Annals of Hematology
https://doi.org/10.1007/s00277-020-04188-y

ORIGINAL ARTICLE

Manual erythroexchange in sickle cell disease: multicenter validation of a protocol predictive of volume to exchange and hemoglobin values

Barbara Gianesin[1] · Valeria Maria Pinto[1] · Maddalena Casale[2] · Paola Corti[3] · Carmelo Fidone[4] · Sabrina Quintino[1] · Vincenzo Voi[5] · Gian Luca Forni[1]

Received: 16 March 2020 / Accepted: 15 July 2020

Abstract

Manual erythroexchange (MEEX) was proven to be effective and safe in the management of sickle cell disease (SCD). The goal is to quickly reduce the percentage of hemoglobin S (*HbS%*). A national survey of the Italian Society for Thalassemia and Hemoglobinopathies (SITE) observed a great variability among MEEX protocols none of which were found to be predictive of the values of *HbS%* and hemoglobin (*Hb*) after the exchange. Two equations to estimate the *HbS%* and *Hb* values to be obtained after MEEX were developed based on the results of the MEEX procedures in place in the centers participating in the present study. A standard protocol was subsequently defined to evaluate the volumes to exchange to obtain the target values of *HbS%* and *Hb*. The protocol was tested in 261 MEEX performed in SCD patients followed in the 5 participating centers that belong to the Italian Hemoglobinopathy Comprehensive Care Network, with the support of the SITE. The results showed a correlation between the estimated and measured values of *HbS%* and *Hb* (R_p 0.95 and 0.65 respectively, $p < 0.001$). A negligible bias was found for the prediction of *HbS%* and a bias of 1 g/dl for *Hb*. From consecutive MEEX, a rate of increase of *HbS%* between two exchanges of around 0.4% per day ($p < 0.001$) was measured. This protocol was shown to be effective and safe, as all patients reached the target value of *HbS%*. All the MEEX procedures were carried out with single venous access. No adverse events or reactions such as hypotension or electrolyte imbalance were reported nor were any complaints concerning the procedures received from patients.

Keywords MEEX · Sickle cell disease · Volumes exchanged · HbS% · Hb

Introduction

Sickle cell disease (SCD) is the most relevant hemoglobinopathy worldwide in terms of frequency and social impact and has recently been recognized as a global public health problem by the World Health Organization and the United Nations [1, 2]. SCD is a hereditary red cell disorder characterized by the presence of a point mutation on the β-globin chain, resulting in the synthesis of the pathological hemoglobin S (*HbS*) [3, 4] which determines entrapment of dense, dehydrated sickle red cells in the microcirculation [5–8], and vaso-occlusive crisis (VOC). Hence, therapeutic strategies aimed at lowering *HbS* level and reducing its negative impact are effective in preventing and managing SCD-related complications [3–5, 9].

Red blood cell (RBC) transfusion represents one of the key treatments for the management of SCD. However, erythroexchange, both automated (AEEX) and manual (MEEX), has advantages over RBC transfusion only, as can lower *HbS%* quicker, with less risk of hyperviscosity and a reduced or even no iron overload. The UK 2018 clinical care standards for adults with SCD [10–12] state that all hospitals that admitting SCD patients should have protocols and training in transfusion for SCD in place, including MEEX

✉ Gian Luca Forni
gianluca.forni@galliera.it

[1] Hemoglobinopathies and Congenital Anemia Center, Ospedale Galliera, Genoa, Italy

[2] Department of Woman Child and General Surgery, University of Campania "Luigi Vanvitelli", Naples, Italy

[3] Pediatrics Unit, Monza e Brianza per il Bambino e la sua Mamma Foundation (FMBBM), Monza, Italy

[4] Thalassemia Center, SIMI Ragusa, Ragusa, Italy

[5] Department of Clinical and Biological Sciences, University of Turin, Turin, Italy

Published online: 21 July 2020

procedures in emergency departments. MEEX requires minimal specialized equipment and little staff training, so that also in terms of cost-effectiveness it is a procedure that could be made much more widely available than AEEX. The latter, although with advantages in terms of clinical outcomes (faster reduction of *HbS%*, longer interval between procedures) and time needed, is not so widely available, and it is problematic in small-weighted children [13].

A national survey by the Italian Society for Thalassemia and Hemoglobinopathies (SITE) (unpublished data) confirmed what reported by others [9, 14, 15] on the use of a number of different protocols for MEEX among specialized centers. Although relatively simple, cheap, and effective, the procedures for MEEX have not yet been standardized [16], and a wide variability in their implementation and results has been reported [10, 15]. Moreover, none of the protocols include a formula to predict the value of *HbS%* and *Hb* after the exchange. To our knowledge, while methods to estimate *HbS%* after RBC transfusion only [17] or the number of RBC units to exchange in AEEX [18] have been reported, there is still no method to predict the reduction in *HbS%* following MEEX available, in spite of its potential usefulness in the management of SCD patients.

We report the results of a multicenter study aimed at defining a standardized protocol to evaluate the volumes to be exchanged to obtain the target values of *HbS%* and *Hb* and provide attending physicians with an effective and safe tool to plan treatment according to defined therapeutic targets (i.e., the desired *HbS%* reduction and *Hb*). We also describe a standardized MEEX procedure including two mathematical equations for the predictive estimation of *HbS%* and total *Hb* after MEEX.

Material and methods

Study design and methods

The results of MEEX performed in one of the centers participating in the present study [19] were used to develop a formula to estimate *HbS%* and *Hb* after MEEX and subsequently to develop a protocol to evaluate the volumes to be exchanged to obtain the desired values of *HbS%* and *Hb*. This MEEX procedure has been used in the aforementioned center for the last 35 years to perform over 1000 MEEX, with no significant adverse events or reactions [19]. The same center coordinated the study.

