Assessment of functional shunting in patients with sickle cell disease

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Supplementary materials

Laboratory parameters

Blood was drawn from a cubital vein on the day of the study visit and an intravenous catheter was placed at the site of cannulation for the administration of ACZ during the MRI study. Hematological laboratory parameters such as hemoglobin (Hb), hematocrit, platelet count, fetal hemoglobin (HbF), sickle hemoglobin (HbS), and markers representing hemolysis, including reticulocytes, lactate dehydrogenase (LDH) and total bilirubin, were assessed using standard laboratory procedures.

Data acquisition

Anatomical scans

In pediatric patients, a 2D T_2-weighted scan was performed with voxel size = 0.45 x 0.45 x 4 mm, FOV = 230 x 205 x 144 mm, TR/TE = 3000/80 ms, flip angle = 90° and scanning duration = 3 minutes. Additionally, a 2D fluid-attenuated inversion recovery (FLAIR) sequence was acquired with voxel size = 0.45 x 0.45 x 3.75 mm, FOV = 230 x 205 x 144 mm, TR/TE = 11000/100 ms, inversion delay = 2600 ms, flip angle = 90° and scan duration 2:56 minutes, to assess white matter lesions. In adults, 3D FLAIR images were acquired with voxel size = 0.98 x 0.98 x 1.12 mm, FOV = 250 x 250 x 180 mm, TR/TE = 4800/356 ms, SPAIR fat suppression, inversion delay = 1650 ms, flip angle = 90° and scan duration = 5:11 minutes.

Functional scans

Arterial spin labeling

Pseudo-continuous arterial spin labeling (PCASL) sequence with a 2D single-shot gradient-echo echo-planar imaging (GE-EPI) readout was used with the following imaging parameters: TR/TE = 4000/17 ms, FOV = 240 x 240 mm^2, voxel size = 3 x 3 mm^2, slice thickness = 7 mm, effective post-label delay = 1525 ms, label duration = 1650 ms, 17 axial slices, flip angle = 90°, background suppression, 75 control-label pairs and a total scan duration of 10 minutes. Some parameters differed in the adult study: TR/TE = 4400/14 ms, effective post-label delay = 1800 ms, label duration = 1800 ms, 19 axial slices, a total of 35 control-

label pairs, and a total scan duration of 5 minutes. In adult participants, 16 mg/kg ACZ dissolved in 20 mL saline, 0.9% NaCl, a flow rate of 0.1 mL/sec was used and it was flushed with 15 mL saline (Diamox®, Mercury Pharmaceuticals Ltd., London, UK). In addition to the ASL scan, an M0 scan was acquired for quantification purposes in adult participants. M0 scans were not available for the pediatric patients, therefore, a single fixed arterial M0 value was used, derived from CSF, as described in a previous study[1].

T_2-prepared tissue relaxation with inversion recovery MRI

For the venous oxygenation measurements in adults, T_2-prepared tissue Relaxation with an Inversion Recovery MRI (T_2-TRIR) sequence[2] was used with a 2D single shot FFE EPI Look-Locker read-out TR/TE/TI1/ΔTI = 150/24/10/130 ms, FOV = 202 x 243 mm^2, voxel size = 2 x 2 mm^2, slice thickness 4 mm, 1 slice perpendicular to the sagittal sinus, flip angle = 95°, 4 dynamics and total scan duration of 50 seconds. The scan was acquired twice, before and approximately 22 minutes after receiving ACZ.

Phase-contrast MRI

For the velocity measurements in the brain feeding arteries, a 2D phase-contrast single-shot gradient-echo T_1 FFE sequence was acquired with the following parameters: TR/TE = 15/6 ms for adults and 15/5 ms for children, FOV = 230 x 230 mm^2, voxel size = 0.45 x 0.45 mm^2, slice thickness = 4mm, flip angle = 15°, VENC = 80 cm/s for adult participants and 140 cm/s for pediatric participants, 1 axial slice perpendicular to the internal carotid and vertebral arteries, based on 2D coronal and sagittal phase-contrast angiograms, and a total acquisition time of 65 seconds. In adult participants, this scan was performed both before and approximately 16 minutes after ACZ administration.

Data analysis

Gray matter CBF

GM CBF was quantified using the dual compartment model[3] with subject-specific arterial transit time (ATT), labeling efficiency and blood T_1 measured in the sagittal sinus using T_2-TRIR. In pediatric patients, an average blood T_1 of 1818 ms was used as previously measured in these patients[4], and a fixed ATT of 1800 ms was used. Gray matter masks were created with gray matter > 25% of the gray matter tissue probability image. For adult participants, the baseline CBF and post-ACZ CBF were quantified from the pre-and post-ACZ ASL scan respectively.

Velocity in the brain feeding arteries

Average velocities of right and left internal carotid arteries and right and left vertebral arteries were multiplied by the fraction of total flow contributed by each vessel and summed for each subject to obtain a weighted velocity.

OEF and $CMRO_2$ measurements

For the OEF measurements, T_2 values were computed from T_2-TRIR and converted to venous oxygen saturation (SvO_2) using an SCD-specific model[5] (HbS model) for the patients and a healthy subject model for controls[6] (HbA model). To study the effect of different calibration models on our regression models, the analyses were repeated using the HbA model for both patients and controls (see below). Arterial oxygen saturation (SaO_2) was assumed to be 98%. OEF was calculated using the following equation:

$$OEF\ (\%) = (SaO_2 - SvO_2)/\ SaO_2 \cdot 100\%. \qquad [1]$$

The oxygen carrying capacity (CaO_2) was calculated using the following equation:

$$CaO_2\ (\mu mol\ O2/\ 100\ mL\ blood) = [(Hb \cdot 1.34 \cdot SaO_2) + (0.003 \cdot pO_2)]/\ 22.4 \cdot 1000$$

In which Hb is the patient-specific hemoglobin, 1.34 is a constant representing the amount of oxygen that can bind to hemoglobin, 0.0031 is the solubility coefficient of oxygen in human plasma and pO_2 is arterial oxygen tension, which is assumed to be 100 mmHg for room air. Unit conversion to molar concentration was performed by dividing by $22.4 \cdot 1000$.

