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ARTICLE

Quantifying the decline in fetal hemoglobin following adult red blood cell transfusions in preterm infants

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Abstract (150 words)

OBJECTIVE: To quantify the effect of transfusing preterm neonates with 20 mL/kg adult red blood cells (RBCs) on fetal hemoglobin (HbF%) and examine associations with outcomes.

STUDY DESIGN: We retrospectively analyzed HbF% before *vs.* after RBC transfusions and investigated associations with retinopathy of prematurity (ROP) and bronchopulmonary dysplasia (BPD).

RESULTS: HbF% in 1147 non-transfused infants were compared with pre- *vs*. post-transfusion values from 23 transfused infants. Each initial transfusion decreased HbF% from normal (mean=**91%**, 95%CI, 86-96%) to abnormal (**49%**, 95%CI, 42-56%, p<0.001). Multiple transfusions progressively decreased HbF% (p<0.001). Infants receiving ≥40 mL/kg before 30 weeks postmenstrual age (PMA) were more likely to develop *severe* ROP and BPD.

CONCLUSIONS: Adult RBC transfusion substantially reduces HbF% in preterm neonates. Low HbF% before 30 weeks PMA is associated with *severe* ROP and BPD. Serially measuring HbF% warrants evaluation as a surrogate endpoint in clinical trials comparing umbilical cord blood *vs.* adult RBC transfusions.

Introduction

Fetal hemoglobin (HbF) is uniquely adapted to transport oxygen from mother to fetus, and to release oxygen appropriately, not excessively, to fetal tissues [1]. To

accomplish these functions HbF binds oxygen more avidly than adult hemoglobin (HbA) and releases oxygen to tissues more sparingly. During their stay in the NICU, extremely low gestational age neonates (ELGANs, <28 weeks gestation) should have HbF, not HbA, as their predominant oxygen transporter during critical periods of organ development [1-3].

A growing number of reports suggest that keeping the HbF% within the normal physiologic range might be protective for both retinopathy of prematurity (ROP) and for bronchopulmonary dysplasia (BPD) [3-20]. Specifically, it has been hypothesized that increasing the proportion of HbA in the blood of preterm infants, from transfusing adult donor red blood cells (RBC), increases oxygen delivery to the developing retina and alveoli, leading to oxidative injury and a higher incidence and severity of ROP and BPD [3-17].

In a previous study we collected HbF% values from infants who were never transfused with adult RBC, or prior to receiving their first RBC transfusion [2]. From those values we created HbF% reference intervals, defining the upper (97.5^{th} percentile) and lower (2.5^{th} percentile) reference values from 23 through 40 weeks post menstrual age (PMA). In the present study, we recorded paired HbF% values from infants before *vs.* after their first RBC transfusion. We also determined the magnitude of fall according to the cumulative volume of RBC transfusions received during their NICU stay and the PMA at which each transfusion was given. We also recorded ROP and BPD outcome measurements from medical records and evaluated associations between RBC transfusions given, HbF% values measured, and ROP/BPD outcomes recorded.

Methods

Study design, population, and HbF% measurements.

We analyzed HbF% values obtained from infants born between September 2025 and August 2026 at Utah Valley Hospital (Provo, Utah) and McKay-Dee Hospital (Ogden, Utah). The study protocol was approved by the Institutional Review Boards of Intermountain Health (1053682) with a waiver of informed consent. We obtained HbF% measurements from blood gas analyzers (ABL800 FLEX; Radiometer America, Brea, CA) and entered these in REDCap (Research Electronic Data Capture; Nashville, TN).

For each HbF% measurement, we recorded the PMA when the blood sample was drawn. We also recorded each RBC transfusion administered and the HbF% before and after, including the number of hours before and after completing the transfusion when the HbF% was drawn. We only included post-transfusion values if they were measured within 72 hours of the transfusion. We plotted paired before *vs.* after transfusion values on our reference interval chart to determine whether each value was normal (within the reference interval) or abnormal (below the lower reference limit) [2]. For each infant in our HbF% database, whether they received RBC transfusions or not, we recorded the results of the ROP examination and the BPD score at 36 weeks, as described below.

RBC transfusions and darbepoetin administration

All transfused RBCs were drawn from volunteer adult donors by whole blood donation or collected by apheresis at the American Red Cross Blood Services. Transfused RBCs were leukoreduced, stored in Nutricel (AS-3) and irradiated.

