Assessment of functional shunting in patients with sickle cell disease


Received: October 19, 2021.
Accepted: May 5, 2022.


Publisher's Disclaimer.
E-publishing ahead of print is increasingly important for the rapid dissemination of science. Haematologica is, therefore, E-publishing PDF files of an early version of manuscripts that have completed a regular peer review and have been accepted for publication. E-publishing of this PDF file has been approved by the authors. After having E-published Ahead of Print, manuscripts will then undergo technical and English editing, typesetting, proof correction and be presented for the authors' final approval; the final version of the manuscript will then appear in a regular issue of the journal. All legal disclaimers that apply to the journal also pertain to this production process.
Assessment of functional shunting in patients with sickle cell disease

Liza Afzali-Hashemi1, Lena Václavů2, John C. Wood3, Bart J. Biemond4, Aart J. Nederveen1,
*Henk J.M.M. Mutsaerts1 and #Anouk Schrantee1

*These authors contributed equally to this work

1Department of Radiology & Nuclear Medicine, Amsterdam University Medical Centers, Amsterdam, The Netherlands
2C.J. Gorter Center for High Field MRI, Department of Radiology, Leiden University Medical Center, Leiden, The Netherlands
3Division of Cardiology, Children’s Hospital Los Angeles, Keck School of Medicine, University of Southern California, Los Angeles, CA, United States
4Department of Hematology, Amsterdam University Medical Centers, Amsterdam, The Netherlands

Author contribution statement

LAH: involved in study conception and design, analysis and interpretation of the data and manuscript drafting; LV: involved in data acquisition, analysis and interpretation of the data and manuscript revision; JCW: involved in analysis and interpretation of the data and manuscript revision; BJW: involved in data acquisition, analysis and interpretation of the data and manuscript revision; AJN: involved in study conception and design, analysis and interpretation of the data and manuscript revision; HJMM: involved in study conception and design, analysis and interpretation of the data and manuscript revision; AS: involved in analysis and interpretation of the data and manuscript drafting.

Running head:
Functional shunting in SCD patients

Corresponding author:
Anouk Schrantee, Ph.D.
Meibergdreef 9
1105 AZ Amsterdam
The Netherlands
Phone: +3125668327
Email: a.g.schrantee@amsterdamumc.nl
Data sharing statement
Data available on request from the authors

Abstract word count: 238
Text word count: 3918
Number of Tables and Figures: 8
Supplementary files: 1

ClinicalTrials.gov identifier: NCT02824406

Acknowledgements
The authors would like to thank all the participants for their time and effort to take part in this study and they are grateful to Dr. Jan Petr for his advices regarding ExploreASL.

The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: National Heart Lung and Blood Institute (1R01HL136484-A1).
Abstract

Silent cerebral infarcts (SCIs) are common in patients with sickle cell disease (SCD) and are thought to be caused by a mismatch between oxygen delivery and consumption. Functional cerebrovascular shunting is defined as reduced oxygen offloading due to the rapid transit of blood through the capillaries caused by increased flow and has been suggested as a potential mechanism underlying reduced oxygenation and SCI. We investigated the venous arterial spin labeling signal (VS) in the sagittal sinus as a proxy biomarker of cerebral functional shunting, and its association with hemodynamic imaging and hematological laboratory parameters. We included 28 children and 38 adults with SCD, and 10 healthy race-matched adult controls. VS, cerebral blood flow (CBF), velocity in the brain feeding arteries, oxygen extraction fraction (OEF) and cerebral metabolic rate of oxygen (CMRO$_2$) were measured before and after acetazolamide administration. VS was higher in patients with SCD compared to controls (p<0.01) and was increased after acetazolamide administration in all groups (p<0.01). VS was primarily predicted by CBF (p<0.01), but CBF-corrected VS was also associated with decreased CMRO$_2$ (p<0.01). Additionally, higher disease severity defined by low hemoglobin and increased hemolysis was associated with higher CBF-corrected VS. Finally, CMRO$_2$ was negatively correlated with fetal hemoglobin, and positively correlated with lactate dehydrogenase, which could be explained by changes in oxygen affinity. These findings provide evidence for cerebral functional shunting and encourage future studies investigating the potential link to aberrant capillary exchange in SCD.

Keywords: Sickle Cell Disease, Functional Shunting, Cerebral Blood Flow, Cerebral Metabolic Rate of Oxygen (CMRO$_2$), Arterial Spin Labeling
**Introduction**

Sickle cell disease (SCD) is characterized by chronic hemolytic anemia resulting in organ damage including silent cerebral infarcts (SCIs)\(^1\,^2\). SCIs are associated with cognitive impairment at an early stage in life, which can result in under- and unemployment as well as lower quality of life\(^2\,^3\). SCIs are primarily localized in deep white matter and are hypothesized to be a result of impaired oxygen delivery\(^4\,^6\). To maintain brain oxygenation, cerebral blood flow (CBF) is increased in patients with SCD\(^7\,^9\). However, despite the preservation of normal global oxygen delivery at rest\(^10\), the incidence of SCIs continues to rise throughout the lifetime of these patients and is prevalent in more than 50% of adult SCD patients by the age of 32 years\(^11\). One potential pathophysiological mechanism that has been proposed suggests that SCIs result from localized impairments in oxygen delivery caused by cerebral functional shunting\(^10\,^12\,^13\). Cerebral functional shunting is defined as the rapid transit of blood through the brain capillaries as a result of increased flow, limiting offloading of oxygen to the tissue\(^13\). However, functional shunting is difficult to assess and more insight into this process is required to understand the underlying cerebral hemodynamics in SCD.