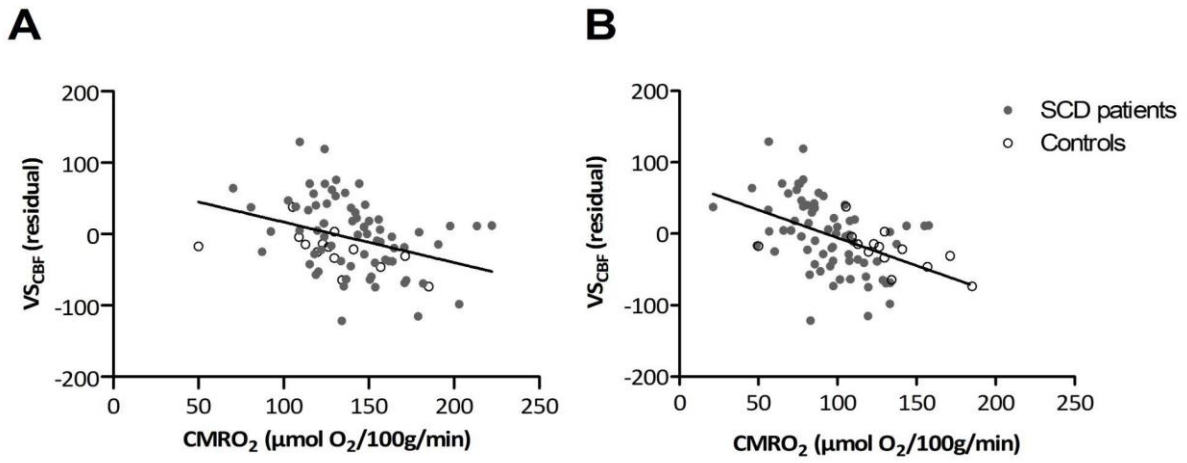
$CMRO_2$ was calculated according to Fick's principle using the previously quantified parameters:

$$CMRO_2\ (\mu mol\ O_2/100g/min) = CBF \cdot OEF \cdot CaO_2 \qquad [2]$$

Calibration model

Multiple calibration models for the calculation of venous oxygenation from T_2 values in SCD and controls have been proposed over the years[5-7]. The first model was the Bovine model, but this model was calculated for much higher hematocrit values than observed in anemic sickle cell patients. Therefore, the

HbA and HbS models were proposed, based on blood measurements from controls and SCD patients, respectively. We decided to use the HbS model, which accounts for the pathological and hematological properties of SCD. However, for completeness and reproducibility purposes we repeated our analyses including OEF and $CMRO_2$ using the HbA model. In line with our findings using the HbS model, VS_{CBF} was significantly predicted by $CMRO_2$ (β=-0,58 F(1,82)=12.1, p<0.001) (Fig. 1), Hb (β=-10.4 F(1,66)=6.6, p = 0.012), and participant group (β=-56.7, F(1,67)=5.9, p<0.018). When separating the analysis for SCD adults and controls, we observed that in addition to $CMRO_2$, Hb and LDH were significant independent predictors of VS_{CBF} in patients with Hb having a negative association (β=-17.2 p<0.001) and LDH having a positive association (β=0.15, p<0.001). Moreover, linear mixed modeling demonstrated that HbF and LDH are significant predictors of $CMRO_2$-HbA when corrected for $VS_{CBF.}$ Higher HbF was associated with lower $CMRO_2$-HbA (β=-1.44, F(1,62)=17.6, p<0.001), and higher LDH was associated with higher $CMRO_2$-HbA (β=0.08, F(1,65)=18.5, p<0.001). In summary, independent of the chosen calibration model, $CMRO_2$ was the strongest predictor of VS_{CBF} and significant predictors of $CMRO_2$ were HbF and LDH.



Supplementary Figure 1: Scatterplots of VS_{CBF} and $CMRO_2$ obtained from the HbA (A) and HbS (B) model before and after ACZ in all adult subjects, showing a significant correlation across all subjects.

Supplementary Table 1: Available sample size for each included parameter

	Pediatric SCD (N)	Adult SCD (N)	Controls (N)
Demographics			
Age	28	38	10
Sex	28	38	10
Clinical parameters			
Hemoglobin (g/dL)	28	37	10
Hematocrit (%)	28	38	10
Reticulocytes (10e9/L)	28	37	8
LDH (U/L 37 ˚C)	28	36	7
Total Bilirubin (mg/dL)	27	37	7
HbF (%)	25	37	10
HbS (%)	25	38	2
Hydroxyurea (N[%])	9	15	-
Exchange transfusion (N[%])	-	3	-
Imaging parameters			
GM CBF (mL/100g/min)			
Pre-ACZ	28	37	10
Post-ACZ	-	38	10
VGR			
Pre-ACZ	28	37	10
Post-ACZ	-	38	10
Weighted velocity (cm/s)			
Pre-ACZ	28	36	10
Post-ACZ		34	10
OEF (%)			
Pre-ACZ	-	36	8
Post-ACZ	-	36	7
$CMRO_2$ (µmol O_2/100g/min)			
Pre-ACZ	-	35	8
Post-ACZ		35	6
Lesion volume (mL)	28	38	10

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