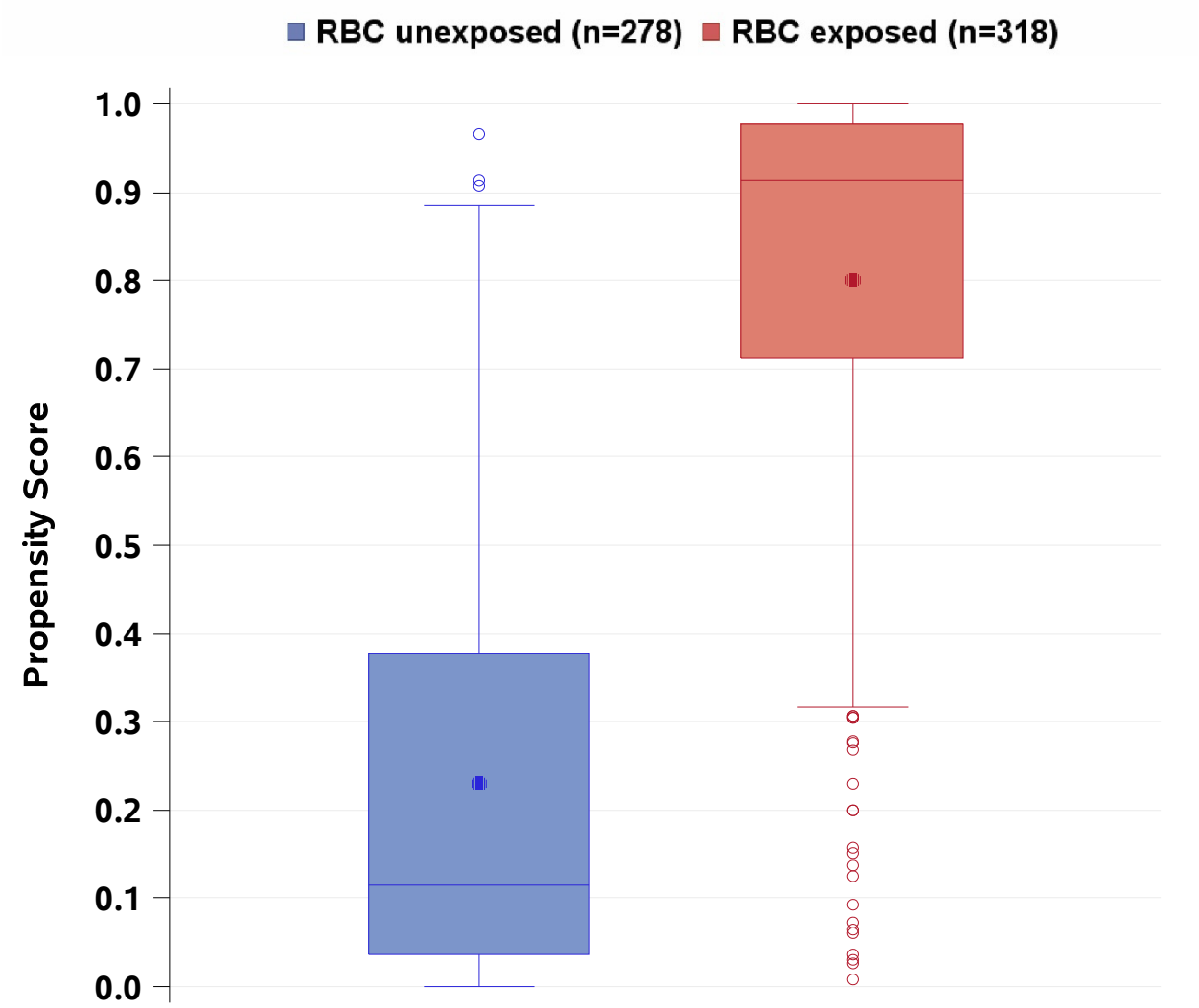
eFigure 2. Timing of RBC transfusion and severe anemia in relation to NEC onset



Individual infants are labelled by gestational age and ordered vertically by (1) survival and (2) age at NEC onset. Of the 44 infants with NEC Bell's stage 2 or greater, 33 (75%) received ≥1 RBC transfusion before onset and 7 (15.9%) received an RBC transfusion within 48 hours before NEC onset. The overall risk of developing NEC within 48 hours after RBC transfusion was 0.49% (7 events following 1,430 RBC transfusions). 8 infants (18.2%) developed severe anemia before NEC onset and 13 infants (29.5%) died.