Arterial spin labeling (ASL) MRI is widely used in research settings as a non-invasive technique for cerebral perfusion measurements in patients with SCD\(^9\,^14\,^15\). ASL applies radiofrequency pulses in the brain feeding arteries that change the magnetization of blood, also known as labeling. Following labeling, the blood travels to the brain and a labeled image is acquired. The same procedure is repeated without labeling to acquire a control image. By subtracting the labeled image from the control image, a quantitative perfusion map is obtained. A requirement of ASL is that images are
acquired after a predefined delay (post-label delay), allowing the labeled blood to travel to the capillary bed, and for the labeled spins to enter the tissue. The difference between labeled spins detected in arterial and venous circulations represents spins that have exchanged with the brain parenchyma. According to the Renkin-Crone model, which relates blood flow to extraction fraction, the amount of unexchanged labeled spins in the venous outflow, i.e. the venous signal (VS), will increase approximately proportionally with CBF. However, in the presence of cerebral functional shunting, a larger volume of the labeled blood will pass unexchanged through the capillaries and may arrive on the venous side at the time of imaging. Indeed, a previous study demonstrated that, with longer post-label delays, the VS in the sagittal sinus can be used to inform on the exchange of spins at the capillary level. As such, VS may provide additional hemodynamic information about oxygen utilization at the capillary level, which may be affected in patients with SCD.

Support for this hypothesis comes from prior work in SCD patients. Juttukonda et al. observed the presence of ASL signal in the venous sinuses at a regular post-label delay (1900ms), which was associated with increased CBF and elevated velocities in the carotid arteries. When administered to adult patients with SCD, acetazolamide (ACZ; a compound that induces vasodilation and increased CBF) led to observed reductions in oxygen extraction fraction (OEF) as well as the cerebral metabolic rate of oxygen (CMRO$_2$), indicating that oxygen consumption can actually deteriorate despite increasing oxygen delivery. Moreover, in SCD patients with SCIs, an inverse relation between VS scores and OEF was found, providing support for a mechanism involving functional shunting. However, whether VS relates to cerebral functional shunting and could therefore be a marker of capillary oxygen exchange efficiency in SCD patients, remains to be investigated.
Therefore, in the present study, we investigated cerebral functional shunting in patients with SCD, by examining the relationship between VS and cerebrovascular imaging parameters of perfusion, cerebral oxygen extraction and metabolism as well as laboratory measures. To directly probe the VS-CBF relationship, we studied the imaging parameters both before and after ACZ administration in adult participants.

Methods

The data were obtained from two studies (one adult and one pediatric study) that were approved by the medical ethics committee of Academic Medical Center in Amsterdam and performed in accordance with the Declaration of Helsinki. Written informed consent was obtained from all adults and from parents/legal guardians of participants in the pediatric study cohort. Sixty-six patients with SCD (59 HbSS and 7 HbSβthalassemia) were recruited from hematology outpatient clinics. Additionally, 10 healthy adult race-matched controls (8 HbAA and 2 HbAS) were recruited. Exclusion criteria were MRI contraindications, history of cerebral pathology, sickle cell crisis at inclusion, hospitalization one month prior to the study day, pregnancy, and ACZ contraindications for adult participants.

Data acquisition

Images were acquired on two 3.0 T MRI systems (Philips Intera and Ingenia, Philips Healthcare, Best, The Netherlands) with an 8-channel head coil for pediatric patients and a 32-channel head coil for adults. Prior to MRI, blood was drawn to quantify
hematological laboratory parameters (for details, see online Supplementary Materials). Anatomical sequences included a 2D T2-weighted and a 2D fluid-attenuated inversion recovery (FLAIR) scan for children and 3D FLAIR for adult participants were used for lesion and gray matter segmentation, and registration purposes.

For VS and CBF measurements, pseudo-continuous arterial spin labeling (PCASL) was used (Table 1). Adult participants received intravenous ACZ (16 mg/kg with a maximum of 1400 mg) 10 minutes prior to the second ASL scan.

To obtain venous saturation for OEF measurements in adult participants’ pre and post-ACZ, T2-prepared tissue Relaxation with an Inversion Recovery MRI (T2-TRIR) sequence was used. Finally, to obtain velocity measurements in the brain feeding arteries, a 2D phase-contrast single-shot gradient-echo T1 FFE sequence was acquired both pre and post-ACZ (Table 1) (for more details on the acquisition parameters, see online Supplementary Materials).

