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Predicting Changes in HbS Following Simple Transfusion using Complete Blood Counts

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Abstract

BACKGROUND: Hemoglobin S (HbS) percentages are used for management of patients with sickle cell disease (SCD). However, HbS measurements are often not routinely or rapidly performed. Rapid and accurate methods to estimate the HbS levels following simple transfusion may improve the care of SCD patients.

STUDY DESIGN AND METHODS: A comprehensive review of the electronic medical record identified 24 stable SCD patients who had simple RBC transfusions and had HbS measurements before and after the transfusion that were less than 72 hours apart. Examination of these patients identified 62 separate transfusions that met our criteria. Three simple equations that utilized CBC values and readily available information from the medical record were used to predict the post transfusion HbS following transfusion. Equation 1: Predicted Post-HbS = Pre HbS X [Pre Hb/Post Hb]; Equation 2: Predicted Post-HbS = Pre HbS X [Pre Hematocrit (Hct)/Post Hct]; Equation 3: Predicted Post-HbS=Pre HbS X Total Pre Hb /[Total Pre Hb+ (RBC Vol x 20)].

RESULTS: The predicted HbS for all three equations showed a highly significant correlation with the measured post HbS value. R^2 values for equations 1, 2 and 3 were 0.95, 0.92 and 0.97 respectively. Predicting the post-HbS values using an estimate of the patient's total hemoglobin and the transfused hemoglobin (Equation 3) was the most precise.

CONCLUSION: Reductions in HbS values in patients with SCD undergoing simple RBC transfusions can be reliably predicted using CBC measurements and simple arithmetic equations.

Keywords

Sickle Cell Anemia; Hemoglobin electropheresis; RBC transfusion

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Conflict of interest

The authors declare that they have no conflicts of interest relevant to this manuscript submitted to TRANSFUSION.

Introduction

Sickle Cell Disease (SCD) is a common genetic hemoglobinopathy caused by a mutation in beta-globin chain at position 6 (glutamic acid to valine). This mutation results in Sickle Cell Disease when found in the homozygous state or in combination with other mutations.[1] SCD includes sickle cell anemia, homozygous for the β^s gene (HbSS), as well as HbSC, Hb β-thalassemia (HbS β° and HbS β^+), HbSD, etc.[2, 3] Management of patients with SCD includes supportive and prophylactic care. Hydroxyurea (HU) and chronic transfusion (simple or exchange) are still the two main disease modifying treatments options for SCD.[3, 4] Over the last three decades, the number of evidence-based indications for RBC transfusion in SCD has been increasing.[3]

Exchange transfusions are used emergently in management of acute complications (stroke, acute chest syndrome, etc) of SCD as well as prophylactically to prevent chronic complications (stoke and pain crisis). Simple RBC transfusions are utilized for routine management of symptomatic anemia as well as used in place of exchange transfusion when it cannot be performed or as part of initial management of acute complication before exchange transfusion can be arranged.[2] Transfusions are also used in perioperative period in patients with SCD to prevent vasoocclusive crises, stroke, and acute chest syndrome after surgery.

RBC transfusions are thought to work by dilution of HbS via addition of normal hemoglobin A (HbA) containing red cells from donor blood. Suppression of erythropoietin release caused by rise in Hb reduces the production of new HbS cells. The transfused HbA has higher oxygen carrying capacity and longer circulating lifespan compared to HbS, which increases oxygen delivery to tissues and reduces symptomatic anemia. The HbS level proportionally decreases with the amount of simple RBC transfused. Exchange transfusion results in significantly larger per unit drop in HbS level compared to simple transfusion due to HbS removal during the procedure on top of the addition of HbA.

HbS percentages are used for diagnosis and management of patients with SCD. Higher HbS levels are usually associated with complications. HbS <30% is frequently used as a target for acute management and chronic care of patients with SCD.[5, 6] Quantitation of HbS levels are usually performed by Hb electrophoresis, Hb fractionation by High Performance Liquid Chromatography (HPLC) or by isoelectric focusing.[7, 8] These methods are technically challenging and may have a long turnaround time. This may require clinicians to make an educated guess about the HbS values following simple RBC transfusions when planning subsequent treatments. In our experience, SCD patients initially managed with simple transfusions may clinically progress to require an emergent RBC exchange. For RBC exchange procedures, the pre-procedure HbS value is required to calculate the fraction of cells remaining (FCR) and determine the number of RBC units needed for the exchange.[9] Methods to accurately estimate HbS values following simple transfusion may aid in the management of these patients. In this study we utilize simple arithmetic equations which use pre-transfusion (pre) HbS percentage, complete blood count (CBC) values, post-transfusion (post) CBC values and height and weight of patient (to calculate blood volume and total

hemoglobin) to estimate the post HbS in patients who have received simple RBC transfusion.