Abbreviations: RBC, red blood cell; NEC, necrotizing enterocolitis; Hb, hemoglobin; w, weeks.

eFigure 3. Evaluation of common support using distributions of propensity scores by RBC exposure

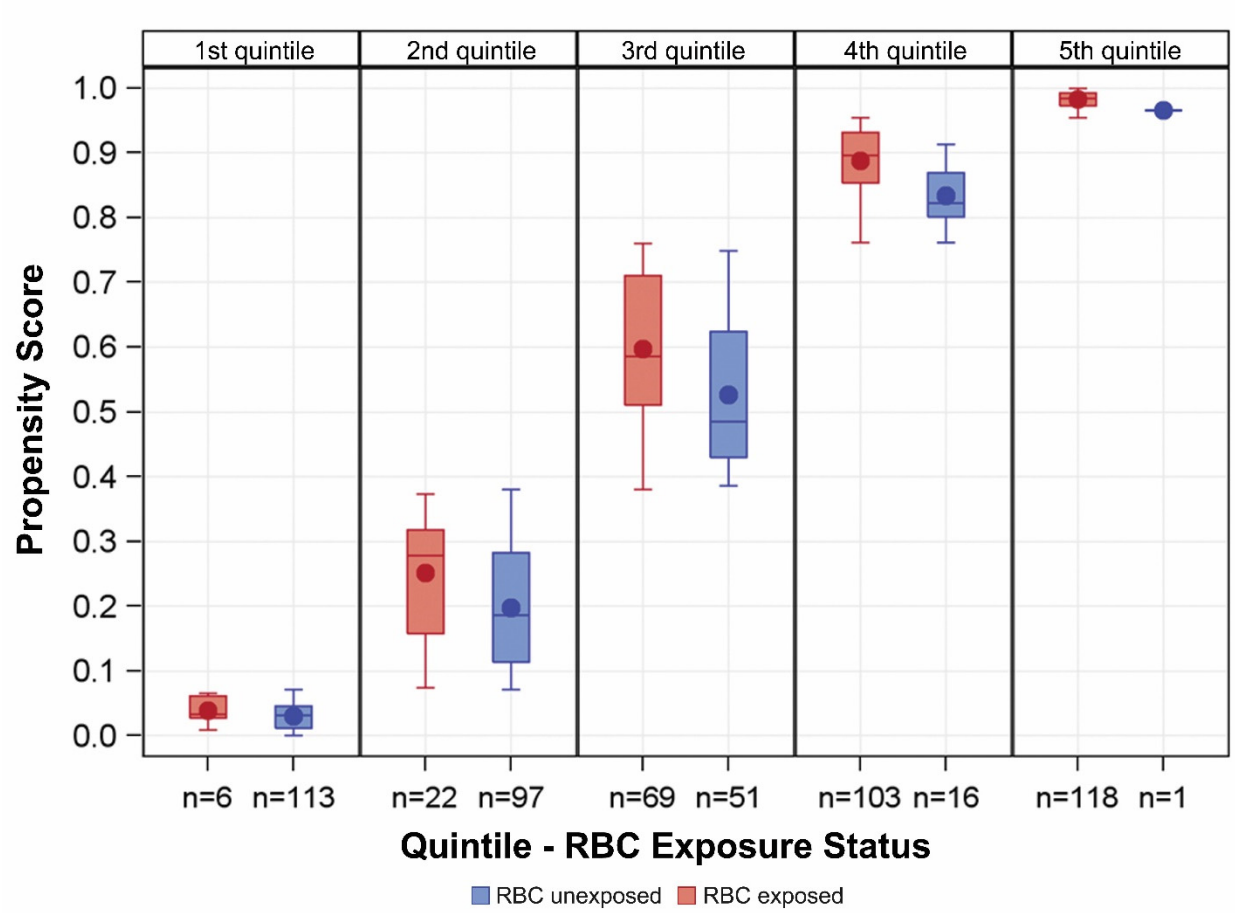


The degree to which the propensity score has been appropriately specified was ascertained through evaluation of common support. Common support is defined by overlapping distributions of propensity scores between RBC exposure groups. Overlap in the propensity score distributions indicates the potential for an infant in the RBC transfusion exposed group to be in the RBC transfusion unexposed group, and that infants with each level of covariates may have either exposure status (i.e. supporting the assumptions of exchangeability and positivity)[7]. A lack of common support, or a complete separation of propensity scores without any overlap between the two exposure groups (i.e. RBC exposed and unexposed) indicates severe differences between the two exposure groups and the possibility that confounding cannot be reduced using propensity methods[8].

This figure demonstrates overlapping ranges of the boxplots of propensity scores between RBC exposed and unexposed infants, which indicates that the propensity model exhibits common support. The propensity score was modeled using the following 8 covariates in a logistic regression fit to the outcome of receipt of at least 1 RBC transfusion: birth weight (continuous), gestational age (continuous), SNAP score (continuous), Apgar score at 5 minutes (continuous), hemoglobin concentration at birth (continuous), mechanical ventilation on the day of birth (yes/no), receipt of ≥1 dose of antenatal steroids (yes/no), and center (categorical). The model demonstrated good fit (Hosmer-Lemeshow goodness of fit statistic P=0.25). Circles within each boxplot denote the mean score. The middle line within