Restrictive RBC transfusion guidelines were in place in both NICUs during the period studied [20]. Briefly, RBC transfusions were considered for a hemoglobin <7 g/dL for infants on room air, <8 g/dL for infants receiving supplemental oxygen or non-invasive respiratory support (e.g. CPAP), and <9 g/dL for those on mechanical ventilation. Individual transfusions were generally 20 mL/kg current patient weight and were typically infused over 2 to 4 hours.

A darbepoetin transfusion-reduction program was in place in each NICU. Briefly, infants with a birthweight <1000 grams, or "critically ill" with a birthweight 1001 – 1500 grams, received darbepoetin 10 μg/kg subcutaneously once per week beginning within 48 hours of birth and continuing until at least 34 completed weeks postmenstrual age [22, 23].

Ophthalmological data

ROP was classified according to the International Classification of Retinopathy of Prematurity (ICROP), third edition (ICROP3) [24], using five stages: 1) A thin demarcation line between vascularized and avascular retina; 2) A ridge at this demarcation line, 3) Abnormal neovascularization extending into the vitreous; 4) Partial retinal detachment; 5) Total retinal detachment.

Ophthalmology exams began no later than 31-33 weeks postmenstrual age, or 4 – 6 weeks after birth, whichever was later, as recommended in the AAP/AAO/AAPOS guidelines [25]. Subsequent inpatient and outpatient exams were conducted based on the findings of the previous examination. The following were recorded for each eye at each exam: 1) highest stage of ROP in the lowest zone, 2) highest stage of ROP in any zone, 3) presence of plus disease, and whether criteria were met for Type 1 ROP.

Retinal examination data were recorded until one of the following endpoints was met: 1) death, 2) No ROP (full vascularization to the ora serrata or vascularization in zone III, without ROP, on two consecutive examinations, 3) Non-severe ROP, listed according to highest stage in either eye, or 4) Severe ROP in either eye, treated with peripheral retinal ablation, vitreoretinal surgery or bevacizumab (or alternative) injection.

Diagnosing and scoring BPD

We defined and scored according to the criteria of Jensen *et al*. at 36 weeks' PMA, regardless of prior or current oxygen therapy [26]. Infants breathing room air without any respiratory support were graded as having no BPD, infants requiring nasal cannula ≤2 L/min had grade 1 BPD, infants requiring nasal cannula >2 L/min or noninvasive positive airway pressure had grade 2 BPD, and infants on invasive mechanical ventilation had grade 3 BPD. We did not make corrections for altitude.

Statistical analysis

Summary statistics (means, standard deviations, medians, quartiles, and proportions) and basic statistical tests (chi-square test for categorical variables; Student’s t-test for continuous variables when means were used, and Wilcoxon rank-sum test when medians were used) were used for variable summaries and comparisons. Linear regression was used to evaluate the relationship between volume transfused and HbF%. Statistical analyses were done in the R language and environment for statistical computing (R Foundation, Vienna, Austria).

Results

Our HbF database contained 1170 infants with 2882 HbF% values. Of those, 110 infants, with 1066 HbF% values, were born before 32 weeks' gestation. Three of the 2882 HbF% values were flagged as probable errors. Each of those was a value <50%, flanked by several values ≥90% before and after the low value (sometimes these were on the same day with no transfusion). We excluded those three HbF% values from the database. **Table 1** is a demographic description of the 1170 infants from whom the remaining 2879 HbF% measurements were made.

Our first hypothesis was that prior to receiving an initial RBC transfusion, infants would have an HbF% value within the normal reference range for PMA, and after the transfusion their HbF% would be below the 2.5th percentile lower limit [19]. Indeed, in all ten infants where we had HbF% levels before and after a 20 mL/kg RBC transfusion, the HbF% values were normal (mean=**91%**, 95%CI: 86 - 96%) before the transfusion, and abnormally low (mean=**49%**, 95%CI: 42 - 56%) after the transfusion (p<0.001) (**Figure 1**).

We next analyzed the cumulative effect of repeated RBC transfusions on HbF%. We found that, after the first transfusion, every additional 20 mL/kg/RBC transfusion resulted in a further lowering of the HbF% by an additional 7% (95%CI: 4 - 8%, P<0.001). **Figure 2** illustrates the cumulative effect of RBC transfusions HbF%.