A prospective study was designed involving 5 centers belonging to the Italian Hemoglobinopathy Comprehensive Care Network to test the protocol. The coordinating center received the patient data (age, sex, weight, height, *Hb*, and *HbS%* recorded on the day of the exchange) and indicated the volumes to be exchanged according to the target values of *HbS%* and *Hb*. When possible, the *HbS%* is 30% or less [4, 20–22] and a reduction of 15–20% is usually chosen when the values of *HbS%* before the MEEX are greater than 50%. The target value of *Hb* is chosen in order to avoid post-transfusion hyperviscosity [20, 21, 23]. Post-MEEX *HbS%* and *Hb* were measured 1 h after the end of the procedure. All data were sent to the coordinating center for processing. The measurement of *HbS%* was measured by high-performance liquid chromatography (HPLC) using the β-Thalassaemia Short Program in VARIANT (VARIANT; Bio-Rad Laboratories, Hercules, CA, USA). MEEX was performed with single venous access as reported by Forni et al. 2010 [19]. A standard procedure is reported in Table 1, considering the hematocrit (*Ht*) of the RBC units transfused equal to 60%, in accordance with reference guidelines and standards [24]. Figures reported in Table 1 should be rescaled according to *Ht* value. Iron overload in patients was assessed by magnetic resonance imaging T2* (MRI) [25].

Patients

The study population consisted of a total of 46 patients affected by SCD, both homozygote HbSS and HbS-Thalassemia followed in the 5 participating centers where the MEEX procedures were carried out. MEEX performed both in chronic and acute therapeutic regimen were considered for analysis. The study was approved by all the local ethics committees, and all SCD patients included in the study gave written informed consent.

Relationship between blood volumes to be removed and transfused and *HbS%* and *Hb* after exchange

Hemoglobin S percentage and *Hb* concentration after MEEX ($HbS\%^{POST}$, Hb^{POST}) can be calculated based on the volumes exchanged during the procedure. $HbS\%^{POST}$ is calculated as the ratio between the total amount of *HbS* and the total amount of *Hb* after MEEX. Hb^{POST} is calculated as the ratio between the total amount of *Hb* and the blood volume (BV) after MEEX, net of the volumes of phlebotomy, transfusion, and hydration. The blood volume before the exchange (BV^{PRE}) was calculated according to reported formulas [17, 26–29] using patient age, sex, and weight. For patients under 12 years of age, the BV was calculated at 75 ml/kg [26, 27]; for adolescents (older than 12 years) and adults, it was calculated at 70 ml/kg for males and 65 ml/kg for females [17, 27]. Appropriate correction was applied to over-weight subjects with body mass index > 25 kg/m^2 [28, 29].

With the whole blood drawn by phlebotomy (V^{ph}), an amount of *Hb* equal to ($V^{ph} \cdot Hb^{PRE}$) is removed from the body, as well as a proportional amount of *HbS* equal to ($V^{ph} \cdot Hb^{PRE} \cdot HbS\%^{PRE}$), where Hb^{PRE} and $HbS\%^{PRE}$ are the values of *Hb* and *HbS%* before the MEEX.

On the other hand, the amount of *Hb* introduced with RBC transfusion is equal to $V^{RBC} \cdot 20\left(\frac{g}{dl}\right)$, where V^{RBC} is the volume of the RBC units transfused and $20\left(\frac{g}{dl}\right)$ is the mean

Table 1 Procedural scheme for Manual Erythroexchange (MEEX)

Hb pre-MEEX (g/dl)	MEEX		Hydration	
	Adult	**Pediatric**	**Adult**	**Pediatric**
< 85 g/L*	Only transfusion (9–12 ml/kg)	Only Transfusion (8–10 ml/kg)	–	–
85–95 g/L (or patient weight ≤ 40 kg)	STEP1: Hydration (500 ml) STEP2: Phlebotomy (10 ml/Kg*) STEP3: Transfusion (9–12 ml/kg)	STEP1: Hydration STEP2: Phlebotomy (6 ml/Kg) STEP3: Transfusion (8–10 ml/kg)	Before 1st phlebotomy: 500 ml Before 2nd phlebotomy: 300 ml	Weight < 10 kg: 3–5 ml/kg (infusion rate 2 ml/s) Weight 10–20 kg: 5–10 ml/kg (infusion rate 5–7 ml/s) Weight 20–30: 10 ml/kg (infusion rate 8 ml/s) Weight 30–40 kg: 8–10 mk/kg (infusion rate 8 ml/s)
95–100 g/L (or patient weight ≤ 40 kg)	STEP1: Hydration (500 ml) STEP2: Phlebotomy (10 ml/Kg*) STEP3: Transfusion (9–12 ml/kg)	STEP1: Hydration STEP2: Phlebotomy 8 ml/Kg STEP3: Transfusion (8–10 ml/kg)		
>100 g/L	STEP1: Hydration (500 ml) STEP2: Phlebotomy (10 ml/Kg*) STEP3: Transfusion (3–5 ml/Kg) STEP1: Hydration (300 ml) STEP4: Phlebotomy (10 ml/Kg*) STEP5: Transfusion (3–5 ml/Kg)	STEP1: Hydration STEP2: Phlebotomy (5 ml/Kg) STEP3: Transfusion (2 ml/Kg) STEP4: Phlebotomy (5 ml/Kg) STEP5: Transfusion (4–6 ml/Kg)		

*Maximum limit of single phlebotomy 700 ml, for a maximum limit of total phlebotomy of 1400 max

Transfusion volume is calculated according to hematocrit of the red blood cell unit 60%

concentration of *Hb* per unit, estimated based on an average *Ht*/unit of 60% and considering the ratio between *Ht* and *Hb*/unit equal to 3 [24]. The total amount of *Hb* after the procedure can be estimated as $\left[Hb^{PRE}\cdot\left(BV^{PRE}-V^{ph}\right)+V^{RBC}\cdot 20\left(\frac{g}{dl}\right)\right]$. Similarly, the total amount of *HbS* after the procedure can be estimated as $[HbS\%^{PRE}\cdot Hb^{PRE}\cdot(BV^{PRE}-V^{ph})]$.

