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Partial manual exchange reduces iron accumulation during chronic red cell transfusions for sickle cell disease

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Abstract

Iron overload is an inevitable consequence of chronic red cell transfusions without erythrocytapheresis or chelation therapy. The effectiveness of partial manual exchange, a technique used to slow iron loading, has not been evaluated. We evaluated all children with sickle cell disease (SCD) receiving chronic transfusion to identify chelation naïve subjects who had quantitative liver iron concentration (LIC) studies. Seventeen chelation naïve children with SCD received a median of 29 transfusions prior to first LIC determination. Serum ferritin concentrations were assessed prior to each transfusion. The mean volume of blood phlebotomized prior to each transfusion was 5.1 ± 1.8 cc/kg, which cumulatively resulted in a calculated median 35.0 mg/kg removal of iron. Using linear regression, pretransfusion phlebotomy resulted in a statistically significant reduction in ferritin (-8.8 ng/mL of ferritin for each mg/kg of iron phlebotomized, $P = 0.02$). A reduction in LIC from pretransfusion phlebotomy could not be established ($P = 0.4$). Partial manual exchanges appear to be an effective strategy for slowing the pace of iron loading in the setting of chronic transfusion for SCD.

Keywords

Sickle cell; iron loading; transfusion; iron; ferritin

Introduction

Chronic red cell transfusion is a commonly used therapy for primary prevention of stroke and secondary prevention of stroke and other sickle cell disease (SCD) complications. In particular, the success of chronic red cell transfusion for the primary and secondary prevention of stroke has led to an increase in the number of children and adults receiving red cell therapy over the last 20 years¹⁻³. As research into other roles for chronic red cell transfusion in SCD continues, the number of indications for chronic transfusion may continue to increase^{4,5}.

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An inevitable consequence of chronic red cell transfusion without erythrocytapheresis chelation therapy is iron overload. The sequelae of transfusional iron overload on the liver, heart, and endocrine organs are well described, particularly in patients with chronically transfused thalassemia⁶⁻⁸. Automated red cell exchange (erythrocytapheresis) and partial manual exchange are two transfusion methods that have been used to slow the progression of transfusional iron overload. Erythrocytapheresis has been shown to even reverse iron accumulation⁹⁻¹².

Partial manual exchange involves phlebotomy immediately prior to transfusion. In theory, serial partial manual exchanges should maintain low sickle hemoglobin concentrations comparable to chronic simple transfusion, but with less net iron loading; however, there are no data that quantify the effectiveness of this procedure. Partial manual exchange is a widely used technique that consumes time and resources, so data supporting its use are needed. Furthermore, it is conceivable that pre-transfusion phlebotomy could contribute to more pronounced anemia and result in transfusion of larger volumes of blood, minimizing reductions in iron loading.

Our primary hypothesis is that partial manual exchange slows iron accumulation in the setting of chronic transfusion for SCD. The goal of this study is to quantify the effect that partial manual exchange has on the rate of iron accumulation, as measured by liver iron concentration (LIC) measurements and by serum ferritin.

Materials and Methods

Subjects

Subjects were retrospectively identified from the group of patients with SCD receiving chronic red cell transfusion at Johns Hopkins Hospital. Blood bank, pathology, and radiology records were cross-referenced to identify appropriate subjects. As iron chelators would be a significant confounder of the effects of partial manual exchange on iron status, patients with LIC measured by biopsy or MRI were included only if there was no exposure to chelating medication prior to initial iron measurement. Subjects were negative for HIV and viral hepatitis. The study was approved by the Johns Hopkins University School of Medicine IRB.

Transfusion

Phlebotomy was performed by nursing staff using syringes to draw off a pre-specified volume of blood immediately prior to transfusion. Target phlebotomy volumes were 7-10 cc/kg, depending on patient tolerance. Transfusion volume was ordered to be 14cc/kg if pre-transfusion hemoglobin was 9.5-10 g/dL, 16 cc/kg if pre-transfusion hemoglobin was 9.0-9.4 g/dL, and 18cc/kg if pre-transfusion hemoglobin was <9.0. The pre-transfusion targets were hemoglobin of 10 g/dL and percent HbS of <30%. Prophylactic red cell phenotype matching was performed only for subjects with a red cell alloantibody, in which case red cell units were phenotypically matched for all clinically significant red cell antigens¹³.