MATERIALS AND METHODS

Study Design

This project involved a retrospective query of our electronic medical record (EMR) to identify patients who had Sickle Cell disease and had been transfused. The study was reviewed by the University Institutional Review Board (IRB) and was approved with a waiver of consent granted for the study by the IRB (#201410771). Utilizing datamining tool Starmaker[10], the electronic medical record for University of Iowa Hospitals and Clinics (UIHC) patients were mined to identify all patients with non-zero HbS levels from 2010 to 2015. 192 patients were identified with non-zero HbS values during this time. The HbS values (reported as percent HbS) available for each of these patients was then exported for further analysis. A drop in the HbS level between two measurements was used to identify patients who had been transfused. HbS values can be influenced by patient erythropoiesis, bleeding, hemolysis and treatment with drugs such as hydroxyurea. Therefore a window of 72 hr between HbS measurements was chosen to minimize these effects and only transfusions where HbS measurements were less than 72 hours apart were further analyzed. These restrictions results in the identification of 44 patients who had at least one transfusion that met these criteria. A review of the electronic medical record of these patients (dating back to 2003) was performed to determine if the patient had a simple transfusion and was not bleeding, undergoing surgery or had evidence of clinically significant hemolysis. This eliminated 20 patients, most of whom had received either manual or automated exchange transfusions and where thus not eligible for inclusion in this study. This analysis left 24 patients who had simple transfusions that met the above criteria and were analyzed for this study. In total, 62 transfusions in these 24 patients met our criteria and were included in this analysis. All transfusions involved RBC units stored in ADSOL and all Sickle cell patients receive sickledex negative units that. In addition, our policy is to utilize RBCs < 7days old in SCD patients in non-emergent settings so that RBC survival is optimized.

The following parameters (in addition to the HbS (%) values) were recorded from the EMR for each transfusion that met the above criteria. The time and values of pre/post transfusion hemoglobin (g/dl) and hematocrit (%) levels. Patient height (cm) and weight (kg) nearest to the time of the transfusion. The volume (dl) of transfused RBCs between the two HbS measurement was recorded with 3 dl (300 ml) per RBC unit estimated when the actual volume transfused was not documented in the EMR. The patient blood volume (dl) at the time of the transfusion was estimated using the patients sex, height and weight. For pediatric subjects (< 40 Kg), 80 ml/Kg was utilized to estimate the blood volume. This value was selected for pediatric transfusions as this approach is commonly used in our hospital and is also very close to the mean value from a meta-analysis on blood volume for children in this age group[11].

For non-obese (BMI<25) subjects > 40 Kg, 70 ml/Kg was used for males and 65 ml/Kg for females. For subjects > 40 Kg and BMI > 25 the Nadler formula was used to estimate the blood volume[12]. We did not have any subjects less than 40 Kg who had a BMI >25 in this

cohort but we would recommend using the Linderkamp nomogram to estimate the blood volume in these subjects[13] as the Nadler formula has not been validated for pediatric patients[12].

Laboratory Parameters

Patient Hemoglobin (g/dl) and Hematocrit (%) values were measured on the analyzers used in our hospital at the time of the transfusion. The following analyzers were utilized; 2001–2008: Siemens Advia Hematology Systems (Malvern, PA, USA) and from 2008: Sysmex XE-5000 Automated Hematology System (Lincolnshire, IL, USA). The HbS measurements (reported as a percentage) were performed on the following analyzers; 2001–2012: Bio-Rad HPLC Systems (Hercules, CA, USA) and from 2012: Sebia Capillarys 2 (Norcross, GA, USA). For some transfusions only the Hb level was measured so the post transfusion HbS level could not be estimated using equation 2 since no Hct level was measured. In one case, only the Pretransfusion Hb level was measured so only Equation 3 could be used to estimate the post transfusion HbS level. Each laboratory measurement and the time it was drawn were documented for each transfusion that met the criteria.