Base on the above figures, the formula to preliminarily estimate *HbS%* after the exchange turns out to be:

$$HbS\%^{POST}=\frac{HbS\%^{PRE}\cdot Hb^{PRE}\cdot\left(BV^{PRE}-V^{ph}\right)}{Hb^{PRE}\cdot\left(BV^{PRE}-V^{ph}\right)+V^{RBC}\cdot 20\left(\frac{g}{dl}\right)} \quad (1)$$

Likewise, the formula for the preliminary estimation of *Hb* after the exchange is:

$$Hb^{POST}=\frac{Hb^{PRE}\cdot\left(BV^{PRE}-V^{ph}\right)+V^{RBC}\cdot 20\left(\frac{g}{dl}\right)}{BV^{PRE}-V^{ph}+V^{RBC}+V^{Hy}} \quad (2)$$

where V^{Hy} is the volume of hydration administered during the procedure.

We evaluate the volumes V^{ph} and V^{RBC} from the weight of the patient and V^{Hy} according to the procedural scheme reported in Table 1. All the volumes are then checked to respect the limits on the maximum values of volumes to exchange depending on whether the patient is adult or pediatric. The values of $HbS\%^{POST}$ and Hb^{POST} are estimated with Eqs. 1 and 2 in order to verify that a suitable couple of these variables is obtained. If necessary, adjustments of the volumes to exchange are performed to obtain optimal results for both $HbS\%^{POST}$ and Hb^{POST}.

The MEEX protocol at a glance

A summary of the protocol is presented in the flow chart in Fig. 1. The main steps are:

- Step #1—planning: the volumes to exchange are defined from the procedure reported in Table 1 and from the values of *HbS%* and *Hb* post MEEX estimated with Eqs. 1 and 2.
- Step #2—performing: the MEEX is performed with single venous access following the guide reported in Table 1.
- Step #3—verification: the values of *HbS%* and *Hb* after MEEX are estimated with Eqs. 1 and 2 in consideration of the real volumes exchanged during the MEEX; these values are compared with those measured.

Statistics

Descriptive analysis was performed with means and standard deviations (SD) or medians and interquartile range

(IQR—25th and 75th percentile). The measured and estimated values of *HbS%* and *Hb* were compared and subjected to linear regression analysis. The Pearson correlation coefficient R_p was used to evaluate the correlation between variables. The methods were also compared using the Bland-Altman plots [30, 31]. The correlation coefficients and linear fit coefficients are reported with a 95% confidence interval (CI). Excel (Microsoft, Seattle, WA, USA) was used for data management. Statistical analysis was performed in R for statistical computing and graphics [32]. Estimation of *HbS%* and *Hb* after MEEX was performed with a tool developed using Microsoft Excel.