Data analysis

VS was assessed both qualitatively and quantitatively. Qualitatively, ASL images were visually inspected by an observer (LA) for the presence or absence of the venous ASL signal. Quantitatively, we scaled the perfusion-weighted signal (control-label) from the ASL images, by the group-based T1 of blood (patients = 1818 ms and controls = 1650 ms), subject-specific estimates of labeling efficiency based on velocity, and M0 in ExploreASL. The superior sagittal sinus and straight sinus were manually segmented in three group-average (children and adults with SCD and healthy control group) images of all participants in standard MNI space (Figure 1). The segmented average
image of each group was resampled to the native ASL space of each individual and used as an ASL mask from which the average VS is measured. In case the segmented venous sinuses were not correctly aligned after resampling, manual correction was applied to only include the VS. In addition, subject-specific GM masks were used in the native space to measure the mean signal in the gray matter (GM). For the comparison between children and adults, the proportion of signal in the venous ROI relative to GM was calculated in the VS images (VS/GM). This ratio is referred to as VGR henceforth. Taking the ratio rather than VS cancels out global physiological perfusion confounders and imaging acquisition differences and allows a comparison between the pediatric and adult group. However, the VS was used to study the associations between VS and other hemodynamic parameters within each group.

CBF, OEF and CMRO$_2$ were calculated as described in the previous studies$^{10,21}$ and are illustrated in Figure 2 and described in the Supplementary Materials, together with the analysis of the velocity in the brain feeding arteries. White matter lesions were delineated on FLAIR images using manual segmentation and quantified as previously described$^6,21$.

**Statistical analyses**

Statistical analyses were performed in SPSS v26 (IBM, NY, USA). P<0.05 was considered statistically significant. Independent t-tests and paired-sample t-tests (or non-parametric alternatives in case of non-normality) were performed to test differences between the groups and to test the statistical differences of VGR before and after ACZ administration. We performed the correlation analysis to assess the relationship
between VS and CBF. In order to test if VS contained additional hemodynamic information independently from CBF, the residuals of the regression between VS and CBF (\(\text{VS}_{\text{CBF}}\)) were used as the dependent variable in stepwise multiple linear regression analyses against age, sex, participant groups, hydroxyurea, hemoglobin (Hb; as a measure of anemia) and LDH (as a measure of hemolysis) for the baseline data. To test the role of oxygen metabolism and ACZ on VS in adult participants, linear mixed-effects modeling was performed, in which OEF, CMRO\(_2\) and ACZ condition were added as additional predictors.

To further explore the relationship between parameters of oxygen metabolism and hemodynamic and laboratory parameters in adult SCD patients, we performed linear mixed-effects modeling with CMRO\(_2\), corrected for \(\text{VS}_{\text{CBF}}\) as the dependent variable. The predictors tested in the model were CBF, VS, Hb, LDH, HbF, and ACZ condition.

**Results**

Demographic, clinical and imaging parameters of 28 pediatric patients with SCD, 38 adult patients and 10 healthy controls are presented in Table 2. Supplementary Table 1 shows the available sample size for each included parameter. Of this sample, 9 (32%) pediatric and 15 (39%) adult patients were using hydroxyurea. In the adult SCD group, 3 (8%) patients received regular blood exchange transfusions and were studied 3-28 days since their last transfusion.

**Venous ASL signal**
Venous ASL signal was observed in 27 (96%) pediatric patients, 36 (95%) adult patients and 1 (10%) healthy control. After ACZ administration, VS was observed in all adult participants (Figure 3A). VGR was significantly higher in patients compared to controls ($Z = -4.24, p < 0.01$), but not between pediatric and adult SCD patients ($t = -1.78, p = 0.08$) (Figure 3B). After ACZ administration, VGR increased in adult patients ($t = -6.10, p < 0.01$) and controls ($Z = 2.80, p < 0.01$) compared to baseline VGR (Figure 3B). No significant VGR differences were found between patients receiving hydroxyurea and those not receiving this intervention.

**Associations of VS with other parameters**

We observed a positive relationship between baseline CBF and baseline VS ($R^2=0.59; F(1,73)=104.4, p<0.001$; Figure 4A). After ACZ administration, these associations remained significant ($R^2=0.57; F(1,45)=59.4, p<0.001$; Figure 4B). No significant association was found between VS and lesion volume in patients with SCD ($\beta=0.002, p=0.89$). Subsequently, we tested associations with baseline $V_{SCBF}$ in all participants. Stepwise multiple regression analysis demonstrated significant associations with Hb ($\beta=0.26, p<0.001$) and participant group ($\beta=0.55, p=0.004$; total model $R^2=0.23$). Subsequent analyses demonstrated a negative correlation between Hb and $V_{SCBF}$ in the combined patient groups ($\beta=-0.45, p<0.001$), but not in the control group ($\beta=0.02, p=0.85$) (Figure 5A). After splitting the patient groups, the association between Hb and $V_{SCBF}$ remained significant in both adults ($\beta=-0.43, p<0.001$) and children ($\beta=-0.45, p=0.02$) with SCD.