the box represents the median, the top line represents the 75th percentile and the bottom line represents the 25th percentile. The upper fence is defined as the third quartile (represented by the upper edge of the box) plus 1.5 times the IQR. The lower fence is defined as the first quartile (represented by the lower edge of the box) minus 1.5 times the IQR. Observations outside the fences are identified with an open circle.

Abbreviations: RBC, red blood cell; SNAP, score for neonatal acute physiology; IQR, interquartile range.

eFigure 4. Distribution of propensity scores by quintiles and RBC exposure



Boxplot demonstrates distribution of propensity scores among RBC transfusion exposed and unexposed infants by quintiles of propensity scores. Circles within each boxplot denote the mean score. The middle line within the box represents the median, the top line represents the 75th percentile and the bottom line represents the 25th percentile. The upper fence is defined as the third quartile (represented by the upper edge of the box) plus 1.5 times the IQR. The lower fence is defined as the first quartile (represented by the lower edge of the box) minus 1.5 times the IQR. The propensity score, in order to estimate the probability of RBC transfusion, was modeled using the following 8 covariates in a logistic regression fit to the outcome of receipt of at least 1 RBC transfusion: birth weight (continuous), gestational age (continuous), SNAP score (continuous), Apgar score at 5 minutes (continuous), hemoglobin concentration at birth (continuous), mechanical ventilation on the day of birth (yes/no), receipt of ≥1 dose of antenatal steroids (yes/no), and center (categorical). The model demonstrated good fit (Hosmer-Lemeshow goodness of fit statistic P=0.25).

Abbreviations: RBC, red blood cell; IQR, interquartile range; SNAP, Score for Neonatal Acute Physiology.