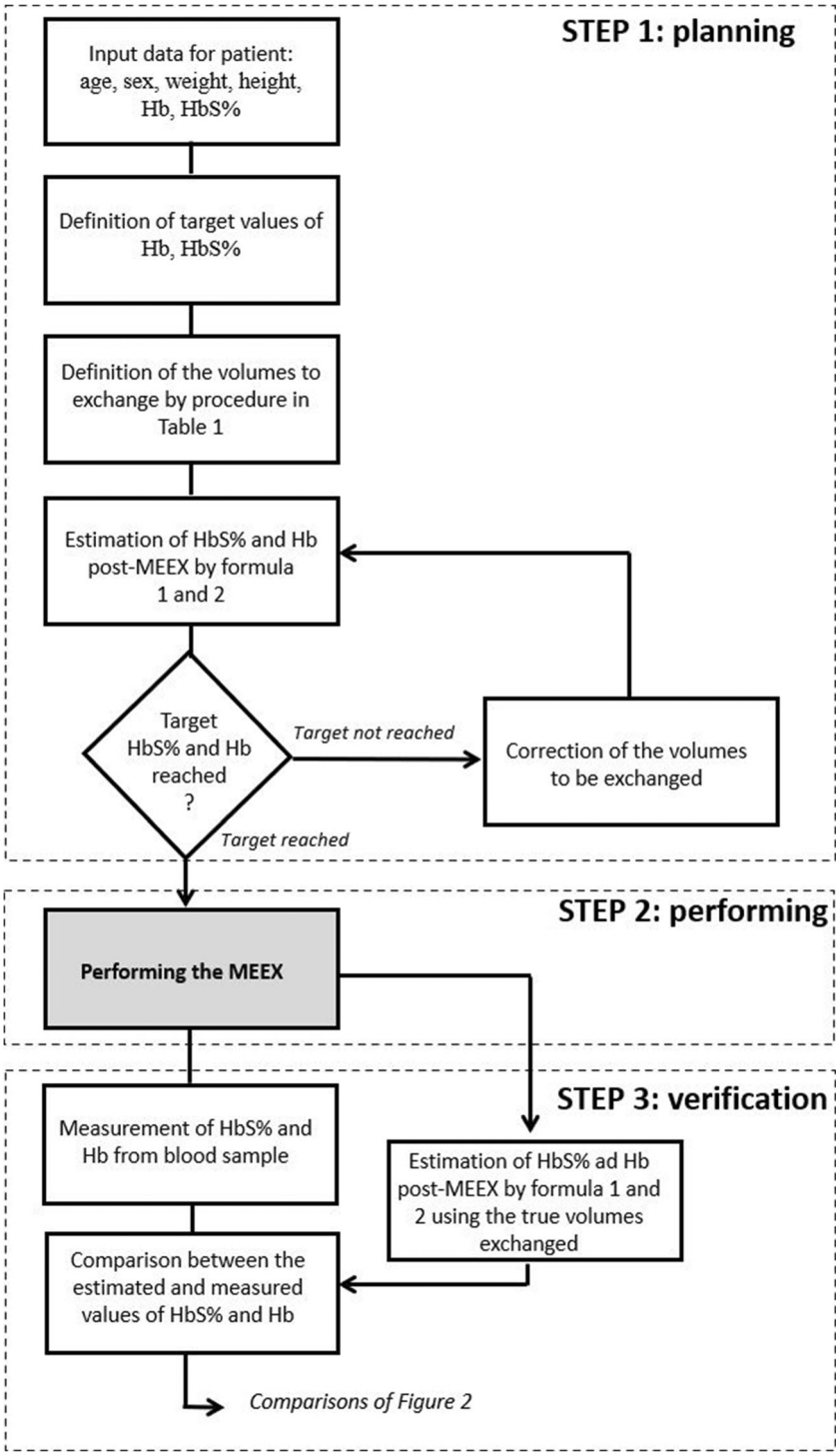

Fig. 1 Flow chart representing the protocol

Results

A total of 261 MEEX procedures performed according to the reported protocol were analyzed, resulting from data collected on the procedures carried out on 22 adult (64% males, median age 42 years IQR 22–52 years) and 24 pediatric (54% males, median age 9 years, IQR: 7–12 years) SCD patients. Analysis included, MEEX procedures performed both in chronic (89%) and in acute (11%) therapeutic regimens. A single MEEX was documented for 27 out of 46 patients and multiple MEEX for 19 patients (median number of procedures 7; IQR: 3–15). Table 2 reports patients' demographics and clinical characteristics and details of the MEEX procedures, the volumes exchanged, and *Hb* and *HbS%* values before and after MEEX. Iron overload evaluated by MRI is reported for 27 of the 32 patients on a chronic therapeutic regimen. Twenty-two (81%) patients showed no iron overload, with a liver iron concentration (LIC) < 3 mg/g of liver dry weight tissue, while 5 patients (19%) had LIC around 5 mg/g of liver dry weight tissue. No cardiac iron overload was detected.

No adverse events or reactions, such as hypotension or electrolyte imbalance, were reported nor were any complaints concerning the procedures received from patients.

Fig. 2a reports the comparison between the measured values of *HbS%* and those estimated by Eq. 1 utilizing the volumes exchanged during the procedure: a high degree of correlation between these two variables is shown [$R_p = 0.95$ (95% CI:0.94–0.96, $p < 0.001$)]. The differences between the measured and estimated *HbS%* values were plotted against their averages in a Bland-Altman plot (Fig. 2b). The mean of differences between the measured and estimated values (bias) is −0.5 (95% CI: −0.84 - −0.18), which corresponds to a median agreement of 1% between estimates and post-MEEX values (SD of distribution 2.7%). Figure 2c reports the comparison between the measured values of *Hb* and those estimated by Eq. 2, in consideration of the volumes exchanged during the procedure: correlation coefficient between these two variables $R_p = 0.65$ ($p < 0.001$). Figure 2d reports the Bland-Altman plot for *Hb* values which detects a bias of 1.0 g/dl (95% CI: 0.9–1.1) in the preliminary estimate of *Hb* (SD of distribution of the differences 0.7 g/dl). These results show that the Eqs. 1 and 2 can be used to estimate both *Hb* and *HbS%* after MEEX on the basis of the volumes exchanged and patient data (age, sex, weight, height, pre-MEEX *Hb*, and *HbS%*).

Our data show that the measured variation of *HbS%* before and after the MEEX correlates with *HbS%* value before the exchange ($R_p = -0.41$, $p < 0.001$). Table 3(A) reports the median decrease in *HbS%* for patients with *HbS%* pre-MEEX greater and lower than 50%, respectively. The reduction in *HbS%* was greater in the subset with pre-MEEX *HbS%* > 50%values ($p < 0.001$). Likewise, we found a correlation between measured variation of *Hb* and its value pre-MEEX ($R_p = -0.63$, $p < 0.001$). Table 3(B) reports the median increase in *Hb* for the three subsets of patients with values of *Hb* before the exchange 8.5–9.5 g/dl, 9.5–10 g/dl, and > 10 g/dl, showing that there is less variation in *Hb* when pre-MEEX values of *Hb* are greater ($p < 0.001$).

Furthermore, from 145 pairs of consecutive MEEX procedures, it was possible to evaluate the mean increase in *HbS%* between two consecutive procedures. Figure 3 plots the differences between *HbS%* measured after the MEEX and before the subsequent exchange as a function time laps (days) between the two procedures. Linear regression analysis ($R_p = 0.64$, $p < 0.001$) estimated an increase in *HbS%* over time of 0.39%/day (95% CI: 0.31–0.47). These data could help establish suitable intervals between procedures.