For adult participants, we added OEF, CMRO$_2$ and ACZ condition as additional parameters and used a linear mixed model to accommodate the repeated measures
dependencies. The strongest predictor of \( V_{SCBF} \) was \( \text{CMRO}_2 \) \((\beta=-0.79, \ F(1,81)=-24.5, \ p<0.001)\), demonstrating that subjects with lower \( \text{CMRO}_2 \) showed higher \( V_{S} \), independent of CBF (Figure 5B). Subsequently, participant group (adult SCD vs. controls) in combination with either Hb (model 1) or LDH (model 2) were significant additional predictors (model 1: \( \text{CMRO}_2 \) \( \beta=-0.75, \ F(1,82)=19.8, \ p<0.001 \) / group \( \beta=-69.6, \ F(1,72)=11.5, \ p=0.001 \) / Hb \( \beta=-11.6, \ F(1,78)=11.7, \ p=0.001 \); model 2: \( \text{CMRO}_2 \) \( \beta=-0.92, \ F(1,78)=27.8, \ p<0.001 \) / group \( \beta=-40.1, \ F(1,78)=5.4, \ p=0.023 \) / LDH \( \beta=0.11, \ F(1,79)=10.0, \ p=0.002 \)). When splitting the group into SCD adults and controls, we observed that in addition to \( \text{CMRO}_2 \), Hb and LDH were significant independent predictors of \( V_{SCBF} \) in patients (but not in controls) with Hb being a negative predictor \((\beta=-14.2 \ p<0.001)\) and LDH being a positive predictor \((\beta=0.13, \ p=0.002)\). We repeated our statistical analyses excluding the 3 patients receiving exchange transfusion and found comparable results.

To assess further contributing factors to \( \text{CMRO}_2 \) in SCD patients, we explored the relationships between \( \text{CMRO}_2 \) and the various hemodynamic and hematological markers. Linear mixed modeling demonstrated that HbF and LDH were significant predictors of \( \text{CMRO}_2 \) when corrected for \( V_{CBF} \). Higher HbF was associated with lower \( \text{CMRO}_2 \) \((\beta=-1.4, \ F(1,56)=23.4, \ p<0.001)\) and higher LDH was associated with higher \( \text{CMRO}_2 \) \((\beta=0.05, \ F(1,61)=8.5, \ p=0.005)\). As HbF is increased by hydroxyurea, we further split the data into hydroxyurea and no hydroxyurea groups to investigate its effects. The associations between HbF and \( \text{CMRO}_2 \), and LDH and \( \text{CMRO}_2 \) were similar across both groups.

Discussion
The purpose of this study was to investigate cerebral functional shunting in patients with SCD, by means of exploring the relationship between the VS intensity in ASL images and cerebral circulation and oxygen metabolism. Our results confirmed prior observations of higher VS in patients with SCD compared to healthy controls, although no differences between pediatric and adult patients were observed. Our VS data showed a strong association with CBF, confirming that venous outflow is indeed strongly dependent on the inflow to the brain. A key finding of this study was that the CBF-corrected VS was negatively associated with CMRO\(_2\), demonstrating that VS contains important information about microvascular oxygen offloading, providing evidence for functional shunting. Additionally, higher disease severity, as reflected by more severe anemia and hemolysis was, independently of CBF, associated with higher VS. Finally, in addition to potential cerebral shunting mechanisms in SCD patients, we found that CMRO\(_2\) was further negatively correlated with HbF and positively correlated with LDH.

The oxygen-carrying capacity of blood in patients with SCD is reduced and as a result, compensatory increases of CBF are observed\(^7\text{–}^9\). Previous studies using both continuous and categorical estimates of VS\(^{13,28,29}\) observed that higher perfusion in SCD patients results in the presence of venous ASL signal at standard post-label delays, and the results of the current study corroborate these findings. In addition to CBF, microvascular permeability affects the fraction of labels arriving in the venous sinuses. In case of endothelial dysfunction, the permeability-surface area product (PS), defined as the flow of molecules through the capillary membranes in a certain volume of tissue, may be altered. At physiologically plausible CBF levels and in subjects with intact microvasculature, labeled spins enter the tissue and barely any VS is observed (Figure 6A). In case of increased CBF (e.g. after ACZ administration) and intact PS, VS
can be observed in the brain (Figure 6B). However, at elevated CBF with low PS, we expect more labeled spins to pass unexchanged into the cerebral venous circulation (Figure 6C), a process we refer to as microvascular shunting. Importantly, our current findings provide empirical evidence for shunting, by showing that higher VS (independently of CBF) is associated with lower CMRO\(_2\). Our results are concordant with decreased OEF values observed in SCD patients with categorically increased venous signal scores\(^{20}\). Taken together, these data suggest that the venous signal, beyond that expected for a given CBF, is a biomarker of cerebral functional shunting, which might be a result of PS reduction.

