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Impact of Voxelotor on Red Blood Cell Exchange Therapeutic Procedures: Evaluation of Multi-Institutional Procedure Data

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ABSTRACT

Hemoglobin S (HbS) polymerization inhibitor drugs such as voxelotor can result in a split peak in HbS as well as additional peaks with hemoglobin A in quantitative methods of HbS measurement. It is unclear how these results should be used to make transfusion decisions. The goal of this study is to compare RBC exchange (RBCX) replacement volumes calculated with HbS-Vox + HbS versus HbS alone. Patients aged 15–58 years who had variant hemoglobin quantitation performed for clinical care purposes with evidence of voxelotor treatment (split peak in HbS and/or additional peaks with hemoglobin A) were identified by investigator review of variant hemoglobin quantitation test results from the clinical laboratory. The RBCX replacement volume calculated with HbS% total (RBCX volume HbS% total) was compared to the RBCX replacement volume calculated with HbS unmod% (RBCX volume HbS unmod%) in each case. The mean difference between RBCX volume total HbS% and RBCX volume HbS% unmod is 398 mL with 95% CI (198, 598) and RBCX volume total HbS is significantly different from RBCX volume HbS unmod (p value = 0.0006). If the HbS total is not used to calculate RBCX replacement volumes in patients taking voxelotor, there is a significantly lower amount of RBC that would be ordered, which would lead to higher HbS after RBCX. Additional studies regarding the role of transfusion in such patients are necessary.

1 | Introduction

Sickle cell disease (SCD) affects approximately 100 000 people in the U.S. Caused by a single amino acid substitution in the β hemoglobin gene, normal hemoglobin A is changed to hemoglobin S (HbS). HbS polymerization leads to sickled, rigid red blood cells (RBC) which produce chronic hemolysis and end organ damage [1].

Voxelotor, which received Fast Track, Orphan Drug, and Rare Pediatric Disease designations from the U.S. Food and Drug Administration in 2019 [2], is a HbS polymerization inhibitor. It reversibly binds to hemoglobin by forming a covalent bond with the N-terminal valine of the α-chain of hemoglobin that increases hemoglobin's affinity for oxygen [3]. The drug's half-life is 61 ± 7–85 ± 7 h, and it exhibits highly specific binding to hemoglobin. A single dose of 1000 mg in a patient with SCD is rapidly

absorbed into plasma, and its terminal $t_{1/2}$ of 50 h makes it suitable for oral, once-daily dosing [3]. Voxelotor was voluntarily withdrawn from the market by the manufacturer in September 2024 because of increased vaso-occlusive crises and fatalities [4].

Variant hemoglobin quantitation is used in patients with SCD to make therapeutic decisions, such as when red blood cell transfusion may be needed in relation to clinical events. Multiple methods are used to measure sickle hemoglobin (HbS); the two most common are capillary zone electrophoresis (CZE) and high performance liquid chromatography (HPLC). Both rely on the separation of different hemoglobin species to provide a percentage of each type.

Automated red blood cell exchange (RBCX), a procedure where the patient's RBC are removed and replaced with donor RBC, is used to rapidly reduce the proportion of HbS in acute and chronic complications of SCD such as acute chest syndrome and stroke prevention [5]. Prior to performing this procedure, the percentage of HbS is used to calculate the replacement volume of donor RBC. Typically, the goal of RBCX is to reduce the HbS proportion to 30% or lower.

However, recent studies have shown that voxelotor can impact HbS quantitation. Voxelotor binding results in a split peak in HbS as well as additional peaks with hemoglobin A [6]. This was also demonstrated in a study by Rutherford-Parker et al. [7] The investigators performed variant hemoglobin quantitation using whole blood samples incubated with voxelotor. Voxelotor modified the α-globin of hemoglobins A, S, C, D-Punjab, E, A2, and F by HPLC and CZE. Thus, in patients taking this drug, multiple hemoglobin types cannot be accurately quantified [7].