Table 2 Characteristics of the study population and of manual erythroexchange (MEEX) procedures. Median and interquartile range (IQR), 25th–75th percentile, are reported for continuous variables

	All	Adults	Children
N (M/F)	46 (27/19)	22 (14/8)	24 (13/11)
Age (yrs)	16.6 (9–38)	41.8 (22–52)	9.5 (7–12.5)
Weight (kg)	54 (30–67)	64 (55–74)	32 (23–45)
Number of MEEX	261	108	153
MEEX/ patients	1 (1–5)	2 (1–7)	1 (1–2)
Volume of phlebotomy (dl)	5 (2.5–7.7)	8 (5–13)	2.6 (2.0–3.1)
Volume of red blood cell transfused (dl)	5.1 (2.7–6.0)	5.8 (5.2–7.6)	2.8 (2.5–4.3)
Volume of hydration (dl)	2.5 (2.5–5)	5.5 (2.5–8)	2.5 (0–2.8)
Hemoglobin pre-MEEX (g/dl)	9.7 (9.0–10.6)	9.8 (9.4–10.6)	9.4 (8.8–10.4)
Hemoglobin post-MEEX (g/dl)	10.8 (10.4–11.3)	10.7 (10.4–11.1)	10.9 (10.4–11.8)
HbS% pre-MEEX	52 (43–57)	47 (41–55)	54 (48–61)
HbS% post-MEEX	38 (29–44)	35 (27–41)	39 (33–47)

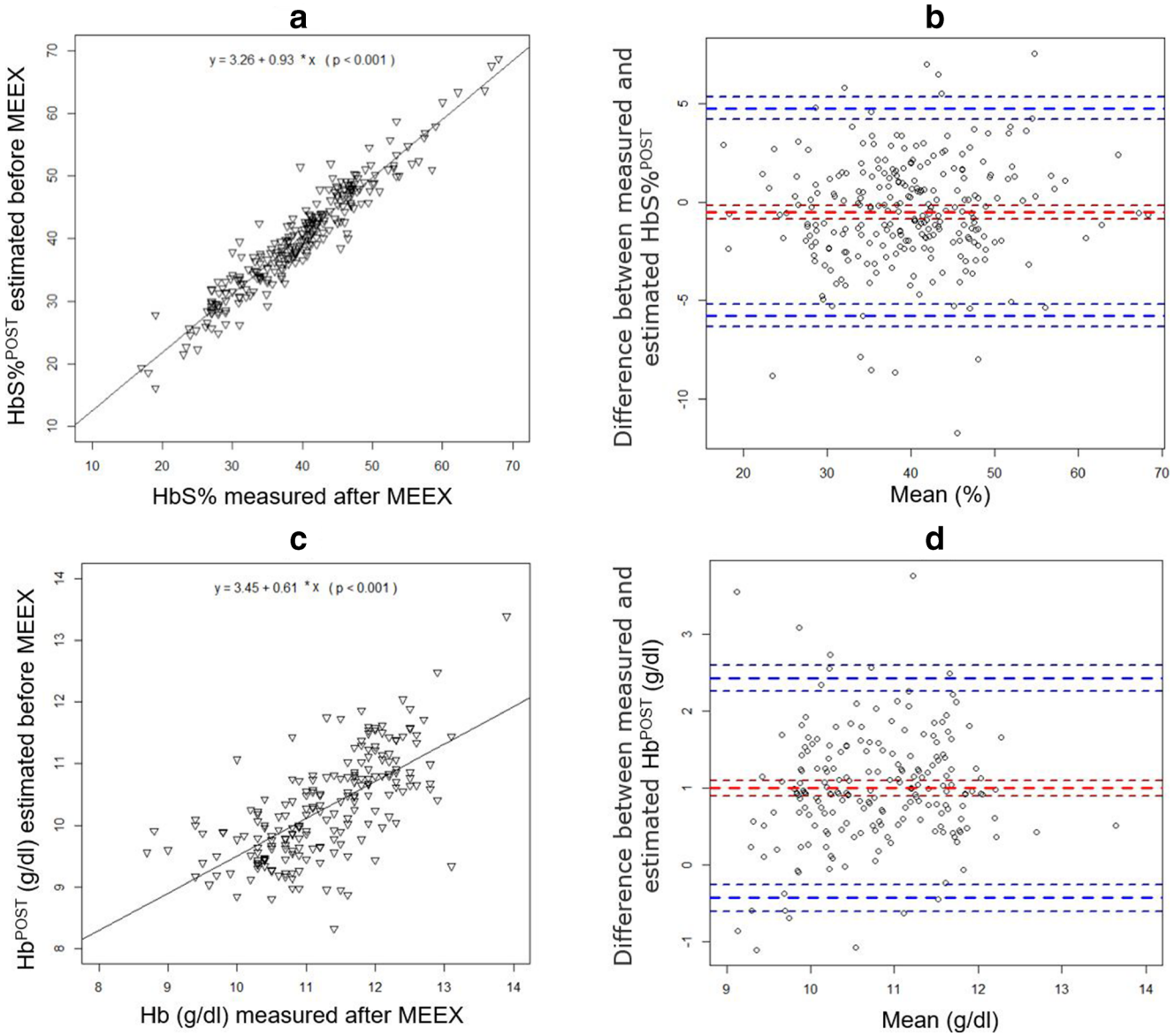

Fig. 2 Comparison between the measured and estimated values of *HbS%* and *Hb* after MEEX. (**a**) Regression analysis between the *HbS%* estimated before MEEX ($HbS\%^{POST}$) and the measured value after the MEEX. (**b**) Bland-Altman plot of differences between the *HbS%* ^{POST} estimated before MEEX and the measured value after the MEEX versus the mean of the two measurements. (**c**) Regression analysis between the *Hb* estimated before MEEX (Hb^{POST}) and the measured value after the MEEX. (**d**) Bland-Altman plot of differences between the *Hb* estimated before MEEX and the measured value after the MEEX versus the mean of the two measurements