Mathematical Formulas for Estimating the Post-transfusion HbS

We utilized equation 1 and 2 below to predict the post transfusion HbS based on the pre-transfusion HbS (%) value and the Hb (g/dl) or Hct (%) values obtain before (pre) and after (post) the transfusion(s). Since Hb changes proportionally with RBC transfusion[14], these equations assume that the change in HbS is inversely proportional to the change in Hb or Hct. It should be noted that these equations do not factor in changes in blood volume likely to occur in patients receiving transfusions and possibly other fluids.

$$\text{Predicted HbS} = \text{Pre HbS} \times [\text{Pre Hb} \ / \ \text{Post Hb}) \quad \text{Equation 1:}$$

$$\text{Predicted HbS} = \text{Pre HbS} \times [\text{Pre Hct} \ / \ \text{Post Hct}) \quad \text{Equation 2:}$$

Equation 3 utilized the pre-transfusion HbS, the pre transfusion Hb and an estimate of the transfused Hb to estimate the HbS level. The patient's total body Hb (g) before transfusion is estimated by multiplying the measured pre-transfusion Hb level (g/dl) by the patient's estimated blood volume (in dl) calculated as described above. The transfused Hb is estimated by multiplying the RBC vol (3 dl was used if the volume was not indicated in the EMR) and the average Hb concentration (20 g/dl) at our center (data not shown). This equation assumes the HbS value would decrease in direct proportion to the ratio of total patient Hb to the transfused Hb.

$$\text{Predicted HbS}(\%) = \text{Pre HbS}(\%) \times \text{Total Pre Hb (g)} \,/\, [\text{Total Pre Hb (g)} + (\text{RBC Vol (dl)} \times 20\text{g} \,/\, \text{dl})]$$

Equation 3:

Statistical Analysis

The measured and predicted HbS values for each of the transfusions were plotted and compared to the 45° identity line. Linear mixed modeling was performed with clustering on the patient level to calculate the best fit line with 95% prediction intervals for new observations. Prediction intervals containing the identity line over the continuous range of predicted HbS would indicate that there is no significant difference between the measured and predicted HbS values.

RESULTS

Study Design and Transfusion Variables

Figure 1 illustrates how the 24 eligible SCD patients who had received a simple RBC transfusion were identified in an unbiased manner from a systematic search of the EMR. A comprehensive search of the electronic medical record of these patients identified 62 transfusion events that met the criteria outlined in the methods. A transfusion event is defined as all the RBC transfusions that occurred in between the two HbS measurements. 58% of the patients were females and 92% had HbSS disease while 8% had HbSC disease. The mean, median and range of the laboratory and patient variables for each transfusion event are shown in Table 1. The majority of the transfusion events were in the pediatric population but patients as old as 51 were included in the analysis and 17 transfusion events were analyzed in subjects that weighed at least 50 Kg.

The number of transfusion events for each patient ranged from 1 to 16 with 18 of the patients having just one or two transfusion evetns that met our criteria for further analysis (Figure 2A). The transfusion volume generally involved 1 unit or less and only 2 transfusion events involved 3 or more units (Figure 2B). Regarding the timing of the laboratory measurements, most HbS percentages were recorded within 24 hours of each other and only 6 transfusion events involved HbS measurements more than 48 hours apart (Figure 2C).

Predicting HbS percentage using Pre and Post CBC Values

The predicted post-transfusion HbS value calculated using equation 1 and 2 showed statistically significant correlation with the measured post HbS value. The correlation was slightly better when patient Hb values were used (Eq.1; R^2= 0.95) compared to Hct values (Eq. 2: R^2= 0.91) but results with either laboratory parameter were comparable (Figure 3A,B). Equation 1 and 2 slightly underestimate the drop in HbS compared to the measured post HbS value by an average 3.34% using Hb and 3.73% using the Hct. The precision of this equation did appear to be reduced as the interval between CBC measurements was increased. In 7 transfusions with an interval of 48–72 hours between CBC measurements

equation 1 overestimated the HbS value by an average of 7.41% and the R^2 value was only 0.53 (data now shown). The accuracy of predicting the post transfusion HbS value was not affected by the initial HbS level in the patient (**data not shown**).

Predicting HbS values using transfused Hb and patient blood volume

Equation 3 predicts post HbS based on the estimated change in total hemoglobin in the patient following transfusion. The patients pre-transfusion total body Hb is based on an estimate of the patients blood volume combined with the patients Hb concentration prior to transfusion. The transfused Hb is estimated from the actual or estimated transfused volume multiplied by the average Hb concentration of RBC units at our institution (20 g/dl). Predicting post-transfusion HbS with this equation also demonstrated a highly statistically significant correlation with the measured post HbS value, R^2= 0.97 (N=62) **(Figure 3C)**. Equation 3 was more precise as the mean difference between predicted and measured HbS value was only 0.19% (range −7.7–8.5).