It is unclear if HbS-voxelotor complexes are clinically equivalent to HbS. The implications for apheresis machine settings and replacement volume calculation in RBC exchange are unknown.

The purpose of this study is to identify what impact the calculation of replacement RBC volume using the total HbS fraction (HbS-Total, which includes HbS-voxelotor (HbS-Vox) and HbS not bound to voxelotor) versus only the HbS fraction not bound to voxelotor (HbS-Unmod) would have on the amount of RBC replacement volume necessary.

2 | Materials and Methods

Patients at the University of Illinois at Chicago, Washington University in St. Louis, and Virginia Commonwealth University with SCD were included. This study was approved by the IRB at the University of Illinois at Chicago as the primary site with a waiver of consent; there was additional IRB approval with a waiver of consent at all participating sites. Patients were aged 15–58 years who had variant hemoglobin quantitation ordered and performed for clinical care purposes.

Sickle cell patients receiving voxelotor therapy were included in the study if the visual analysis of cation exchange HPLC (CE-HPLC) or CZE demonstrated an abnormal pattern of HbS elution into two different peaks that can be quantified (Figure 1). Unmodified HbS (HbS-Unmod) was measured by calculating the area percentage at the expected HbS retention time (4.32 retention time CE-HPLC and 4.42 for CZE). Quantitation of total HbS (HbS-total) was performed by addition of the HbS-Unmod percentage with the percentage of the additional peak of voxelotor-bound HbS.

A RedCap database was established to collect data. Cases identified occurred between November 1, 2020 and December 1, 2021.

The difference in RBCX volume when HbS-Total is used compared to HbS-Unmod was considered significant if it was quantified to be 300 mL or more. The average unit of RBC is approximately 300 mL, and this difference would significantly impact preparation for RBCX in this setting. The volume of donor RBC for RBCX

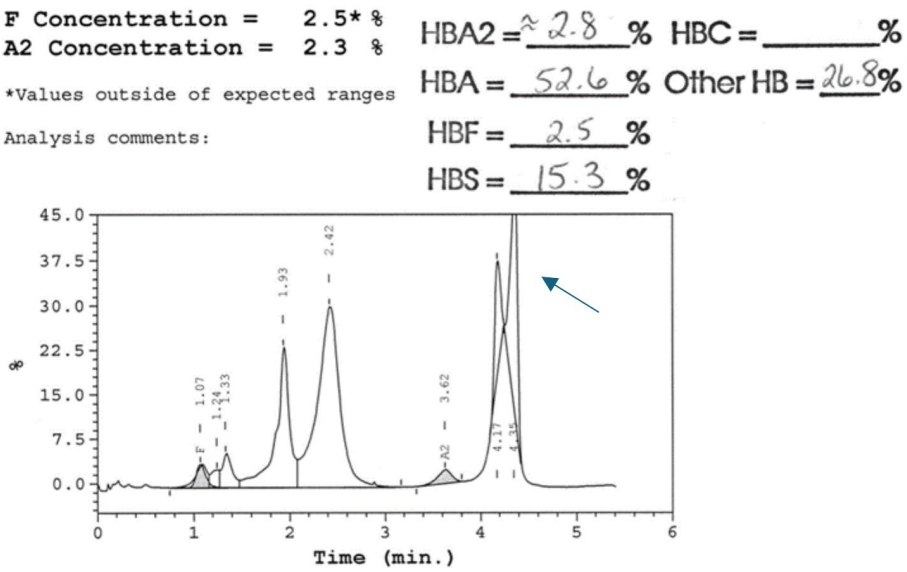

FIGURE 1 | HPLC pattern of hemoglobin elution in patient taking voxelotor demonstrates split peaks at HbS region at 4.17 (15.3% of hemoglobin) and 4.35 (26.8% of hemoglobin) minutes (arrow). The specimen was collected prior to red blood cell transfusion from a patient receiving monthly automated red blood cell exchange.