eMethods. Supplemental methods

Bootstrap bagging

Bootstrap bagging was used to identify stable and reliable predictors of necrotizing enterocolitis (NEC) and mortality[9]. A dataset was constructed of size equal to the original (598 infants) by random sampling of cases with replacement (bootstrap sampling). On average, approximately one-third of infants were not sampled, whereas some infants were sampled more than once. The bootstrap sample was analyzed using the Cox model with an automated forward stepwise algorithm with entry criterion of $p < 0.10$ and a retention criterion of $p < 0.05$. The result was stored. This process of sampling, automated analysis and storing was repeated 1000 times. The number of times a risk factor appeared in these 1000 analyses was taken as reflection of the reliability (signal). Following Breiman's median rule (devised to balance type I and type II errors), risk factors were determined to be reliably associated with the outcome if they appeared in at least 50% of the models[10]. The reliability analysis accounted for the direction of the association (i.e. cause specific hazard ratio <1.0 or >1.0) in each of the models. The cause-specific hazard ratio and its 95% confidence interval were calculated for each factor in the presence of the others in the final model identified with bootstrap bagging. Bootstrap bagging was also used to identify the predictors of mortality.

Longitudinal modeling of hemoglobin values over time

Repeated-measures analysis of hemoglobin (g/dL) was performed using a means model with SAS Proc Mixed (version 9), providing separate estimates of the means by time on study (8 time intervals: 0-2, 3-5, 6-9, 10-17, 18-24, 25-34, 35-48, 49-90 days) and NEC and red blood cell (RBC) transfusion status. These results are reported in **eTable 10**. The model included group (NEC, non-NEC with RBC transfusion and non-NEC without RBC transfusion), time on study, the statistical interaction between these two predictors and birth weight as covariates. For infants who developed NEC, only hemoglobin measurements collected before the onset of NEC were analyzed. A compound-symmetric variance-covariance form among the repeated measurements was assumed for the outcome and robust estimates of the standard errors of parameters were used to perform statistical tests and construct 95% confidence intervals[11]. The model-based means (least-squares means) are unbiased with unbalanced and missing data, so long as the missing data are non-informative (missing at random). All specific statistical tests were done within the framework of the mixed effects linear model using t-tests to compare hemoglobin differences at each time interval between infants who developed NEC and non-NEC infants that received at least 1 RBC transfusion.

Propensity score modeling

In addition to the competing risks analysis, propensity scoring was used as a robust approach to reduce the effects of covariate confounding when estimating the adjusted hazard of NEC relative to RBC transfusion status. To account for potential confounding by indication for RBC transfusion or selection bias (i.e., systematic differences in demographic and clinical characteristics between infants in the two RBC exposure groups), propensity scores or balancing scores were estimated using logistic regression with RBC transfusion status (exposed vs. unexposed) as the outcome variable (**eTable 11**). The eight independent variables for the propensity score model included covariates potentially associated with RBC transfusion status, study outcomes or both[12]. The covariates included: birth weight (continuous), gestational age (continuous), score for neonatal acute physiology score (continuous), Apgar score at 5 minutes (continuous), hemoglobin concentration at birth (continuous), mechanical ventilation on the day of birth (yes/no), receipt of ≥1 dose of antenatal steroids (yes/no), and center (categorical). The propensity or probability of requiring RBC transfusion was calculated for each infant conditional on the covariates until an optimal balance on these covariates was achieved (**eFigures 3 and 4**). Next, three methods were used to estimate the effect of RBC transfusion on NEC and in-hospital mortality: 1) inverse probability of treatment weighting (IPTW); 2) covariate adjustment using the propensity score; 3) stratification on the propensity score[2-3,5]. Standardized differences were used to assess the balance of confounders between the two RBC exposure groups (**eTables 12-14**). A standardized difference < 0.1 suggests negligible difference in the mean or prevalence of a covariate between transfusion groups[4]. In time-to-event analyses, applying propensity scores using inverse probability of treatment/exposure weights minimizes bias relative to other methods of applying propensity scores[6]. To adjust for potential selection bias due to RBC transfusion status, each infant was assigned a "weight" or influence when estimating the effect of transfusion status on NEC. The weight for each infant was inversely proportional to the probability of receiving RBC transfusion (IPTW). In order to reduce the influence of outlying weights (i.e. those observations with a very high or very low propensity score), stabilized weights (standardized) were calculated for each exposure group[8]. These standardized weights were then used in a propensity score-weighted competing risks analysis to determine the time dependent effects of RBC transfusion and severe anemia on the hazard rate for NEC.

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