To more clearly illustrate the precision and variation of each of these equations in predicting post transfusion HbS values, the measured HbS was subtracted from the predicted HbS for all 3 equations and this difference is illustrated in histogram format (Figure 4). These data demonstrate that equations 1 and 2 both tend to underestimate the drop in HbS level. Thus the predicted HbS percentage tends to be higher than the measured HbS percentage and therefore the predicted minus measured values trend in the positive direction. For equation 1, only 3 of 61 transfusions (4.9%) overestimated the drop in HbS value by more than 2%. Similarly for equation 2, only 3 of 42 transfusions (7.1%) overestimated the drop in HbS value by more than 2%. In contrast, the histogram of the data for equation 3 is centered around zero and in 55 of 62 (88%) transfusions the predicted HbS value was within 5% of the measured value.

DISCUSSION

In this study we performed a retrospective analysis of stable non-bleeding Sickle Cell Disease patients who had undergone a simple transfusion in which both the pre and the post HbS value had been measured. Three simple arithmetic equations were examined that utilized CBC parameters, the pre-transfusion HbS value and patient’s sex, height and weight to estimate the post-transfusion HbS level. Our data demonstrate that all 3 equations could reliably estimate the post transfusion HbS level. Equations 1 and 2 which used pre and post transfusion CBC values to estimate the HbS value tended to under estimate the reduction in HbS percentage. We speculate that this underestimation is due to expanded blood volume of the patient that likely occurs during transfusion. This volume expansion would result in an underestimate of the increase in patients hemoglobin as the equation assumes that the blood volume remains unchanged. Regardless of the reason, this approach provides a more conservative estimate for the clinicians and in only rare cases did this approach significantly overestimate the drop in HbS.

Equation 3 utilized estimates of the patient’s total body Hb and the total transfused Hb to predict the drop in HbS percentage following transfusion. This method does not require any post transfusion testing and can therefore be used to estimate the drop in HbS levels before

the transfusion even occurs. We suspect that much of the variation observed using this equation may come from differences in hemoglobin levels among different units and possibly imprecise estimates of the transfused blood volume. The measurement of the hemoglobin in each individual unit and careful assessment of the volume of each unit would likely improve the precision of this equation but those values were not available for this study. Other variation with this method could come from our the estimates of blood volume as significant variation occurs in subjects blood volume [12, 13]. However, equation 3 did provide the most precise estimate of the drop in HbS levels as the mean difference in predicted and measured HbS values was near zero.

We envision several scenarios where using these equations might prove beneficial. SCD patients in crisis often receive simple transfusion before RBC exchanges can be arranged or are necessary to perform. In this setting these equations can be used to estimate the HbS level prior to the RBC exchange. For these procedures, the pre-procedure HbS level is important in determining the fraction cells remaining (FCR) and the FCR is required to determine the number of RBC units needed for the procedure. Since HbS levels are often not rapidly available, utilizing the equations described here might assist with the clinicians estimate of this value. Another scenario would be patients with SCD who have very low Hb levels and the clinical team is deciding between a simple and an exchange transfusions. For example, if a patient Hct is 14% and a HbS value of 60%, equation 2 could be utilized to conservatively predict that transfusing up to a Hct of 30% would have a high probability of reducing the HbS value to 30%. In this scenario, utilizing equation 2 we could predict the HbS value as follows: 60% x (14%/30%) = 28%. Since simple transfusions generally don't require a central line while exchange transfusions do, the costs and potential complications of placing a central line could be avoided in a subject similar to this. Finally, if a provider transfusing a SCD patient has an end HbS target in mind, they could utilize equation 3 to determine how much blood to transfuse in order to achieve a specific target HbS value. For example, if a pediatric patient weighs 25 Kg (est blood volume of 25×80= 2000 ml), has a Hb value of 5.0 g/dl and a HbS of 50% the amount of transfused blood required to drop the HbS value to 30% could be calculated. In this scenario, the pre-transfusion total body Hb would be estimated to be 100 gm (20 dl x 5 g/dl). Transfusing 325 ml (3.25 dl x 20 g/dl= 65 g estimated Hb) would be predicted to drop the HbS to 30% based on the following calculation: 50% x 100/(100+65)= 30%. We conclude that there are a variety of clinical scenarios where these simple mathematical formulas may help in the management of patients with SCD.