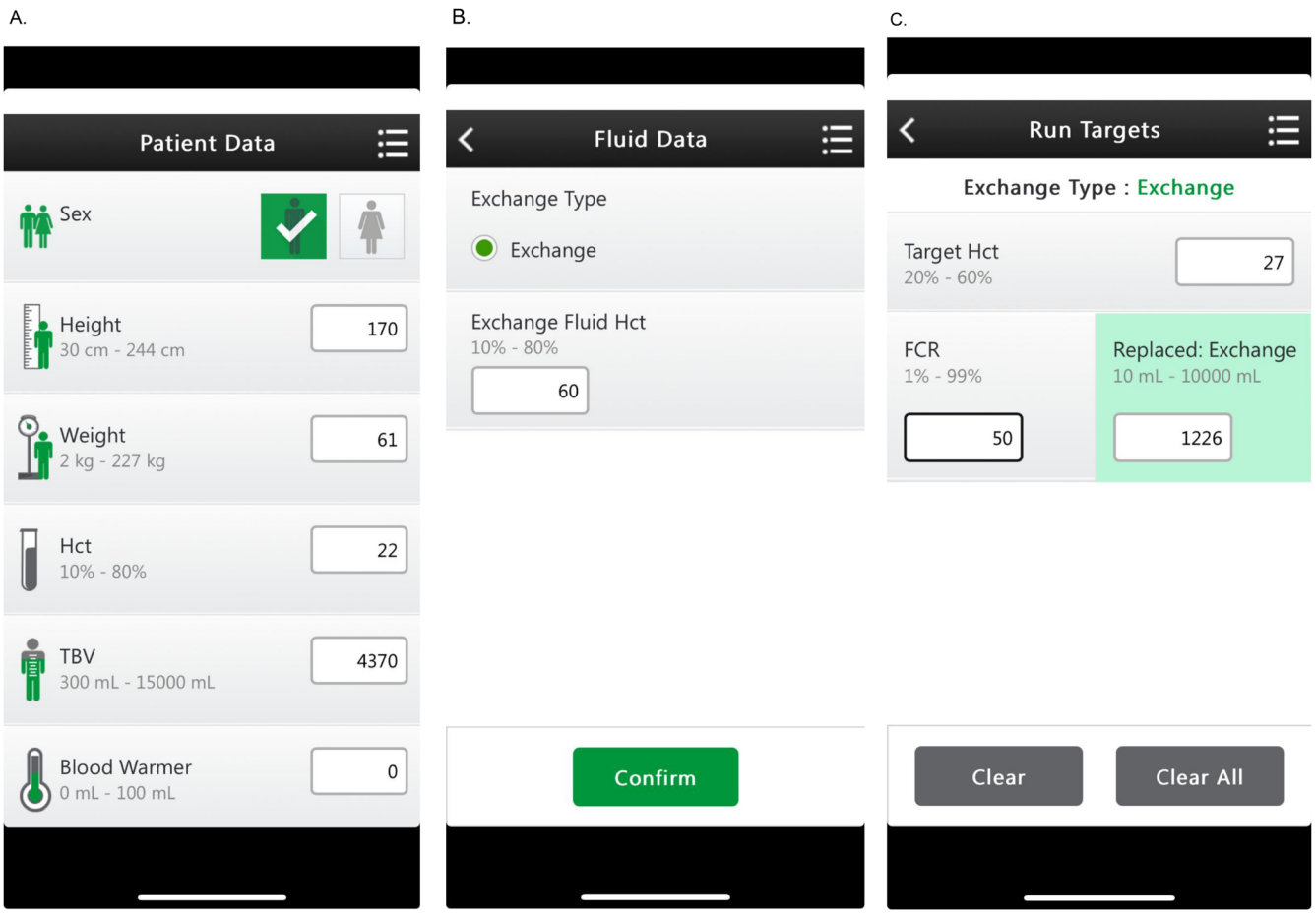

FIGURE 2 | RBCX calculation tool application from Terumo BCT for the Spectra Optia Apheresis System (Lakewood, CO) was used to calculate the volume of donor RBC needed based on data entered into the application. (A) Initial patient data is entered, including sex, height, weight, and starting hematocrit. (B) The type of exchange procedure and hematocrit of exchange fluid are entered. (C) The target hematocrit and FCR are entered, and the volume of donor RBC needed (Replaced: Exchange) is shown.