Figure 3 shows the PMA when each RBC transfusion was given, along with the ROP outcome, among the subset of infants born <32 weeks' gestation who received at least one RBC transfusion (n=17). The five infants who did not develop ROP either had

no transfusions before reaching 30 weeks PMA (n=3) or at most had only one (n=2). In contrast, the 12 who developed ROP (any stage) had a total of 29 transfusions before reaching 30 weeks PMA. RBC transfusion histories were different among the five infants who developed mild to moderate ROP *vs.* the seven who developed treatment-requiring ROP (TrROP). Those with mild to moderate ROP had fewer "early" transfusions (4 transfusions among 5 infants) than those who developed *severe* ROP (25 transfusions among 7 infants).

Figure 4 shows the same 17 infants illustrated in figure 3, scored for BPD at 36 weeks PMA. The ten infants who did not develop *severe* BPD had a total of 16 RBC transfusions administered before they reached 36 weeks PMA (1.6±1.1 transfusions/infant mean ± SD). The seven who did develop *severe* BPD had a total of 26 RBC transfusions before they reached 36 weeks PMA (3.9±1.7 transfusions/infant, p=0.004).

Discussion

Fetal erythrocytes differ from adult erythrocytes in fundamental and profound ways, including differences in oxygen binding, transport, and release to tissues, antioxidant defenses, cellular deformability, metabolism, and size [1]. When ELGANs receive several weeks of intensive care involving repeated phlebotomies for laboratory studies and multiple transfusions of adult erythrocytes, the physiology of their erythron can become significantly perturbed [1-20]. Replacing their native HbF with donor HbA likely results in the release of supraphysiologic and damaging amounts of oxygen to developing tissues, which has been associated with stepwise increases in the risks of developing ROP and BPD [3-20]. Consequently, multiple investigators have

hypothesized that ELGANs should maintain a physiologically appropriate proportion of their hemoglobin as HbF, perhaps at least through 30 to 36 weeks PMA [1-20].

In the present study we tabulated HbF% values before *vs*. after RBC transfusions. Our first observation was that every initial RBC transfusion administered – where we had HbF measurements before and after – resulted in a decline in the HbF% from a normal to an abnormal level, far below the lower reference interval for PMA. It is intuitive that transfusing adult RBC to a newborn infant would result in a fall in HbF%. However, the present findings are, to our knowledge, the first observations that transfusing 20 mL/kg of adult RBC results in a decrement in HbF from a normal value to one that is below the lower 2.5th percentile reference interval [2]. A somewhat similar report to ours was recently published by van Daelen et al [3]. Among neonates born before 30 weeks gestation, they found that administering a, adult RBC transfusion was associated with a sharp decline in HbF%, and that prematurity-related adverse outcomes were more common in those with a steeper HbF decline [3]. Moreover, Dani *et al.* and Hellstrom *et al.* reported that when Radiometer HbF% readings fell below approximately 50%, the risks of ROP and BPD increased [6, 7, 13, 17].

We also found that infants who received RBC transfusions *before* reaching 30 weeks PMA were more likely to develop ROP than were infants who received the same number of transfusions but only *after* reaching 30 weeks PMA (**Figure 3**). That observation is consistent with the findings of Teofili *et al*. who reported that a red blood cell transfusion before 28 weeks predicted severe ROP with a sensitivity of 64% and a specificity of 78% and that receiving a second transfusion before 29 weeks predicted severe ROP with a sensitivity of 75% and a specificity of 69%. They also found, by

multivariate analysis, that the “PMA at second transfusion” outperformed all other variables in predicting *severe* ROP, with an odds ratio of 4.55 (95% CI, 1.33-15.57, p = 0.016) [20].

The HbF% is likely to be lower after multiple RBC transfusions of 20 mL/kg than after a single transfusion. Thus, premature neonates who receive multiple transfusions before reaching 30 weeks would likely have a lower HbF%, and reciprocally a higher HbA%, than those who receive only one transfusion before 30 weeks. Mechanistically, the higher HbA% in multiply transfused neonates might cause more oxidative damage to the developing retinal and pulmonary microvasculature than occurs in neonates transfused only once. In keeping with that assumption, we observed, as did Hellstrom *et al*. [4, 6, 7, 15], Dani *et al.* [13, 16, 17], Prasad *et al.* [26], van Daelen *et al.* [3], Teofili *et al.* [14], and others; that more RBC transfusions were associated with more ROP [27-31] and more BPD [32-39].

