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Fetal hemoglobin levels in premature newborns. Should we reconsider transfusion of adult donor blood?

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ABSTRACT

Purpose: The aim of this study was to assess the percent decrease in fetal hemoglobin (HbF) after transfusion of adult-derived donor packed red blood cell (pRBC) units in extremely low gestational age newborns (ELGANs).

Methods: Control percent fetal hemoglobin (%HbF) levels were measured in newborn cord blood or peripheral blood samples in non-transfused patients prior to elective surgery. ELGANs were followed prospectively and %HbF was measured on residual post-test complete blood count (CBC) specimens. ELGAN %HbF values were compared to the control population and transfusions were recorded.

Results: Initial mean %HbF in ELGANs (n=16) was 92.2±1.3% (range 90.2-95.1%), which is similar to the control group (n=25). Mean levels dropped to 61.1±11.1% (range 34.2-73.2%) after a single pRBC transfusion (n=9) and to a mean of 35.6±6.3% after an additional transfusion (n=5). %HbF levels trended upwards if no additional transfusions were given, but levels still remained lower than expected for gestational age through discharge (n=85 samples).

Conclusions: Percent fetal hemoglobin concentrations in ELGANs decrease precipitously after transfusion with adult donor pRBCs. Further studies are needed to evaluate the benefit of maintaining higher fetal hemoglobin concentrations in these patients and whether administration of HbF rather than adult donor pRBCs would improve patient outcomes.

1. Introduction

Prematurity is a leading cause of morbidity and mortality amongst newborns, with extremely low gestational age newborns (ELGANs, born less than 28 weeks' gestation) at highest risk [1–3]. Transfusions of packed red blood cells (pRBC) are common in this population but are not without risk [4]. Frequent blood transfusions in preterm neonates are associated with numerous morbidities and have been linked with the predictable complications of prematurity, including necrotizing enterocolitis, bronchopulmonary dysplasia, retinopathy of prematurity and intraventricular hemorrhage [5]. Adult donor blood is typically used for transfusion. However, despite the common practice of transfusing ELGANs with adult hemoglobin, no studies have evaluated how this practice impacts the physiologic makeup of ELGANs' circulating hemoglobin.

There are important biological and chemical differences between the transfused adult hemoglobin and the neonates' intrinsic fetal hemoglobin [6,7]. The fetal hemoglobin tetramer is made up of two alpha and two gamma globulin subunits (HbF, $\alpha 2\gamma 2$), which is distinct from the adult hemoglobin tetramer containing two alpha and two beta subunits (HbA, $\alpha 2\beta 2$) [6]. This structural difference is important because it alters chemical and biological behavior. For example, 2,3-diphosphoglycerate (2,3-DPG), a metabolic intermediate that results in reduced oxygen affinity, does not bind as effectively to the γ chain of HbF. This altered binding results in a left shift in the Hgb dissociation curve, allowing HbF to hold on to oxygen more tightly than HbA [8]. The presence of fetal hemoglobin *in utero* is necessary to facilitate transfer of oxygen from the mother to the fetus. After birth, HbF is gradually replaced by HbA, with less than 2% of total hemoglobin being HbF by one year of age [9]. In preterm neonates, HbF remains the major form of hemoglobin produced up to approximately term corrected gestation [10]. Differences in oxygen binding affinity between HbF and HbA have major implications for tissue oxygena-

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tion, and could contribute to some of the morbidities experienced by ELGANs, although this has yet to be studied.

We aimed to investigate how transfusion of ELGANs with adult-derived donor packed red blood cells (pRBCs) impacted the constitution of their circulating blood volume. In this pilot study, we evaluated the percent HbF (%HbF) in a control group of non-transfused infants and compared it to %HbF in ELGANs. We hypothesized that ELGANs who received adult blood transfusions would have a lower percentage of fetal hemoglobin and a higher percentage of adult hemoglobin than ELGANs of the same gestational age who were not transfused.