The inverse relation between CBF and the parameters OEF and CMRO\(_2\) in chronic anemia might seem paradoxical, but can be explained by physiological mechanisms at the capillary level. Low Hb levels trigger reciprocal increases in CBF to preserve resting oxygen delivery. However, elevated CBF results in decreased capillary transit times, potentially promoting trans-capillary pressure, and increasing heterogeneity in transit times across capillaries in the vascular bed\(^{30,31}\). As oxygen extraction is dependent on the time the blood resides in the capillary network, elevated CBF can result in lower OEF and CMRO\(_2\). However, it is important to consider our findings with respect to the existing body of literature. Studies using TRUST have shown increased, unaffected or decreased OEF values in patients with SCD compared to controls\(^{13,19,20,32,33}\), depending on the calibration model used for the SCD patients. Inconsistent OEF values were also found using other techniques, with a previous PET study showing no differences in OEF and CMRO\(_2\) between patients and controls\(^7\), whereas studies applying susceptibility MRI techniques reported both increased or decreased OEF values in SCD patients\(^{34-36}\).
Our findings cannot be fully attributed to microvascular shunting per se, as we did not assess shunting locally at the capillary level, but rather used VS as a proxy. Nevertheless, although anatomical shunts in this population have been reported\textsuperscript{37,38}, microvascular shunting seems the most likely explanation, given the relation between VS and CMRO\textsubscript{2} in the current study, and a previous report showing a reduction of VS after blood transfusion\textsuperscript{39}. Interestingly, a very recent study using multi-TI ASL showed that despite lower bolus arrival times in the tissue, much longer arrival times were found in the sagittal sinus of patients with SCD compared to controls, suggesting that hyperperfusion in the arterial tree may be accompanied by altered capillary and/or venular micro-circulatory flow patterns, potentially due to microvascular resistance\textsuperscript{29}. Future studies are needed to further elucidate these processes in SCD at the capillary level.

Increased VS beyond that expected for a given CBF (VS\textsubscript{CBF}) might represent a reduction in PS that may be pathological. We demonstrated an inverse relationship of VS\textsubscript{CBF} with Hb in both pediatric and adult patients. In adults, VS\textsubscript{CBF} was also highly correlated with LDH. This may indicate that SCD patients with higher disease severity, i.e. more anemia and hemolysis, have lower functional cerebral microvascular surface area. Further studies are needed to confirm this hypothesis and to investigate if such changes represent permanent damage or a reversible physiologic phenotype.

We demonstrated that besides VS, CMRO\textsubscript{2} was also influenced by HbF and LDH. Whereas HbF reduces HbS polymerization, which diminishes the complications of sickle cell disease, we found a negative association of HbF with CMRO\textsubscript{2}. This negative association is likely a result of the higher oxygen-binding affinity of HbF compared to HbA\textsubscript{40}, potentially resulting in lower oxygen extraction and CMRO\textsubscript{2} for the same CBF.
Notably, the HbF effect was not driven specifically by patients on hydroxyurea. The positive association between LDH and CMRO₂ most likely reflects the impact of dense red blood cells (DRBC) on the Hb-dissociation curve. DRBCs are an important biomarker of SCD severity because of their predisposition for polymerization and hemolysis. Dense cells have markedly low oxygen affinity⁴¹ which would facilitate oxygen extraction. Indeed, another study demonstrated that, on multivariate analysis, LDH was the strongest predictor of DRBC, with bilirubin and HbF levels also retained in the model⁴².

VScBF was comparable between pediatric and adult SCD patients, despite differences in absolute CBF. On the one hand, one might postulate higher VScBF in children because of their increased cerebral metabolic rates and greater risk for ischemic injury⁴³. Alternatively, one might expect progressive microvascular damage and loss of cerebrovascular cross-sectional area with increasing age, similar to microvascular disease in other organs in SCD patients. A preliminary report assessing blood-brain barrier permeability demonstrated that children with SCD have higher PS than controls, despite lower extraction, suggesting increased functional capillary exchange area⁴⁴. However, this remains to be confirmed in future studies that are encouraged to incorporate OEF and CMRO₂ measurements from pediatric patients as well as age-matched healthy controls. This is important because SCIs are also present in pediatric patients with SCD and it is, therefore, important to understand potential age-dependent underlying mechanisms⁴⁴,⁴⁵.

We were unable to demonstrate any link between VScBF and white matter damage, however, the lesion load was relatively low, and no patients with overt stroke were included. Previous studies incorporating these samples also showed no
association between lesion volume and hemodynamic parameters. Although evidence from neurodegenerative disorders and vascular disease suggest that there is a link between hypoxia and white matter lesions, it remains to be determined how lower OEF and CMRO₂, and potentially functional shunting mechanisms are related to such lesions in patients with chronic anemia. A previous study demonstrated a relationship between VS and OEF only in patients with a significant lesion load, but not in patients with overt stroke. However, they also did not correlate VS to lesion volumes.