The recent umbilical cord-derived RBC transfusion studies from Barcelona, Spain used HbF% as a surrogate endpoint [41-43]. For three reasons we consider that to be a very useful, pragmatic, and forward-looking approach. First, multiple observational studies have linked low HbF levels before 30 weeks PMA with increased risks of developing severe ROP and BPD [3, 6-20]. Second, validated surrogate endpoints can substantially improve clinical trial efficiency by reducing the initial study duration, cost, and sample size, compared with trials requiring long-term clinical outcomes. Established examples of pragmatic surrogate endpoints include using bone mineral density as a surrogate in studies aimed at preventing osteoporosis-related fractures [44] and using LDL cholesterol reduction as a surrogate in studies intending to prevent death from

cardiovascular disease [45]. Third, studies from Valencia, Spain using causative association analyses strongly suggested that it is HbA replacing HbF, and not the RBC transfusions themselves as an independent variable, which results in ROP risk among preterm infants [46, 47].

We recognize various shortcomings in our approach, and we realize limitations in our conclusions. For instance, as a retrospective study, our data lacks consistent timing of HbF values drawn before and after transfusion. Protocolizing the HbF drawing times in a prospective study would result in greater precision in the magnitude of HbF decrement. Also, our HbF measurements were by Radiometer, as part of clinically ordered blood gas analyses, based on HbF's unique optical absorption characteristics in multiwavelength spectrophotometry. This is a practical method for assessing HbF% because it requires no additional blood drawing or laboratory charges. Also, there is precedent for this, as Radiometer HbF readings were used for this purpose by Hellstrom *et al.* [6, 8, 9, 17], Dani *et al*. [15, 18, 19], van Daelen [3], and Torrejón-Rodriguez *et al*. [28]. Admittedly the Radiometer method lacks the accuracy of measuring HbF using high-performance liquid chromatography (HPLC) [6, 10] or capillary electrophoresis [41-43]. Although HPLC or CE methods could be used in prospective studies, it would likely require additional phlebotomy and increased costs.

In conclusion, our findings add to growing evidence implicating HbA from adult RBC transfusions as the cause of transfusion-related ROP and BPD risk. We suggest that a mechanistic explanation involves HbA-mediated release of excessive and damaging amounts of oxygen to tissues that have immature antioxidative capabilities and are vulnerable to oxidative damage [48]. Thus, we speculate, as have others, that

keeping the HbF% of ELGANs within the physiological reference range [2, 3], at least through 30 weeks PMA, could reduce the incidence of severe ROP and BPD.

Strategies to maintain a physiologically high HbF level in ELGANs early during their NICU stay include minimizing transfusion exposure through delayed cord clamping at birth; obtaining initial laboratory samples from otherwise discarded umbilical cord blood; implementing and monitoring restrictive RBC transfusion practices; and using erythropoiesis-stimulating agents and iron according to standardized protocols [22, 23]. When RBC transfusions are necessary to treat anemic ELGANs, transfusing RBC that are high in HbF and low in HbA is a potentially beneficial practice. This approach can be evaluated using umbilical cord blood from healthy term births as in the BORN trial [16], the Barcelona studies [41-43], the Valencia studies [28, 46, 47], the Netherlands studies [3, 49, 50], or our U-BET studies [51-53]. Another potential strategy is to transfuse ELGANS exclusively with adult RBC containing >90% HbF, obtained from healthy adult donors who are homozygous for deletional β-globin variants associated with hereditary persistence of fetal hemoglobin [54-58]. Although the methodologies used across these studies differ, a growing body of evidence suggests that successfully maintaining physiologic HbF levels through 30 or more weeks PMA may reduce the risk of severe ROP and BPD.

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DATA AVAILABILITY Inquiries regarding data access can be addressed to the corresponding author.

CODE AVAILABILITY Computer code used for statistical analysis is available upon written request to the corresponding author.

AUTHOR CONTRIBUTIONS Conceptualization, BBZ, TMB, RKO, RDC. Methodology, TMB, RDC. Original Draft Preparation, RDC. Data assembly and display, BBZ, TMB, RDC. Data analysis TMB, RDC. Review and Editing, BBZ, TMB, EFS, REM, JLA, SE, RKO, RDC.

COMPETING INTERESTS The authors declare no competing interests.

ETHICS APPROVAL The protocol for conducting this retrospective, deidentified, records review was approved by the Intermountain Health Institutional Review Board (1053682) and the University of Utah Institutional Review Board (00199750) with waiver of informed consent. All methods were performed in accordance with United States Department of Health and Human Services regulations, including the Health Insurance Portability and Accountability Act (HIPAA) and the Federal Policy for the Protection of Human Subjects (Common Rule, 45 CFR 46).

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Table 1. Characteristics of 1170 infants who had 2879 HbF% values recorded.