Data

Data were extracted from the hospital medical record and transfusion data systems. All data were validated against the paper transfusion shadow chart and flow sheets. Sporadic transfusion prior to the start of chronic transfusion, if applicable, was accounted for in the total transfusional iron burden.

Statistical analysis

The main analyses evaluated the effect of phlebotomized iron on LIC and serum ferritin. The amount of iron phlebotomized was estimated for each transfusion as follows:

$$\text{volume phlebotomized (dL)} * \text{pre-transfusion hgb (g/dL)} * 0.003463$$

The factor 0.003463 is the fraction of hemoglobin mass that is iron, based on the stoichiometry of 4 mols of iron (223.38g) per 1 mol of hemoglobin (64,500g). The iron content of transfused blood is estimated to be 0.75 mg per cc transfused (225 mg iron per 300cc unit), which is calculated from a red cell unit hematocrit of 65% (hemoglobin 21.7g/dL) and the stoichiometric iron content factor of 0.003463. Normalization of transfusion data for body weight was performed using the weight at the time of transfusion. The assumption was made that erythrocytapheresis procedures had no net effect on iron balance.

Linear regression was used to estimate the effect of partial manual exchange on LIC, for which a single value was available for each subject. Partial manual exchange variables were defined as the amount of iron phlebotomized prior to transfusion and the amount of iron transfused. Longitudinal linear regression was used to estimate the effect of partial manual exchange on repeated measures of ferritin, until a ferritin of 1,000 ng/mL was reached. Spearman correlations were used to assess the relationship between ferritin values and iron loading or transfusion volumes. For correlation analysis using ferritin, a running average of three consecutive ferritin values (ferritin value at pre-transfusion testing and values ± 1 month) was used to reflect more stable trends in ferritin.

Results

Clinical characteristics

Seventeen subjects were chelation therapy naïve at the time of initial LIC assessment and were included in the analysis. The 17 subjects received 487 transfusions (median 29 transfusions, range 18-49) before the initial LIC assessment. Subjects underwent pre-transfusion phlebotomy in 410 (84.2%) transfusions. The average ($\pm$ SD) pre-transfusion hemoglobin was 9.4 ± 0.9 g/dL with an average pre-transfusion HbS% of $24.9 \pm 14.7\%$. The amount of pre-transfusion phlebotomy varied across patients. Individuals received an average of 2.2 to 6.6 cc/kg phlebotomized per transfusion over the course of each patient's transfusion course. Of the 487 transfusions, 19 (3.9%) were erythrocytapheresis procedures, 13 of which were performed at initiation of chronic transfusion therapy. The demographic and clinical features of these subjects are shown in Table 1. The youngest patient (0.7 years) experienced a code event and stroke in the setting of a severe pulmonary infection. All subjects had one LIC measurement: 11 had liver biopsies with iron concentrations determined by inductively coupled plasma-mass spectrometry, and 6 had liver MRI (Ferriscan)¹³ for quantitation of liver iron burden.

Effect of pre-transfusion phlebotomy on LIC

The median LIC was 11.0 mg iron/g dry liver weight (range 5.1-27.8 mg/g). A summary of iron transfused and phlebotomized is shown in Table 2. The ratio of iron transfused to iron phlebotomized was similar using both absolute and weight normalized iron values. Using linear regression and adjusting for the amount of iron transfused per kg, there was no statistically significant effect of pre-transfusion phlebotomy on LIC (95% CI -0.2 to 0.47 mg/g change in dry liver weight for each mg/kg iron removed through pre-transfusion phlebotomy, $P=0.4$). Inclusion of the entire partial exchange cohort ($n=30$), including 13 children on chelation prior to LIC determination, did not materially change the estimate of

the effect of pre-transfusion phlebotomy: (95% CI -0.06 to 0.19 mg/g change in dry liver weight for each mg/kg iron removed, $P=0.3$)

Net iron loading (iron transfused/kg – iron phlebotomized/kg) is an additional measure that can be correlated with LIC. Among cumulative transfusion metrics, accounting for iron removed through mg/kg of net iron loading resulted in the most accurate correlate of LIC (Table 3).