In this study, T₂-TRIR instead of T₂-relaxation-under-spin-tagging (TRUST) was used to obtain venous oxygenation estimates for OEF calculation. T₂-TRIR enables T₁ measurements in addition to T₂ measurements. Given this advantage and a previous study showing OEF values in the same range as the values obtained from TRUST, we opted for T₂-TRIR here. However, a recent study from our group demonstrated that the OEF values obtained from T₂-TRIR were significantly lower compared to OEF values obtained from the TRUST sequence (despite comparable reproducibility), which may explain our lower OEF and CMRO₂ values compared to previous studies using the TRUST sequence. Additionally, we here used the HbS calibration model introduced by Bush et al. to obtain the venous oxygenation in patients with SCD (although see Supplementary Materials, showing comparable findings using the HbA model for completeness). Recently, a novel sickle cell specific calibration model, the Bush-Li model, was introduced, based on a larger range of hematocrit. However, they did not show statistical superiority compared to the currently used Bush model, and therefore, we do not expect that our conclusions would be different using the Bush-Li model.
An important strength of this study is the fact that we used a continuous estimate of VS, which allowed us to conduct regression analyses to investigate the relation between VS, hemodynamic measures and hematological parameters. However, our sample size is relatively small for multiple regression analyses, which is why we have carefully chosen our included predictors. Future studies should confirm our regression analyses using larger sample sizes, to further explore the presence of functional shunting and the associations between shunting and the parameters of oxygen metabolism. Furthermore, our pediatric sample did not include age-matched healthy controls. However, previous studies have already shown that CBF is higher in children with SCD compared to healthy children\textsuperscript{9,15}. In addition, Wu et al\textsuperscript{28} showed that the signal in the sagittal sinus was lower in healthy children compared to children with SCD. Furthermore, the MRI scanner, the head coil and multiple scan parameters differed between the pediatric and adult patients, which precluded direct comparison of CBF and lesion volume estimates. Individual M0 scans were not acquired in our pediatric cohort, because the scanning was done prior to the M0 consensus recommendations in 2014\textsuperscript{24}. Instead, we used a single fixed M0 value to quantify CBF. In a post-hoc analysis in adults, we found comparable coefficients of variation for GM CBF obtained from a group average M0 and from a subject-specific M0. Moreover, using a group average M0 did not change the associations between VS and the parameter GM CBF and the markers Hb and LDH. Nevertheless, using a single M0 value does not account for slight spatial differences in magnetization, which is why a subject-specific M0 was used in our adult participants and why this is also recommended for future studies. Additionally, the $T_2$-TRIR scan was not available for the pediatric study, and therefore OEF and CMRO$_2$ estimates could not be obtained in pediatric patients.
In the adults, only a small proportion of patients were on chronic exchange transfusion, and therefore its effect could not be estimated in this study. Although hydroxyurea did not appear to affect the relationships found in this study, future studies in larger samples could shed light on the effects of hydroxyurea treatment on functional shunting. Our control group included two sickle cell trait participants whose hematological parameters and T2 values were in the same range as those of the HbAA controls, in line with previous studies\textsuperscript{19,50}. This suggests that they were appropriately included as control subjects.

In summary, our findings suggest that higher CBF-corrected venous signal may reflect the loss of capillary exchange area as shown by its relationship to CMRO\textsubscript{2}, providing evidence for cerebral functional shunting. Moreover, we found that higher disease severity is related to higher VS and that CMRO\textsubscript{2} is additionally influenced by HbF and LDH. These findings indicate that the venous signal on ASL images can be considered as a complementary biomarker of cerebral perfusion and oxygen metabolism.
References


<table>
<thead>
<tr>
<th></th>
<th>pCASL</th>
<th>Phase contrast</th>
<th>T2-TRIR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>children</td>
<td>adults</td>
<td>children</td>
</tr>
<tr>
<td>TR (ms)</td>
<td>4000</td>
<td>4400</td>
<td>15</td>
</tr>
<tr>
<td>TE (ms)</td>
<td>17</td>
<td>14</td>
<td>5</td>
</tr>
<tr>
<td>Flip angle</td>
<td>90</td>
<td>90</td>
<td>15</td>
</tr>
<tr>
<td>2D / 3D</td>
<td>2D</td>
<td>2D</td>
<td>2D</td>
</tr>
<tr>
<td>FOV (mm)</td>
<td>240x240x119</td>
<td>240x240x133</td>
<td>230x230x4</td>
</tr>
<tr>
<td>Voxel size (mm)</td>
<td>3x3x7</td>
<td>3x3x7</td>
<td>0.45x0.45x4</td>
</tr>
<tr>
<td>PLD (ms)</td>
<td>1525</td>
<td>1800</td>
<td>-</td>
</tr>
<tr>
<td>Label duration (ms)</td>
<td>1690</td>
<td>1800</td>
<td>-</td>
</tr>
<tr>
<td>VENC (cm/s)</td>
<td>-</td>
<td>-</td>
<td>140</td>
</tr>
<tr>
<td>Scan duration (mm:ss)</td>
<td>10:12</td>
<td>5:00</td>
<td>1:05</td>
</tr>
</tbody>
</table>
**Table 2:** Demographic, clinical and imaging summary of participants.