Discussion and conclusion

RBC transfusion is among the life-saving treatments for the management of some acute events in SCD patients, such as acute ischemic stroke, multi-organ failure, acute sickle hepatopathy, acute chest syndrome, and acute priapism. Moreover, it is indicated as a long-term treatment for primary or secondary prevention of acute events [13]. The goals of RBC transfusion are the reduction of *HbS%* to safe levels (< 30–50%) and improvement of oxygen-carrying capacity. These goals must be achieved as rapidly and effectively as possible, as acute and life-threatening is the complication, without inducing post-transfusion hyperviscosity that could exacerbate vaso-occlusive events. MEEX represents the mainstay of treatment of acute events. It is easy to perform at the patient's bedside without specialized equipment and is effective in rapidly reducing *HbS%*. All hospitals admitting SCD patients should have protocols and training in transfusion for SCD, including manual exchange procedures, in place [10–12]. On the other hand, the chronic transfusion regimen is indicated in the primary and secondary prevention of neurological and non-neurological complications (i.e., recurrent acute chest syndrome, recurrent priapism, pulmonary hypertension) where hydroxyurea is

Table 3 Decrease in measured HbS% and increase in measured Hb according to the pre-manual erythroexchange (pre-MEEX) values. Median and interquartile range (IQR), 25th–75th percentile, are reported

(A) HbS%

HbS% range	N	HbS% pre-MEEX (g/dl) median (IQR)	Decrease in HbS% median (IQR)	*p* value*
≤ 50%	92	44 (41–47)	13.0 (10.0–15.0)	
> 50%	165	57 (54–61)	14.0 (11.1–17.3)	<0.001
(B) Hb				
Hb range (g/dl)	**N**	**Hb (g/dl) median (IQR)**	**Increase in Hb (g/dl) median (IQR)**	**p value***
8.5–9.5	62	9.2 (8.9–9.4)	1.4 (1.1–2)	
9.5–10	45	9.8 (9.7–9.9)	1.1 (0.6–1.6)	0.0024
> 10	149	11.4 (10.6–11.8)	0.3 (−0.2–0.8)	< 0.001

*Calculated according to the difference between the previous subset

contraindicated, not tolerated or ineffective [13]. In the chronic transfusion regimen, manual, or automated erythroexchange is preferable than transfusion only, in order to reduce the risk of iron overload and the subsequent increase in mortality and morbidity in SCD patients [33, 34]. AEEX has clear advantages over MEEX in terms of rapidity, duration of the interval between procedures, and better control of *HbS%* and iron overload.

However, AEEX is limited by the need for specialized equipment and staff, for multiple or central venous accesses, and a considerable demand for blood. In cases in which AEEX is not available or feasible, MEEX should always be considered in the chronic transfusion regimen of SCD patients due to the obvious advantages over transfusion only [13]. One of the biggest problems encountered by physicians in adopting

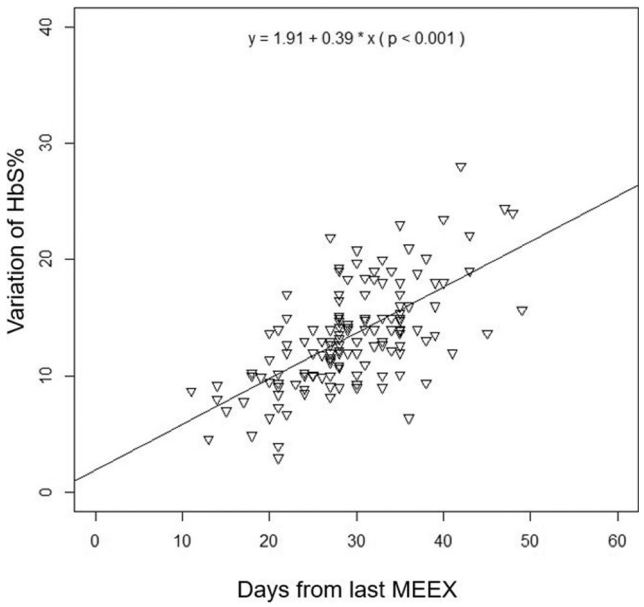

Fig. 3 Variation of *HbS%* between consecutive MEEX versus the time interval between the exchanges

MEEX in clinical practice is the lack of standardized and validated protocols, leading to greater variability in post-MEEX *HbS%* and *Hb* because of different treatment approaches adopted [16]. A survey carried out in 32 academic centers in North America [15] showed that the *HbS%* target during the transfusion regimen was difficult to achieve and maintained in the clinical practice. The same survey reported that the *HbS%* target was reached and maintained more effectively with a unit-based MEEX protocol rather than a weight-based approach. In spite of this, the desired *HbS%* value was not reached in around half of the patients, showing the need for substantial improvements in the MEEX protocol. Moreover, it was proposed that achievement of the *HbS%* target was affected by patient age, duration of transfusion therapy pre-transfusion *Hb* value, and compliance to the transfusion regimen [15]. In this study, we have demonstrated that the pre-transfusion *HbS%* level has a significant impact on the outcome of exchange procedures and that the rate of *HbS%* increase is 0.39% per day, supporting the view that an evidence-based scheduling of transfusions is among the most significant factors to be considered in order to reach the *HbS%* target [15].