2. Methods

This prospective cohort study measured the percentage of fetal hemoglobin (%HbF) in three different cohorts of babies between November 2018 and May 2020. Approval was obtained through the University of Michigan Institutional Review Board (HUM00150811). The first two cohorts made up a control group that consisted of blood samples from non-transfused infants, including post-partum cord blood from newborns of various gestational ages and blood taken when intravenous (IV) lines were placed on healthy infants under 6 months of age undergoing elective operations. The third cohort, the study group, consisted of blood from extremely low gestational age newborns (ELGANs) who were born prior to 28 weeks' gestational age and who were being treated in our neonatal intensive care unit (NICU). Samples were all assigned a unique study code in order to de-identify the data.

2.1. Control groups

Samples from the cord blood cohort were obtained from the University of Michigan's Biorepository for Understanding Maternal and Pediatric health (BUMP). A study team member was notified by Obstetrics and Gynecology research staff if a postpartum cord blood sample was available. Samples were eligible for this study if they were from term or preterm infants who did not have a prenatal history of anemia or cardiac defects. After eligibility was verified, the sample was collected and processed within 24 hours. Samples from 4 different gestational age ranges were collected: 24-28 weeks', 29-32 weeks', 33-36 weeks', and 37+ weeks' gestation age.

For the elective operation cohort, the surgery schedule at C.S. Mott Children's Hospital was reviewed 1-2 times weekly to screen for eligible subjects who were undergoing elective surgery and who were otherwise healthy infants. If an eligible subject was identified, a study member would approach the parent/guardian(s) of the patient to obtain consent for participation in the study. If consent was obtained, the study member would ask the anesthesiology providers to draw approximately 1 mL of blood off the IV line of the patient. If there was no plan for an IV line or the draw was unsuccessful, the patient was eliminated from the study. Successfully collected samples were sent to the clinical chemistry laboratory immediately for %HbF testing. Each enrolled subject was assigned a unique study code in order to de-identify the data. Patients enrolled in this cohort were <6 month of age. Exclusion criteria included patient age older than 6 months of age, history of cardiac anomalies, history of anemia with or without need for transfusions, and lack of parental/guardian consent.

2.2. Study group

For the study cohort of ELGANs, NICU admissions were screened 2-3 times weekly for eligible subjects, which included those born <28 weeks' gestation without a congenital cardiac diagnosis. Patients were excluded if blood transfusion occurred prior to attempted enrollment. When a subject was identified, a study team member would approach the parent/guardian(s) to obtain consent. After consent was confirmed, the charts were screened for complete blood count (CBC) sample collections. To avoid oversampling, each patient ideally had only a single CBC sampled per week unless there was a transfusion noted. If a transfusion occurred, attempts were made to capture the CBC sample directly before and after. The team would request that any residual blood from the samples were sent to the clinical chemical laboratory for %HbF quantification. All pRBC transfusions that were administered to the ELGANs were recorded and timing of the transfusions with the corresponding CBC samples was documented. No non-routine additional blood draws were performed for the expressed purpose of collecting %HbF data from this study cohort.

2.3. Data analysis

For all samples, %HbF was measured by high performance liquid chromatography (HPLC) on a Bio-Rad Variant II Hemoglobin Testing System using the β-thalassemia short program (Bio-Rad Laboratories, Inc: Hercules, CA) at the University of Michigan Hospital System Laboratory in Ann Arbor, MI. MiChart (Epic Systems: Verona, WI) was used to obtain data from patients' electronic health records (EHR). Data was collected in Microsoft Excel (Microsoft Corporation: Redmond, WA) for each enrolled subject. GraphPad Prism 8.0 (GraphPad Software: San Diego, CA) and SPSS 26 (IBM Corp: Armonk, NY) were used for data analysis. The two control sources were combined and a bivariate logistic regression curve was fitted to the data using SPSS 26 to determine the dependence of %HbF on gestational age for otherwise healthy, non-transfused infants. The equation is as follows: $Y = 1 / (1/u + (\beta_0 * (\beta_1{}^t)))$ where u=100; $\beta_0 = 4.73 \times 10^{-6}$; β_1 =1.18; t=individual gestational age. The ELGAN data was graphed next to the interpolated control %HbF for comparison and this data was superimposed on graphs using PRISM. Paired Student t-tests were used to determine the significance between observed and predicted %HbF within each subgroup (transfused versus non-transfused records from the study cohort) [11]. Analyses of significance were conducted using SPSS 26 with $p<0.05$.