<table>
<thead>
<tr>
<th></th>
<th>Pediatric SCD</th>
<th>Adult SCD</th>
<th>Controls</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N=28</td>
<td>N=38</td>
<td>N=10</td>
<td>PSCD vs ASCD vs CTL</td>
</tr>
<tr>
<td><strong>Demographics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>12.7 ± 2.3</td>
<td>32.1 ± 11.2</td>
<td>36.4 ± 15.9</td>
<td>&lt; 0.01 0.74</td>
</tr>
<tr>
<td>Sex</td>
<td>9 F (32%)</td>
<td>14 F (37%)</td>
<td>4 F (40%)</td>
<td>0.69 0.85</td>
</tr>
<tr>
<td><strong>Clinical parameters</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>8.4 ± 1.1</td>
<td>8.8 ± 1.4</td>
<td>13.9 ± 1.2</td>
<td>0.14 &lt; 0.01</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>23 ± 3</td>
<td>26 ± 4</td>
<td>42 ± 3</td>
<td>&lt; 0.01 &lt; 0.01</td>
</tr>
<tr>
<td>Reticulocytes (10⁹/L)</td>
<td>277.7 ± 106.7</td>
<td>260.2 ± 108.2</td>
<td>63.1 ± 24.4</td>
<td>0.60 &lt; 0.01</td>
</tr>
<tr>
<td>LDH (U/L 37°C)</td>
<td>514 ± 110</td>
<td>460 ± 160</td>
<td>181 ± 41</td>
<td>0.12 &lt; 0.01</td>
</tr>
<tr>
<td>Total Bilirubin (mg/dL)</td>
<td>3.5 ± 2.2</td>
<td>3.5 ± 2.8</td>
<td>0.7 ± 0.48</td>
<td>0.85 &lt; 0.01</td>
</tr>
<tr>
<td>HbF (%)</td>
<td>9.8 ± 5.1</td>
<td>9.1 ± 7.6</td>
<td>-</td>
<td>0.20 -</td>
</tr>
<tr>
<td>HbS (%)</td>
<td>85.8 ± 4.6</td>
<td>80.4 ± 15.1</td>
<td>37.0 ± 0.4</td>
<td>0.52 0.02</td>
</tr>
<tr>
<td>Hydroxyurea N [%]</td>
<td>9 [32%]</td>
<td>15 [39%]</td>
<td>-</td>
<td>0.44 -</td>
</tr>
<tr>
<td>Ex transfusions N [%]</td>
<td>-</td>
<td>3 [8%]</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Imaging parameters</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CBF (mL/100g/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pre-ACZ</td>
<td>96.8 ± 14.7</td>
<td>85.8 ± 15.4</td>
<td>52.7 ± 3.15</td>
<td>&lt; 0.01 &lt; 0.01</td>
</tr>
<tr>
<td>post-ACZ</td>
<td>-</td>
<td>113.3 ± 22.0</td>
<td>88.9 ± 9.9</td>
<td>- &lt; 0.01</td>
</tr>
<tr>
<td>VGR</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pre-ACZ</td>
<td>2.4 ± 0.8</td>
<td>2.0 ± 0.9</td>
<td>0.6 ± 0.5</td>
<td>0.08 &lt; 0.01</td>
</tr>
<tr>
<td>post-ACZ</td>
<td>-</td>
<td>2.8 ± 0.7</td>
<td>2.0 ± 0.6</td>
<td>- &lt; 0.01</td>
</tr>
<tr>
<td>Weighted velocity (cm/s)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pre-ACZ</td>
<td>27.5 ± 5.3</td>
<td>24.5 ± 4.7</td>
<td>17.5 ± 2.6</td>
<td>0.03 &lt; 0.01</td>
</tr>
<tr>
<td>post-ACZ</td>
<td>-</td>
<td>30.7 ± 5.0</td>
<td>24.2 ± 4.9</td>
<td>- &lt; 0.01</td>
</tr>
<tr>
<td>OEF (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pre-ACZ</td>
<td>-</td>
<td>27.2 ± 4.4</td>
<td>35.4 ± 3.4</td>
<td>- &lt; 0.01</td>
</tr>
<tr>
<td>post-ACZ</td>
<td>-</td>
<td>19.0 ± 5.8</td>
<td>20.1 ± 7.6</td>
<td>- 0.64</td>
</tr>
<tr>
<td>CMRO₂ (µmol O₂/100g/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pre-ACZ</td>
<td>-</td>
<td>99.8 ± 24.2</td>
<td>128.6 ± 20.3</td>
<td>- &lt; 0.01</td>
</tr>
<tr>
<td>post-ACZ</td>
<td>-</td>
<td>90.7 ± 28.6</td>
<td>127.5 ± 46.0</td>
<td>- 0.01</td>
</tr>
<tr>
<td>Lesion volume (mL)</td>
<td>1.3 ± 4.1</td>
<td>3.9 ± 8.1</td>
<td>0.2 ± 0.3</td>
<td>&lt; 0.01 0.03</td>
</tr>
<tr>
<td>Lesion presence N [%]</td>
<td>17 [61%]</td>
<td>31 [82%]</td>
<td>8 [80%]</td>
<td>0.06 0.91</td>
</tr>
</tbody>
</table>