	No RBC Tx (n=1,147)	1 RBC Tx (n=11)	>1 RBC Tx (n=12)
Gestational age (w)	38.3 (36.4, 39.3)	30.6 (25.8, 35.3)	25.1 (23.9, 26.5)
Male	615 (53.6%)	4 (36.4%)	5 (41.7%)
No BPD	918 (80.0%)	2 (18.2%)	1 (8.3%)
Moderate BPD (Grade 2)	41 (3.6%)	2 (18.2%)	2 (16.7%)
Severe BPD (Grade 3)	0 (0.0%)	0 (0.0%)	7 (58.3%)
No ROP	1141 (99.5%)	9 (81.8%)	2 (16.7%)
Moderate ROP (Stage 2)	0 (0.0%)	1 (9.1%)	3 (25.0%)
Severe ROP (Stage 3)	0 (0.0%)	0 (0.0%)	6 (50.0%)

W, weeks; RBC, red blood cell; Tx, transfusion, BPD, bronchopulmonary dysplasia; ROP, retinopathy of prematurity.

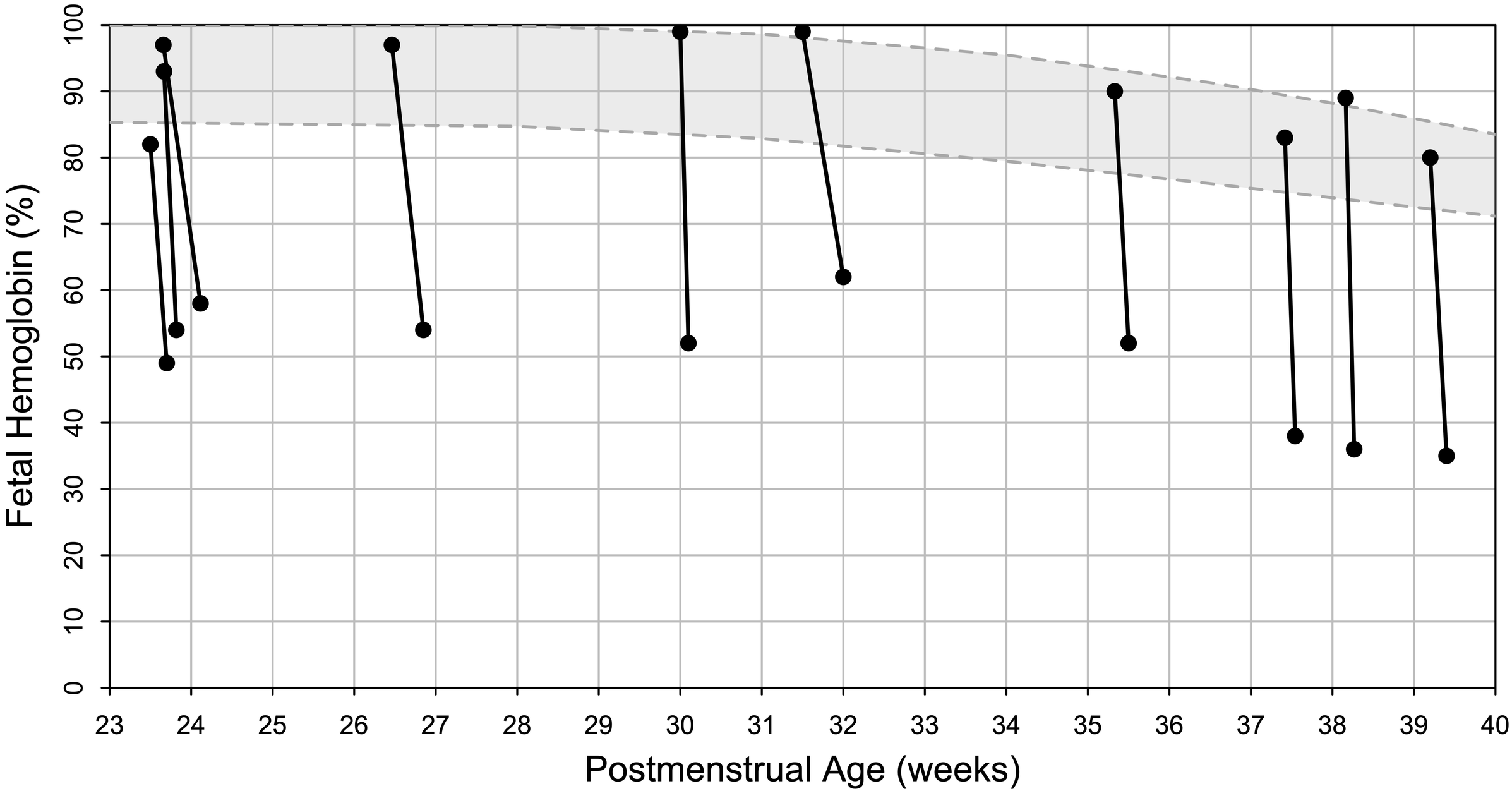
FIGURE LEGENDS

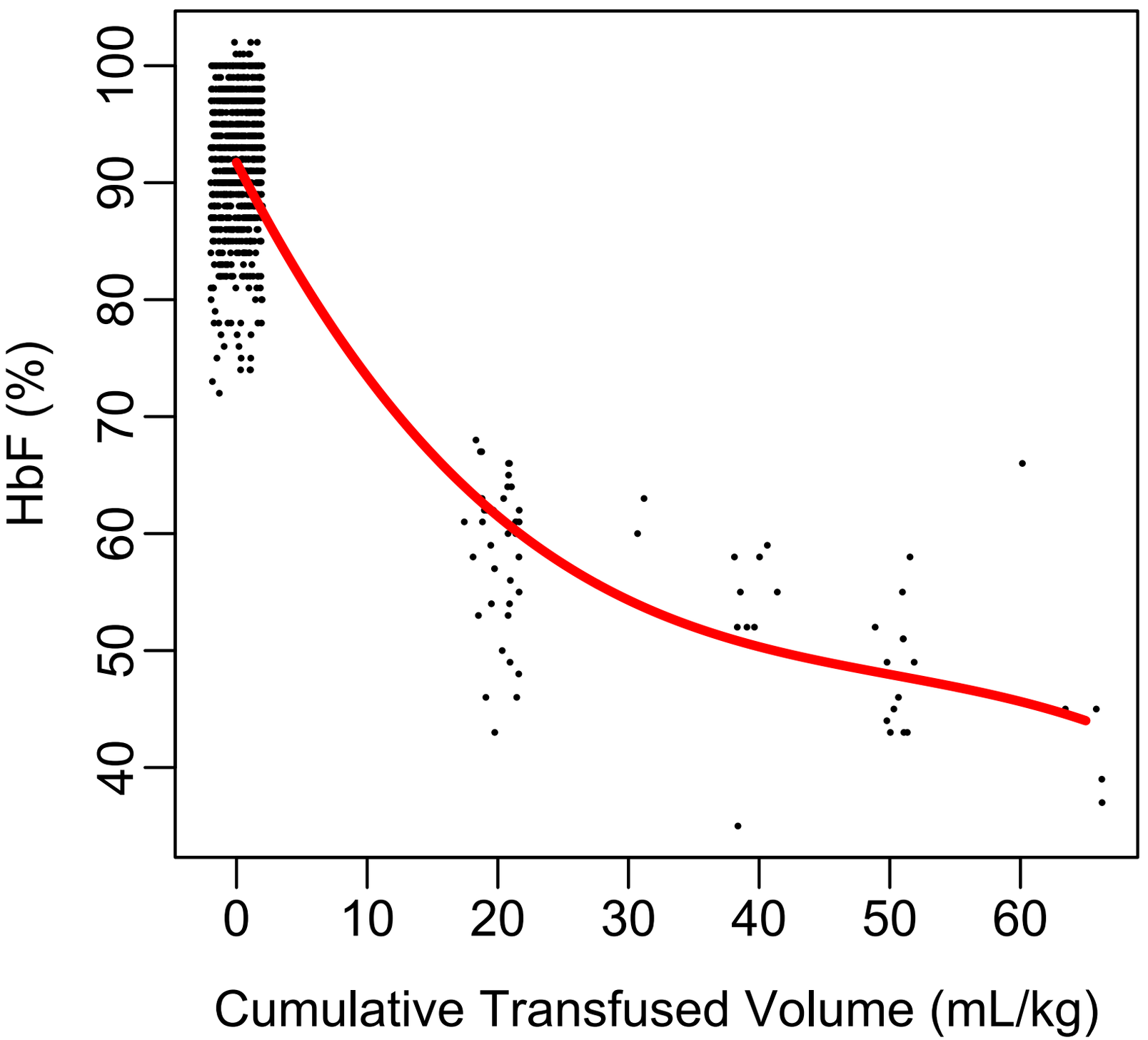
Figure 1. The effect of an initial RBC transfusion (20 mL/kg) on the HbF% value of ten infants. The ten paired values (before *vs*. after transfusion) are connected by a line. The post-transfusion HbF% varied from immediately following, up to three days after the transfusion The paired values overlay the reference interval for Fetal Hemoglobin % among non-transfused infants, shown by the shaded area [2].