3. Results

3.1. Control group

The cord blood samples (n=17) and the elective surgery samples (n=8) yielded 25 %HbF values to be plotted against gestational age in non-transfused infants (**Fig. 1**). The interpolated lo-

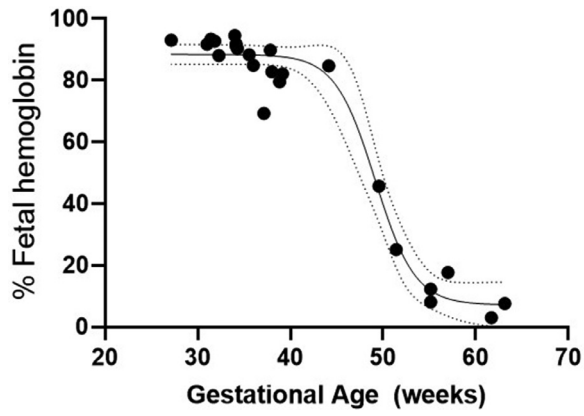

Fig. 1. Graphical representation of cord blood (n=17) and elective surgery samples (n=8) resulting in a control curve of %HbF at various gestational ages.

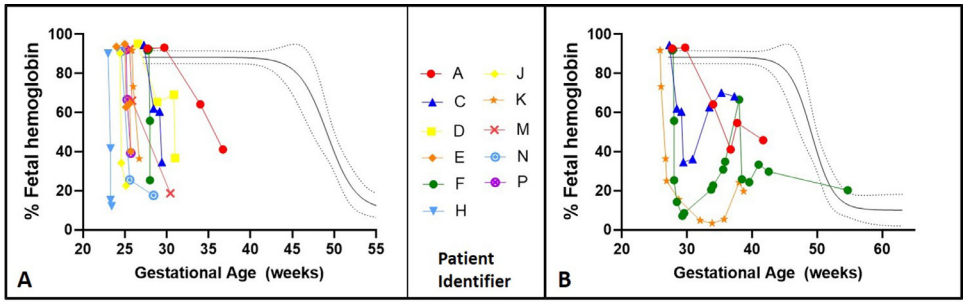

Fig. 2. Graphical representation of the study group of ELGANs (n=11) after initial transfusions (A) and respresentative examples of longitudinal data through discharge (B, n=4). Each line represents a single ELGAN patient and patient identifiers are noted in the middle.

gistic curve had a 95% confidence band and an $R^2 = 0.93$, indicating adequate fit to the control data. These results are consistent with normal %HbF for gestational age reported in the literature [12]. This method also created a calibrated standardized curve such that the %HbF of both study group and control data were obtained, stored, processed and calculated in the same process within the same facility. This resulting curve was then used to interpolate the expected %HbF for each gestational age of the study group data.

3.2. Study group

Sixteen eligible ELGANs were identified and included in the study cohort from April 2019 through March 2020. Median age was 25.8 weeks (range 22w6d-27w1d) and 63% (10/16) were female. The mean initial %HbF (reported with standard deviation here and subsequently) for the group, prior to any transfusion, was 92.2 ± 1.3% (range 90.2-95.1%). Two ELGANs (A and B) had specimens collected from both cord blood (included sample in "control group") and postnatal CBC (included sample in "study group"). These samples were separated by 4 days and were similar as the infants were not transfused in the first 4 days of life (cord blood 93.0 and 93.1% with CBC at 4 days of life 92.7 and 92.5%, respectfully). There were no other crossover patients.