was calculated using the RBCX Calculation Tool application (version 1.0.6) from Terumo BCT for the Spectra Optia Apheresis System (Lakewood, CO) (Figure 2) The application assumes an inlet:AC ratio of 13.0, AC infusion rate of 0.8, and 100% fluid balance. The patient hematocrit at the time of specimen collection for electrophoresis was used as the starting hematocrit for calculation of RBC volume. The exchange replacement fluid hematocrit used for calculation was 55% for all cases. The maximum target hematocrit allowed by the calculation tool based on the patient starting hematocrit was used in each case. The fraction of cells remaining (FCR) was set to reduce the HbS to less than 30; the FCR was approximately 50 if the HbS was 40 or lower, 40 if the HbS was between 41 and 49, and 30 if the HbS was 50 or greater.

3 | Results

Data collected from 17 patients across all three study sites is shown in Table 1. The median age was 45 years (range 15–58 years). Four out of seventeen (24%) patients were male. Fifteen out of seventeen (88%) patients had Hb SS. One patient (6%) had HbSβthal, and one patient (6%) was HbSC. The median patient height was 167 cm (range 149–173) and the median weight was 61 kg (range 49–78). The quantitation method for the hemoglobin variant most used was HPLC for 14 patients (82%); CZE was used for 3 patients (18%). The median total HbS was 81% (range 31–92). The median HbS identified as voxelotor bound was 16% (range 2–36), and the median unbound HbS was 48% (range 16–90).

The median hematocrit used as the starting hematocrit was 23% (range 15–35).

The median RBCX volume calculated using total HbS was 2206 mL (range 1129–2790 mL). The median RBCX volume calculated using HbS unmodified (not voxelotor bound) was 1663 mL (range 505–2715 mL). The difference in RBCX volume ranged from 0 to 1175 mL.

The mean difference between RBCX volume calculated using HbS-Total and RBCX volume calculated using HbS-Unmod is 398 mL with 95% CI (198–598). RBCX volume using HbS-Total significantly differs from RBCX volume using HbS-Unmod (p value = 0.0006). Table 1 shows the milliliter volume and percent difference between procedures calculated with HbS-Total and HbS-Unmod.

4 | Discussion

Most often employed in the treatment of acute chest syndrome, acute stroke, and prevention of first or recurrent stroke, RBCX

TABLE 1 | Red blood cell exchange volume calculation details.

Patient gender	SCD	Height	Weight	Hb quant method	HbS% total	HbS% Vox	HbS% Unmod	Starting Hct	FCR total	Target Hct total	FCR HbS Unmod	Target Hct Unmod	RBCX volume total HbS	RBCX volume HbS Unmod
F	SS	160	60	CZE	92	2	90	20	32	26	33	26	2206	2206
M	SS	183	53	CZE	85	4	81	23	35	28	37	28	2795	2715
F	SS	166	57	CZE	90	3	87	15	33	22	34	22	1942	1885
F	SS	167	78	HPLC	90	20	70	28	30	30	30	30	2790	2790
F	SS	149	63	HPLC	32	16	16	33	40	31	50	31	1822	1378
F	SS	170	77	HPLC	38	20	18	17	40	24	50	24	1540	1140
F	SB	168	76	HPLC	92	2	90	27	30	30	30	30	2718	2718
M	SS	170	61	HPLC	61	13	48	22	40	30	50	27	1868	1363
F	SS	170	74	HPLC	54	14	40	21	40	30	50	27	1831	1336
F	SS	164	49	HPLC	81	33	48	35	30	32	50	32	2454	1413
F	SS	162	69	HPLC	81	23	58	21	30	30	40	30	2185	1663
F	SS	163	67	HPLC	81	36	45	22	30	30	40	30	2212	1683
M	SS	167	50	HPLC	73	32	41	34	30	31	50	31	2770	1595
F	SS	163	54	HPLC	68	31	37	28	30	30	50	30	2212	1274
F	SS	170	63	HPLC	95	13	82	25	30	30	30	30	2396	2396
F	SC	152	54	HPLC	95	18	77	23	30	30	30	30	1843	1843
M	SS	173	58	HPLC	31	6	25	22	51	25	50	25	1129	505
Median		167	61		81	16	48	23	30	30	40	30	2206	1663
IQR		163–170	54–69		61–90	6–23	40–81	21–28	30–33	28–30	33–50	27–30	1843–2454	1363–2206
P														0.0006