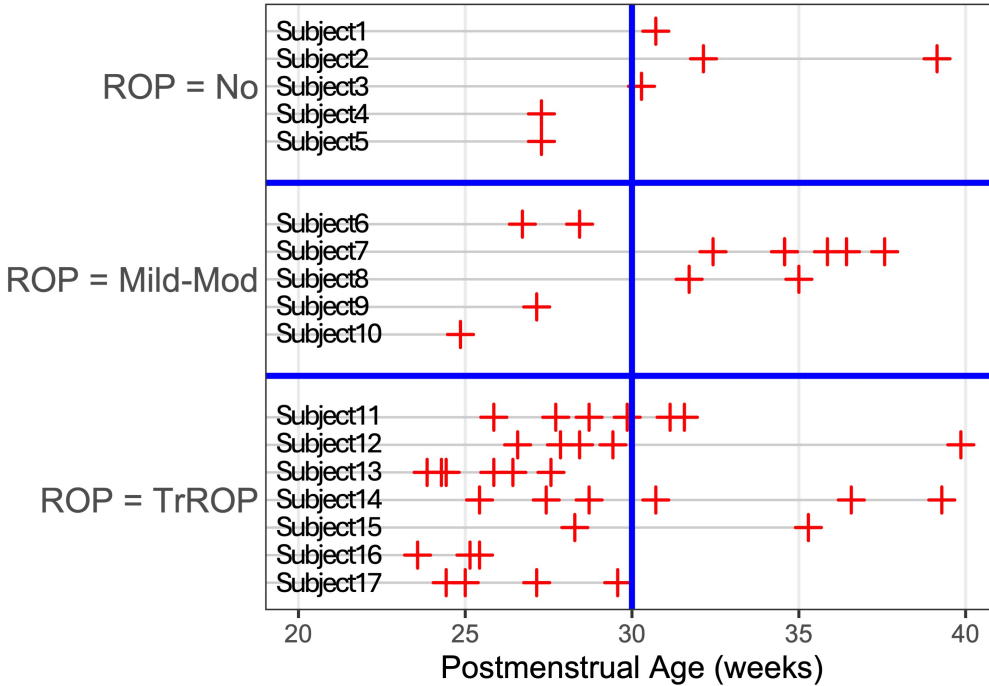
Figure 2. Cumulative effect of the initial and subsequent RBC transfusions on HbF% values of infants in a NICU. Each dot represents the HbF% measured on one patient. The red line estimates an identity relationship between HbF% and total volume (up to 60 mL/kg) of adult RBC transfusions administered during the NICU stay. The post-transfusion HbF% measurements varied from immediately following, up to three days after, transfusions.