Of the sixteen enrolled patients, four did not have follow up data due to early death, transfer, or COVID-19 pandemic-related suspension of certain research activities. The remaining twelve have follow-up blood samples that allowed for continued %HbF measurements before and after pRBC transfusions were given. Nine ELGANs had a single pRBC transfusion with mean %HbF dropping to 61.1 ± 11.1% (range 34.2-73.2%). Five ELGANs had a second single pRBC transfusion with mean %HbF dropping to 35.6 ± 6.3% (range 25.4-41.1%) and four had multiple subsequent pRBC transfusions (2-3) dropping the mean %HbF to 29.3 ± 10.1% (range 18.7-39.3%). Two ELGANs (H and N) had a total of 5 transfusions and %HbF dropped to 15.3% and 17.6%, respectively. A single ELGAN (L) never received a transfusion during the hospital stay and %HbF levels remained within the expected range (87.7-91.8%, n=3 blood draws) (**Table 1**). **Fig. 2A** shows the graphical representation of the 11 ELGANs with follow-up %HbF data available after pRBC transfusions. **Fig. 2B** shows a representative sample of 4 ELGANs that were followed throughout their hospital stay. Once transfusions stopped, the %HbF increased to levels toward the control curve until the next transfusion, a finding that is particularly evident with patients C, E, F, J, M, and N (**Supplemental Figure 1**). This strongly suggests that transfusion is the major cause of low %HbF levels in these ELGANs rather than a complication of their prematurity. Of note, even with pauses in transfusion, %HbF levels did not recover to normal ranges for gestational age by the time of discharge, likely due to persistence of transfused adult donor RBCs. **Table 1** lists the highest %HbF for each patient after the initial drop with transfusion showing that many increased their %HbF while hospitalized.

To demonstrate the effects of pRBC transfusions on %HbF, a two-sample paired t-test was performed which compared each ELGAN's observed %HbF to the predicted normal range %HbF within transfused and non-transfused data subgroups. Statistical differences were detected for each group but the mean difference was notably larger in the transfused group. Prior to transfusions, the ELGAN data points (n=24 patient samples) had a mean difference of -3.67% ($p < 0.001$) (**Fig. 3A**). However, after transfusions, the ELGAN data points (n=85 patient samples) had a mean difference between observed and predicted %HbF of -52.75% ($p < 0.001$) (**Fig. 3B**).

4. Discussion

Fetal hemoglobin (HbF) is the predominant hemoglobin throughout fetal development. HbF makes up 90% of the circulating hemoglobin around 24 weeks' gestation, declining to 70-80% during the third trimester [6]. After birth, there is a switch from production of HbF to HbA, and HbF levels decline to <2% of total hemoglobin levels by 6-12 months of age, commensurate with adult levels [13]. When babies are born prematurely, their fetal hemoglobin percentage is comparable to a similar gestational-age fetus [14]. Extremely low gestational age newborns (ELGANs, born <28 weeks' gestation) are born with approximately 90% HbF, as expected for their gestational age [15].

All newborns experience a transient decline in total hemoglobin levels, known as physiologic anemia of infancy. This decline persists for the first couple of months of life [6]. For ELGANs, this decrease in total hemoglobin is much more pronounced [16]. Severe anemia can be life-threatening, so many ELGANs receive transfusions of adult donor pRBCs to mitigate this risk. These transfusions are associated with many known complications of prematurity, including necrotizing enterocolitis, bronchopulmonary dysplasia, retinopathy of prematurity (ROP) and intraventricular hemorrhage [17]. This study suggests that even one transfusion of adult blood significantly changes the physiologic makeup of an ELGAN's circulating blood by causing a substantial decrease in the %HbF in the blood and subsequent increase in the percentage of adult hemoglobin (%HbA) [17]. The introduction of HbA to the premature infant and the subsequent increase in %HbA and decrease in %HbF could result in excessive unloading of oxygen to the tissues due to differences in oxygen binding affinity. This could produce oxidative stress and reduce angiogenesis, contributing to the development of the afore-mentioned morbid conditions, the so-called 'oxygen radical disease of neonatology' [18,19]. In fact, one pilot prospective cohort study demonstrated that a higher ratio of adult hemoglobin to fetal hemoglobin in very preterm neonates was correlated with the development of ROP. This study found that preterm neonates were more likely to develop ROP if they received more frequent blood transfusions during their stay in the NICU ($p < 0.001$) and if they had an overall lower mean %HbF ($p < 0.001$) [20].