Note: p value: Comparison of RBCX volume using HbS-Total compared to RBCX volume using HbS-Unmod.
Abbreviations: CZE, capillary zone electrophoresis; FCR, fraction of cells remaining; HbS% total, total percentage of hemoglobin S; HbS% Unmod, percentage of hemoglobin S not bound to voxelotor; HbS% Vox, percentage of hemoglobin S bound to voxelotor; Hct, hematocrit; HPLC, high performance liquid chromatography; IQR, interquartile range (25th–75th percentiles); RBCX, red blood cell exchange; SCD, sickle cell disease.

plays an important role in the treatment and management of SCD. In most chronically administered RBCX, the pre-procedure hemoglobin fractionation is known, which is used to set the FCR that determines the end procedure HbS percentage. Voxelotor promotes HbS binding to oxygen and decreases polymerization and hemolysis [8]. This drug also produces split peaks in HbS and additional peaks with HbA on variant hemoglobin quantification by HPLC and CZE methods. This interference can result in quantification errors, such as underestimation of total HbS, and potentially lead to unfavorable effects on patient care [9].

Depending on the clinical setting, the goal of RBCX is to reduce the HbS fraction to 30%–50%. Thus, for patients on chronic RBCX, it is important to have accurate information on the starting HbS prior to RBCX. To alert laboratory staff that a patient is taking voxelotor when hemoglobin fractionation is ordered, some institutions have instituted an automated order inquiry asking the provider if the patient is taking this medication. When this alert is received, the laboratory uses a modified peak integration approach and issues a presumptive HbS% [9].

Voxelotor has been used in place of transfusion when compatible RBC are unavailable due to alloimmunization or administration of transfusion is difficult due to low inventory during the COVID-19 pandemic [10, 11]. However, there were patients who still required chronic transfusion while receiving this medication. It is unclear if removal of a portion of the HbS-voxelotor after RBCX negatively impacts patient response to this drug as the portion of transfused HbAA RBC decreases before the next scheduled RBCX.

Automated RBCX technology does not allow separation of HbS containing RBC from HbAA RBC and certainly does not currently allow separation of drug bound HbS RBC from other RBC. For example, if a patient receives a simple transfusion of HbAA RBC prior to RBCX, some of these HbAA RBC will be removed along with HbS RBC; similarly, towards the end of a RBCX procedure, a portion of HbAA RBC given at the beginning of the exchange is also removed. HbS containing RBC are denser than HbAA RBC, and an attempt to take advantage of this to preserve non-sickled RBC during RBCX has been investigated. Using the effluent bag from RBCX procedures as a simulated patient, Thibodeaux et al. [12] demonstrated the impact of targeting denser RBC. By programming half the actual hematocrit as the starting hematocrit and end hematocrit, these investigators effectively targeted denser RBC and more effectively reduced HbS than using the actual hematocrit of the effluent bag.

5 | Conclusions

Our study shows that if the HbS total is not used to calculate RBCX replacement volumes in patients taking voxelotor, there is a significantly lower amount of RBC that would be ordered, which would lead to higher HbS after RBCX. Although this specific drug is no longer available, there are other hemoglobin polymerization inhibitors, such as GBT021601 [13] that may lead to a similar impact if patients taking these drugs require RBCX. Additional studies regarding the role of transfusion in such patients will be necessary.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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