Effect of pre-transfusion phlebotomy on rate of ferritin rise

Ferritin was measured monthly as part of routine pre-transfusion testing and provides additional data for assessing the effect of pre-transfusion phlebotomy on iron loading. Ferritin values increase with iron loading, particularly at lower values¹⁴. For 15 subjects, the initial ferritin values were less than 1,000 ng/mL, and these data were used in a longitudinal linear regression, accounting for amount of iron transfused. There was a statistically significant relative decrease of -8.8 ng/mL of ferritin for each mg/kg of iron phlebotomized (95%CI -16.0 to -1.7 ng/mL, $P=0.02$).

Discussion

This study provides evidence that supports the effectiveness of the partial manual exchange technique to reduce the rate of iron loading. The rate of ferritin increase is lowered when pre-transfusion phlebotomy is used. LIC is better correlated with net iron loading than total iron loading.

We did not find an association between extent of phlebotomy and changes in LIC despite finding the hypothesized inverse association between phlebotomy and ferritin. The absence of an association may be due to the small number of LIC values used in analysis compared to the number of ferritin values. Additionally, the association of ferritin and LIC is indirect, with weak linear correlation^{7,14-17}, so there is not necessarily a robust association between ferritin and LIC.

Partial manual exchange is a chronic transfusion technique that is feasible in any center that provides chronic transfusion. How widely the procedure is used varies with venous access of the patient and time available to staff to perform the phlebotomy. Of the parameters evaluated in this study, our results suggest that net iron transfused per kg will predict LIC most accurately, and this measure can be easily calculated and followed longitudinally, along with ferritin.

Iron loading through transfusion is concerning because of the potential to cause damage in multiple organs. Of subjects with liver biopsies, all except two had normal histology, and two displayed Ishak grade 1 fibrosis (data not shown); therefore, there were insufficient data to perform a stratified analysis for factors associated with liver injury. Liver fibrosis more likely appears later in the course of chronic transfusion than the follow-up period of this study. The subjects included in this study were chelation naïve and necessarily earlier in the course of transfusion therapy, before chronic injury would be more manifest.

This study has limitations. While all subjects received transfusion through partial manual exchange transfusion, the extent of pre-transfusion phlebotomy was not standardized and was affected by venous access and patient tolerance. It was important for this study to evaluate subjects who had never received iron chelation. Inclusion of subjects on chelation would increase the amount of data for analysis, but the data would be irreconcilably biased by chelation therapy, the efficacy and adherence of which cannot feasibly be assessed in a retrospective study. Erythrocytapheresis was used as a transfusion technique in a small

number of transfusions in this study. The assumption that erythrocytapheresis had no net iron loading effect is generally supported by the literature, but there is individual variation^{9,11,12}. Nevertheless, erythrocytapheresis procedures likely had little impact on overall iron balance in this study, because they comprised <5% of all transfusion and most often were used as the initial procedure.

In summary, partial manual exchange appears to be an effective way to slow iron accumulation during chronic transfusion for SCD. Using net iron loading as a metric of transfusional iron loading may be a useful adjunct to determinations of LIC.

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Table 1
Clinical characteristics of subjects receiving chronic transfusion (n=17)

Hemoglobin SS, n	17(100)
Age at chronic transfusion initiation, years	8.1 (0.7-16.8)
Indication, n	
Stroke, 1° prevention	7 (41.2)
Stroke, 2° prevention	4(23.5)
Severe acute chest syndrome	3 (17.6)
Intractable pain	3 (17.6)

Data are median (range) or n (%), as appropriate

Table 2
Iron balance prior to LIC determination (n=17)

Absolute iron (mg)	Median	Range
Transfused	9,320	2,768 - 22,376
Phlebotomized	1,160	302 - 2,712
Ratio Iron phleb:tx	0.12	
Iron normalized to body mass (mg/kg)		
Transfused	307	181 - 490
Phlebotomized	35.0	20.0 - 63.6
Ratio Iron phleb:tx	0.11	

Table 3
Transfusion predictors of LIC (n=17)

	r^2	P
Transfusion volume/kg	0.24	0.04
Net transfusion volume/kg	0.16	0.11
Iron transfused/kg	0.24	0.04
Net iron transfused/kg	0.41	0.005