Table 1
Demographics of study participants with percentage of fetal hemoglobin (%HbF) before and after transfusions.

PatientIdentifier	Gestational Age at birth	%HbF prior to transfusion(GA)	After initial transfusion(# of transfusions)	After subsequent transfusion(# of subsequent transfusions)	Highest %HbFafter initial transfusions(GA)	Follow-up
Single Transfusion followed by 2nd Single Transfusion						
A	27w1d	93.2 (29w5d)	64.2 (1)	41.1 (1)	54.7 (37w5d)	Discharged 42w4d
C	25w6d	94.5 (27w2d)	62 (1)	34.6 (1)	70.1 (35w2d)	Discharged 37w6d
D	26w4d	95.1 (26w4d)	65.4 (1)	36.7 (1)	59.4 (35w1d)	Discharged 37w2d
E	23w6d	95.0 (25w0d)	62.8 (1)	40.2 (1)	77.8 (37w4d)	Discharged 41w4d
F	27w4d	92.3 (27w6d)	55.8 (1)	25.4 (1)	66.6 (38w0d)	Discharged 60w5d
Single Transfusion followed by Multiple Transfusions						
J	24w3d	90.3 (24w3d)	34.2 (1)	22.6 (2)	64.8 (38w5d)	Discharged 42w5d
K	25w5d	91.9 (25w6d)	73.2 (1)	36.4 (2)	24.2 (38w0d)	Discharged 51w0d
M	24w4d	93.3 (25w5d)	66.1 (1)	18.7 (3)	48.9 (40w3d)	Discharged 40w5d
P	25w0d	92.2 (25w1d)	66.6 (1)	39.3 (2)	39.3 (25w5d)	Discharged 49w6d
Multiple Transfusions						
H	22w6d	90.2 (23w0d)	41.6 (2)	15.3 (3)	n/a	Died
N	24w4d	91.3 (24w4d)	25.5 (3)	17.6 (2)	41.7 (40w3d)	Discharged 41w5d
Never Transfused						
L	27w1d	91.2 (27w4d)	n/a	n/a	91.8 (30w5d)	Discharged 36w5d
Lacking follow-up due to death, transfer, or COVID-19						
B	27w1d	92.5 (27w5d)	n/a	n/a	n/a	Died
G	22w6d	91.3 (23w0d)	n/a	n/a	n/a	Died
O	25w6d	92.1 (25w6d)	n/a	n/a	n/a	Transferred 28w1d
Q	27w1d	91.9 (27w2d)	n/a	n/a	n/a	Discharged 40w4d

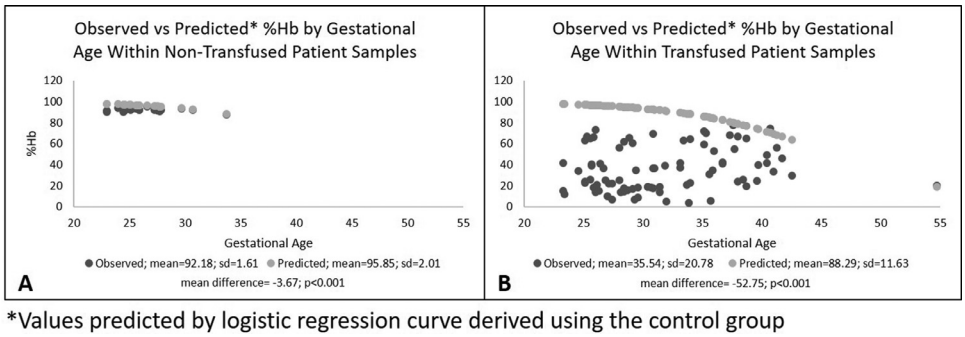

Fig. 3. Graphical representation of the observed versus predicted %HbF by gestational age in ELGANs before (A, n=24 blood draws) and after (B, n=85 blood draws) transfusion.