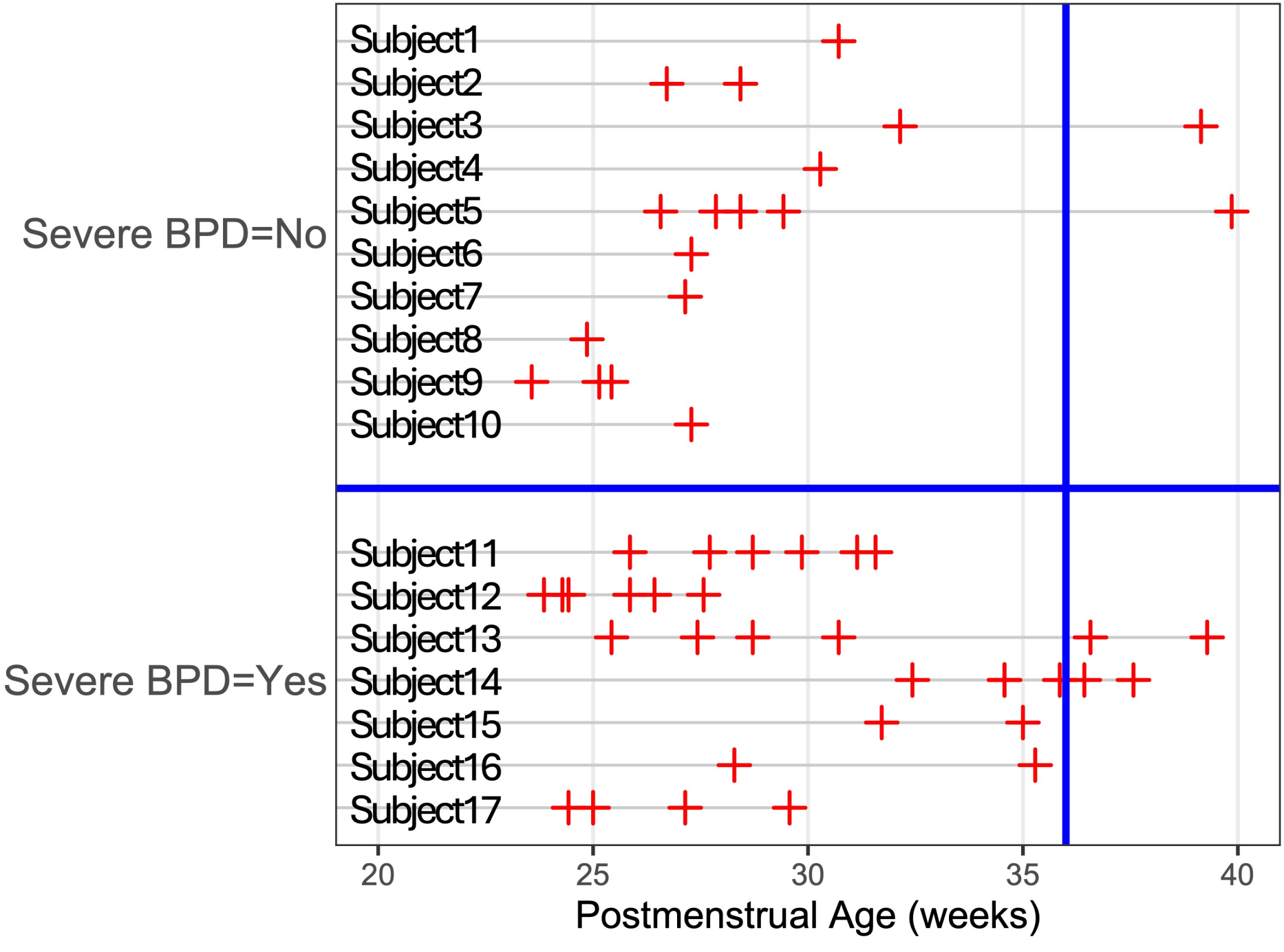
Figure 3. RBC transfusion histories and ROP outcome for 17 infants born <32 weeks' gestation who received at least 1 transfusion. Each line represents a different infant. The postmenstrual ages (PMA) at which each transfusion was administered are shown by red crosses. Five infants did not develop ROP (upper portion), 5 developed mild-moderate ROP not requiring treatment (middle portion), and 7 developed severe, treatment-requiring ROP (TrROP, lower portion). Infants who did not develop ROP had at most one transfusion before reaching 30 weeks PMA. With the exception of one, infants who developed severe, TrROP had multiple transfusions before reaching 30 weeks PMA (and several were before 28 weeks).

Figure 4. RBC transfusion histories and BPD outcome (determined at 36 weeks PMA) for 17 infants born <32 weeks' gestation who received at least 1 transfusion. Ten infants did not develop severe (Grade 3) BPD (upper portion) and seven did (lower portion). The postmenstrual ages at which each transfusion was administered are shown by red crosses. The 10 infants who did not develop severe BPD had a total of16 transfusions (mean 1.6 each) before reaching 36 wks PMA. The 7 who developed severe BPD had a total of 26 transfusions (mean 4.1 each) before reaching 36 wks.


Fetal Hemoglobin (%)
0
10
20
30
40
50
60
70
80
90
100
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
Postmenstrual Age (weeks)


HbF (%)
40
50
60
70
80
90
100
0
10
20
30
40
50
60
Cumulative Transfused Volume (mL/kg)


ROP = No
ROP = Mild-Mod
ROP = TrROP
Subject1
Subject2
Subject3
Subject4
Subject5
Subject6
Subject7
Subject8
Subject9
Subject10
Subject11
Subject12
Subject13
Subject14
Subject15
Subject16
Subject17
20
25
30
35
40
Postmenstrual Age (weeks)


Severe BPD=No
Severe BPD=Yes
Subject1
Subject2
Subject3
Subject4
Subject5
Subject6
Subject7
Subject8
Subject9
Subject10
Subject11
Subject12
Subject13
Subject14
Subject15
Subject16
Subject17
20
25
30
35
40
Postmenstrual Age (weeks)