The strengths of this study include the relatively large number of transfusions examined and the unbiased acquisition of data as all transfusions that met pre-defined criteria were analyzed. Another strength is that the patients studied included a wide variety of ages who received a variable number of transfusions. Thus the results should apply to many clinical scenarios involving simple transfusions of stable non-bleeding sickle cell patients. One weakness of the study was the retrospective nature of the study and the relatively broad inclusion criteria which allowed measurements to be up to 3 days apart. A prospective analysis with a tighter laboratory measurement would be useful to better assess the clinical utility of these equations. Another weakness is all three equations require that the pre-transfusion HbS percentage be available before applying the equations. In some cases this

information is not available and the equations can only be used to provide an estimate of how much the HbS percentage would be reduced. In this scenario the patients past transfusion history and laboratory measurement could be used to conservatively estimate the HbS value prior to transfusion. Then the equations could be used to estimate how much the HbS value would drop. Overall we conclude that the application of these equations may aid the in the acute or chronic management of patients with SCD.

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Abbreviations:

FCR	Fraction Cells Remaining
Hct	Hematocrit
Hb	Hemoglobin
HbS	Hemoglobin S
SCD	Sickle Cell Disease
RBC	Red Blood Cell

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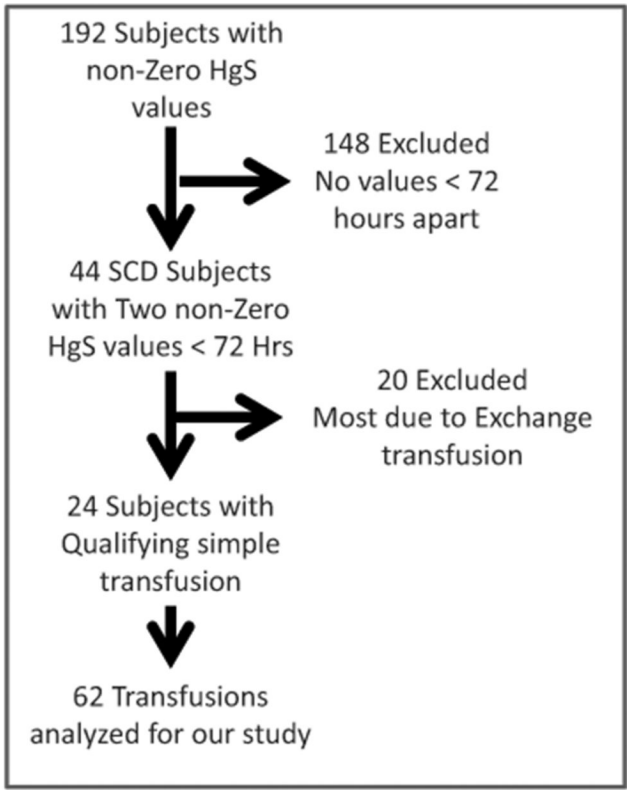

Figure 1. Study design and data acquisition.

This schematic summarizes the design of this study and shows how the 24 patients were identified who had a total of 62 transfusions that met the criteria for this study.

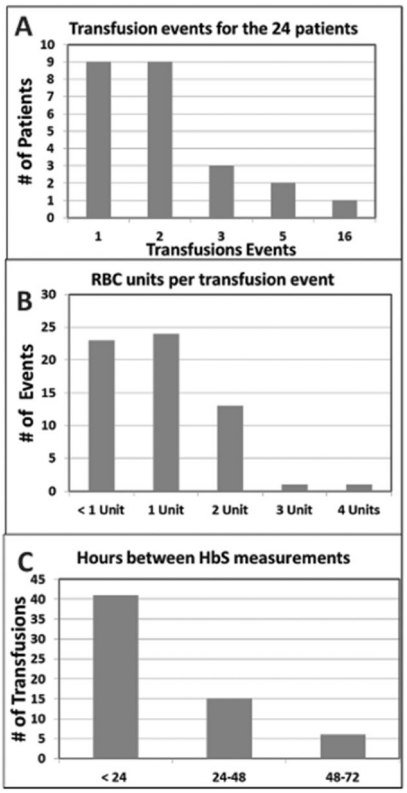

Figure 2. Transfusion parameters included in this study.