Fetal and adult hemoglobin have numerous distinct molecular properties in addition to differences in oxygen binding affinity. Fetal hemoglobin is less likely to oxidize, has a greater capacity to create nitric oxide, and is less likely to undergo DNA cleavage. All of these properties may be of benefit to a fragile premature infant. Additionally, residual leukocytes found in umbilical cord blood have phenotypes that are more immunotolerant and less inflammatory compared to those found in adult donor blood [18]. Our results demonstrate that premature neonates develop a significant drop in the percentage of HbF after only a single transfusion, so it is plausible that these molecular and biologic distinctions could contribute to transfusion-associated complications experienced by premature infants. We also demonstrate that premature infants have an increase in %HbF after transfusions cease, which implies that a high concentration of HbF is biologically appropriate for this patient population.

To avoid the physiologic change in %HbF associated with pRBC transfusions, others have attempted to either avoid transfusion or utilize umbilical cord blood for transfusion. The practice of delayed cord clamping (DCC), in which fetal blood is allowed to continue to flow from the placenta and the umbilical cord into the infant for more than 30 seconds after birth, results in an increase in HbF in the preterm infant. A Cochrane review found that DCC in preterm infants resulted in decreased need for blood transfusion, decreased intraventricular hemorrhage, and lower risk of necrotizing enterocolitis [21]. DCC and the administration of erythropoietin are strategies that have reduced the quantity of adult red blood cell transfusions given to premature infants, but have not eliminated them. The collection of autologous umbilical cord blood from a premature infant poses many logistical challenges, and typically does not produce adequate volumes of blood to meet a single premature infant's transfusion requirements [22,23]. Another novel alternative is the transfusion of allogeneic umbilical cord blood that is collected and stored after the birth of healthy term neonates. Bianchi, et al. have demonstrated the safety and feasibility of this therapy in Italy, but larger studies are needed to demonstrate efficacy and evaluate long-term outcomes [24].

While our study demonstrates that %HbF levels significantly drop after ELGANs are transfused with adult donor pRBC, it is not without limitations. Our sample size is not large enough to asso-

ciate the drop in %HbF with any physiologic changes. All institutional research was suspended in March 2020 due to the COVID-19 pandemic and this interrupted our ability to collect follow-up samples on enrolled subjects or recruit any further patients lending to the small sample size. A second limitation is that we were unable to collect and test for %HbF in the control and study group patients at the same gestational ages. Instead, the control value was interpolated from data of otherwise healthy newborns/infants and may not adequately reflect a non-transfused ELGAN in the intensive care unit. We acknowledge that these two groups are not matched other than corrected gestational age. The considerable challenge of obtaining a true prospective control group of non-transfused ELGANs is well recognized, as the vast majority of ELGANs will be transfused during their NICU stay [25,26]. We do bring attention to the single ELGAN, patient L, who was treated longitudinally in the intensive care unit and had %HbF levels that remained on the control curve prior to discharge as an example of a non-transfused ELGAN. Lastly, the CBC collection in the ELGANs was dependent upon when the clinical care team ordered the test and was not uniform within the group. Future investigations could consider comparing post transfusion %HbF after a randomized adult or fetal hemoglobin transfusion to further delineate the effects of the current standard of care. Furthermore, while this pilot study was the first to demonstrate a change in physiologic hemoglobin in ELGANs who received adult donor pRBCs, further investigations are warranted to determine the clinical consequences of these findings.

5. Conclusion

Percent fetal hemoglobin drops precipitously when neonates are transfused with adult donor blood. Fetal hemoglobin levels increase when transfusions cease, suggesting that normal physiology for a preterm neonate includes a high concentration of fetal hemoglobin. In order to maintain this physiology, blood transfusions rich in fetal hemoglobin rather than adult hemoglobin should be explored.

Author contribution

Study Conception and Design. SHL, ARC, JRB, GBM, RBH, EEP
Acquisition of Data. AEG, SHL, EEP
Analysis and Interpretation of Data. AEG, DD, EEP
Drafting of Manuscript. AEG, DD, EEP
Critical Revision of Manuscript. ARC, JRB, SHL, GBM, RBH

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jpedsurg.2021.04.018.

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