In this study, we tested and validated a standardized protocol to evaluate the volumes to be exchanged to obtain the target value of *HbS%* and *Hb* after each MEEX. The protocol is inclusive of two equations for predictive estimation of *HbS%* and *Hb* after MEEX; the results presented showed the efficacy and accuracy of Eqs. 1 and 2 in the estimation of both *Hb* and *HbS%* after MEEX on the basis of the volumes exchanged and patient data (age, sex, weight, height, pre-MEEX *Hb*, and *HbS%*). Moreover, from the measurements collected, it was possible to evaluate the rate of *HbS%* increase between exchanges. In this way, the interval between exchanges and the quantity of blood to exchange can be customized according to the initial *HbS%* value and the individual patient's therapeutic goal, which have been reported as determinant factors in reaching the target *HbS%* value [16]. This means the transfusion regimen can be adapted to the patient's clinical condition and improve the organization of the

exchange program to ensure the consistency of the pre-established therapeutic target. For example, the accurate prediction of the change of *HbS%* and *Hb* in a patient with acute events allows the physicians to plan the number of exchange procedures and the request for RBC units without running the risk of hyperviscosity. Furthermore, the early estimation of the change in *HbS%* and *Hb* can facilitate a more accurate and effective MEEX program in preparation for surgery.

The main causes of errors that affect the estimations of *HbS%* and *Hb* with Eqs. 1 and 2 are the assessment of BV, the hydration of the patient, and particular cases of splenomegaly and hypersplenism. Another factor to be considered is that the *Ht* of RBC units is not consistent among the transfused units. In our analysis we use a standard value of 60%, but international standards accept a variation between 50 and 70% [24]. In this study, applying Eqs. 1 and 2 and using the median values of variables measured in our population, it was estimated that an increase in RBC units' *Ht* from 60 to 70% may cause a decrease of approximately 1 g/dl in the estimation of *Hb* and of 1.7% in the estimation *HbS%*. We believe that the use of a standardized protocol allows doctors to use MEEX with greater confidence and reduce the margin of errors associated with the procedure.

In conclusion, our study presents a practical standardized MEEX protocol for the evaluation of the volumes to be exchanged to obtain the target values of *HbS%* and *Hb* that can be used both in the chronic transfusion regimen and to manage acute events. *HbS%* and *Hb* values are estimated with two equations from the volumes exchanged and patient data (age, sex, weight, height, and pre-MEEX *Hb* and *HbS%*). This protocol was shown to be effective and safe, as all patients, both adults and children, reached the desired *HbS%* with no adverse events or iron overload. It potentially be used for implementation in different clinical settings and without specialized personnel, with the aim of improving the management of SCD-related complications worldwide, especially in low-resource settings.

Compliance with ethical standards

Conflict of interest Conflict of interest: GLF has received research funding from Novartis, Celgene; consults from Roche and BBB, served an DSMB panel for Apopharma. VMP, VV, MC, CF participated on an Advisory Board for Novartis. Other authors have no conflict of interest to disclose.

References

1. Modell B (2008) Global epidemiology of haemoglobin disorders and derived service indicators. Bull World Health Organ 2008(6): 480–487. https://doi.org/10.2471/BLT.06.036673
2. WHO (2011) Regional Committee for Africa, 60. Sickle-Cell Disease: a strategy for the WHO African Region
3. Ware RE, de Montalembert M, Tshilolo L, Abboud MR (2017) Sickle cell disease. Lancet 390(10091):311–323. https://doi.org/10.1016/S0140-6736(17)30193-9
4. Rees DC, Williams TN, Gladwin MT (2010) Sickle-cell disease. Lancet 376(9757):2018–2031. https://doi.org/10.1016/S0140-6736(10)61029-X
5. Eaton WA, Hofrichter J (1990) Sickle cell hemoglobin polymerization. Adv Protein Chem 40:63–279 Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2195851
6. De Franceschi L, Cappellini M, Olivieri O (2011) Thrombosis and sickle cell disease. Semin Thromb Hemost 37(03):226–236. https://doi.org/10.1055/s-0031-1273087
7. Manwani D, Frenette PS (2013) Vaso-occlusion in sickle cell disease: pathophysiology and novel targeted therapies. Blood 122(24): 3892–3898. https://doi.org/10.1182/blood-2013-05-498311
8. Hebbel RP (2008) Adhesion of sickle red cells to endothelium: myths and future directions. Transfus Clin Biol 15(1–2):14–18. https://doi.org/10.1016/j.tracli.2008.03.011
9. Forni GL, Finco G, Graziadei G, Balocco M, Rigano P, Perrotta S et al (2014) Development of interactive algorithm for clinical management of acute events related to sickle cell disease in emergency department. Orphanet J Rare Dis 9:91. https://doi.org/10.1186/1750-1172-9-91
10. (2018) Standards for the clinical care of adults with sickle cell disease in the UK, 2nd edn. Sickle Cell Society
11. Davis BA, Allard S, Qureshi A, Porter JB, Pancham S, Win N et al (2017) Guidelines on red cell transfusion in sickle cell disease. Part I: principles and laboratory aspects. Br J Haematol 176(2):179–191. https://doi.org/10.1111/bjh.14346
12. Davis BA, Allard S, Qureshi A, Porter JB, Pancham S, Win N et al (2017) Guidelines on red cell transfusion in sickle cell disease part II: indications for transfusion. Br J Haematol 176(2):192–209. https://doi.org/10.1111/bjh.14383
13. Howard J (2016) Sickle cell disease: when and how to transfuse. Hematology 2016(1):625–631. https://doi.org/10.1182/asheducation-2016.1.625
14. Swerdlow PS (2006) Red cell exchange in sickle cell disease. Hematology 2006(1):48–53. https://doi.org/10.1182/asheducation-2006.1.48
15. Mian HS, Ward R, Telfer P, Kaya B, Kuo KHM (2015) Optimal manual exchange transfusion protocol for sickle cell disease: a retrospective comparison of two comprehensive care centers in the United Kingdom and Canada. Hemoglobin 39(5):310–315. https://doi.org/10.3109/03630269.2015.1057734
16. Aygun B, Wruck LM, Schultz WH, Mueller BU, Brown C, Luchtman-Jones L et al (2012) Chronic transfusion practices for prevention of primary stroke in children with sickle cell anemia and abnormal TCD velocities. Am J Hematol 87(4):428–430. https://doi.org/10.1002/ajh.23105
17. Mathur G, Ten Eyck P, Knudson CM (2018) Predicting changes in hemoglobin S after simple transfusion using complete blood counts. Transfusion 58(1):138–144. https://doi.org/10.1111/trf.14371
18. Mandal S, Westra J, Apushkin M, Richa EM, Zibrat SJ, Toney L et al (2014) Automated red cell exchange: a simplified formula for how many red cell units to exchange and validity of haemoglobin S levels measured one to two hours later. Blood Transfus 12(Suppl 1):s145–s146. https://doi.org/10.2450/2013.0267-12
19. Carrara P, Balocco M, Pinto V, Olcese F, Soldà A, Strada P, Forni GL (2010) Manual erythroexchange for chronic transfusion therapy in patients with sickle cell syndromes unresponsive to hydroxyurea: a long-term follow-up. Am J Hematol 85(12):974–974. https://doi.org/10.1002/ajh.21895
20. Bonomo P, Carta M, Forni G, Prati D, Rigano P, Vassanelli A (2014) Raccomandazioni per le strategie trasfusionali nelle