A) The number of transfusions events that the patients had that met the criteria is shown. Each transfusion event involved all RBC transfusions that occurred between the two HbS measurements. Twenty-one of the twenty-four patients had 3 or fewer transfusion events that met the inclusion criteria for the study. **B**) The number of RBC units transfused for each of the 62 transfusion events studies is shown. All but 2 of the transfusion events involved 2 Units or less. **C**) The timing between the HbS measurements in each of the 62 transfusion events is shown. The majority of HbS measurements were within 24 hours of each other.

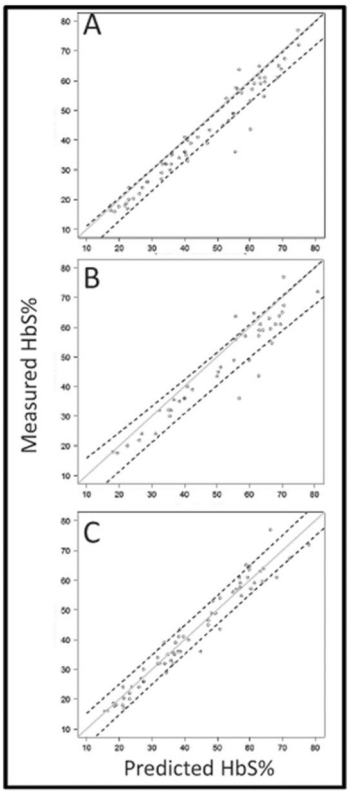

Figure 3. Predicting the post-transfusion HbS (%) levels utilizing simple arithmetic formulas and readily available laboratory information.

The post transfusion HbS value was estimated using the pre and post CBC values as described in the methods. For a number of transfusions, only a post-procedure Hb was ordered so the HbS value could not be estimated utilizing equation 2. Panel A compares measured and predicted HbS levels in 61 transfusions using equation 1 which uses Hb values. Panel B compares measured and predicted HbS levels in 42 transfusions using equation 2 which uses Hct values. Panel C compares measured and predicted HbS levels in 62 transfusion events using equation 3. In all panels the gray solid line represents the line of identity if the predicted HbS value would have matched the measured HbS value precisely. The dashed lines represent the 95% confidence interval calculated as described in the Materials and Methods.

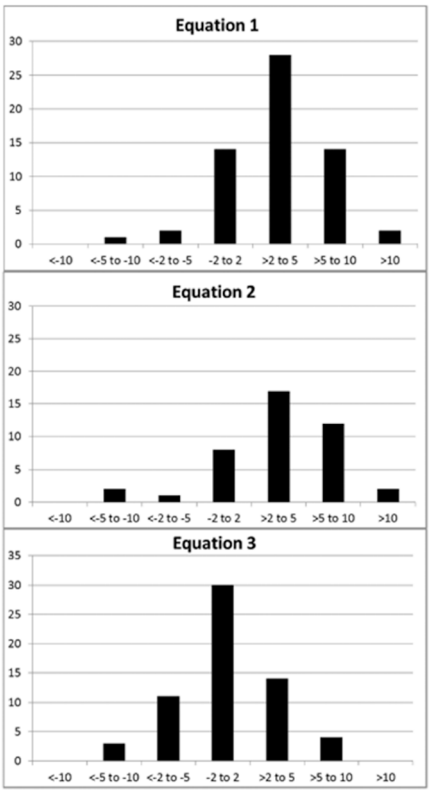

Figure 4. Histograms of predicted minus measured HbS percentage using all three equations.

The measured HbS percentage was subtracted from the predicted HbS percentage for all the transfusion events analyzed from each of the 3 equations. The number of transfusion events that fell within each of the indicated ranges is shown for each equation. The data from equations 1 and 2 skew positive indicating that the predicted HbS percentage tends to be higher than the measured HbS percentage with these equations. The data from equation 3 is centered around zero indicating it provides a more precise estimate of post transfusion HbS percentage.

Table 1.

The mean, median and range for the parameters of the 62 transfusions are shown.

	Mean	Median	Range
Age	11.4	7.7	1.6-51.9
Est Blood Volume (ml)	2391	2064	896-5472
Pre Hb (g/dl)	8.2	8.6	5.5-11.2
Pre Hct (%)	24.1	25.0	16-32
Post Hb (g/dl)	10.2	10.5	6.6-12.6
Post Hct (%)	28.8	29.0	20-36
Time Between HgS (hrs)	18.9	12.0	2.33-67.5
Time Between CBC (hrs)	28.8	13.8	2.33-69.70
Transfused vol (ml)	337.3	300.0	75-1200