Emoglobinopatie. Collana Scientifica S.I.T.E., (3). Retrieved from http://site-italia.org/file/collana_scientifica/libretto_3_2014/download.php?file=Collana_scientifica_SITE_n.3_2014.pdf
21. De Franceschi L, Graziadei G, Rigano P, Cianciulli P, Forni GL (2014) RACCOMANDAZIONI PER LA GESTIONE DEL PAZIENTE ADULTO AFFETTO DA ANEMIA FALCIFORNE della Società Italiana Talassemie ed Emoglobinopatie - SITE. Collana Scientifica S.I.T.E., 2
22. Bartolucci P, Galactéros F (2012) Clinical management of adult sickle-cell disease. Curr Opin Hematol 19(3):149–155. https://doi.org/10.1097/MOH.0b013e328351c35f
23. Josephson CD, Su LL, Hillyer KL, Hillyer CD (2007) Transfusion in the patient with sickle cell disease: a critical review of the literature and transfusion guidelines. Transfus Med Rev 21(2):118–133. https://doi.org/10.1016/j.tmrv.2006.11.003
24. Europe, C. Of (2020) Guide to the preparation, use and quality assurance of blood components. European Directorate for the Quality of Medicines and Healthcare (EDQM), European Committee (Partial Agreement) on Blood Transfusion (CD-P-TS) Retrieved from www.edqm.eu
25. Wood JC, Enriquez C, Ghugre N, Tyzka JM, Carson S, Nelson MD, Coates TD (2005) MRI R2 and R2* mapping accurately estimates hepatic iron concentration in transfusion-dependent thalassemia and sickle cell disease patients. Blood 106(4):1460–1465. https://doi.org/10.1182/blood-2004-10-3982
26. Riley AA, Arakawa Y, Worley S, Duncan BW, Fukamachi K (n.d.) Circulating blood volumes: a review of measurement techniques and a meta-analysis in children. ASAIO J 56(3):260–264. https://doi.org/10.1097/MAT.0b013e3181d0c28d
27. Raes A, Van Aken S, Craen M, Donckerwolcke R, Walle JV, Vande Walle J (2006) A reference frame for blood volume in children and adolescents. BMC Pediatr 6(1):3. https://doi.org/10.1186/1471-2431-6-3
28. Lemmens H, Bernstein D, Brodsky J (2006) Estimating blood volume in obese and morbidly obese patients. Obes Surg 16(6):773–776. https://doi.org/10.1381/096089206777346673
29. Sharma R, Sharma S (2019) Physiology, Blood Volume. StatPearls. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/30252333
30. Bland JM, Altman DG (1986) Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1(8476):307–310 Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2868172
31. Giavarina D (2015) Understanding Bland Altman analysis. Biochem Med 25(2):141–151. https://doi.org/10.11613/BM.2015.015
32. R Core Team (2018) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. R Foundation for Statistical Computing, Vienna. https://doi.org/10.1007/978-3-540-74686-7
33. Kuo KHM, Ward R, Kaya B, Howard J, Telfer P (2015) A comparison of chronic manual and automated red blood cell exchange transfusion in sickle cell disease patients. Br J Haematol 170(3):425–428. https://doi.org/10.1111/bjh.13294
34. Duclos C, Merlin E, Paillard C, Thuret I, Demeocq F, Michel G, Kanold J (2013) Long-term red blood cell exchange in children with sickle cell disease: manual or automatic? Transfus Apher Sci 48(2):219–222. https://doi.org/10.1016/j.transci.2012.09.002