*Mean and standard deviations of each group are displayed unless otherwise indicated. P-values were calculated using independent-sample t-tests or Mann-Whitney test in case of non-normality, or Pearson’s Chi-Square Test for categorical variables. PSCD = pediatric SCD; ASCD = adult SCD; CTL = control; F= female; VGR = VS to gray matter signal ratio.
Figure legends

Figure 1: An example of the semi-automatic segmentation method of the sagittal and straight sinuses. A) Sagittal group-average image in standard space. B) Manual segmentation of the VS in image A. C) Image in native ASL space of a representative patient. D) Red overlay of the segmented VS on the ASL image.

Figure 2: Visual representation of the image analysis steps. Left: The ASL signal was obtained by subtracting the control images from the labeled images. CBF was then quantified using the dual compartment model and the obtained CBF image was multiplied by the gray matter (GM) mask (created from anatomical images not shown here) to obtain the GM CBF image. CBF images were also used for venous signal assessment. Right: T2-TRIR images were used to obtain the T1 and T2 values of venous blood. An automatic localizer tool was used to detect the region of interest in the posterior part of the brain (in red). This tool searched for high-intensity signals in the last 7 phases of the magnitude reconstructed T2-TRIR data to detect the sagittal sinus. Subsequently, a fit was initiated in the sagittal sinus voxels (shown in green on the mask image). The fitted T1 values were used for CBF quantification in adults and the fitted T2 values were converted to venous oxygen saturation using the HbA and HbS calibration models. Subsequently, the venous oxygen saturation and arterial oxygen saturation (assumed to be 0.98) were used to calculate the OEF. CMRO2 was obtained by multiplying the OEF by the CBF and oxygen-carrying capacity (CaO2). For details, see Supplementary Material. ATT = arterial transit time.

Figure 3: VS across groups. A) Group average axial and sagittal ASL images in MNI space before and after ACZ administration. In healthy controls, VS is minimal at baseline (pre-ACZ) but appears after ACZ administration. B) Dot plots and the mean with the standard deviation of VS to gray matter signal ratio (VGR) between the groups.

Figure 4: Association between VS and CBF. Scatterplots of associations between VS and GM CBF before (left) and after (right) ACZ administration.

Figure 5: Predictors of VS_{CBF}. Left: Scatterplot of Hb and VS_{CBF} (the residual of the regression between VS and CBF) for all pre-ACZ data of all subject groups. The
association between Hb and $V_{SCBF}$ was only significant in the patients, but not in controls. **Right:** Scatterplot of CMRO$_2$ and $V_{SCBF}$ for the pre-ACZ and post-ACZ data in all adult subjects, showing a significant correlation across all subjects. Please note that the values for $V_{SCBF}$ differ for both regressions as the pre-ACZ data were analyzed using multiple linear regression, whereas the pre-ACZ vs post-ACZ data were analyzed using linear mixed models to accommodate the repeated measures variation.

**Figure 6: Schematic hypothetical outline of VS in the absence and presence of shunting.** Arterially labeled spins enter brain parenchyma at a rate determined by the product of vascular surface area and intrinsic water permeability (permeability-surface area product). The re-entry of the label was previously shown to be negligible (Lin et al, 2018) and is therefore not displayed. A) In the brain of a healthy control with normal CBF levels, (almost) no VS signal is observed. B) In the brain of a healthy control after acetazolamide administration with increased CBF and intact PS, VS signal can be observed as flow predominates the microvascular uptake. C) In a patient with sickle cell disease with the presence of shunting, blood preferentially passes through short, low resistance capillary pathways leading to functional loss of capillary surface area, further increasing VS and impairing oxygen unloading. HC = healthy control, ACZ = acetazolamide, SCD = sickle cell disease, CBF = cerebral blood flow; PS = permeability-surface area product; VS = venous signal intensity
A: HC

- Normal CBF
- Normal PS
- No VS

B: HC after ACZ

- Elevated CBF
- Capillary
- VS
- Normal PS

C: SCD

- Elevated CBF
- Capillary
- VS
- Lower PS due to shunting
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 T₂-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 mm², voxel size = 3 x 3 mm², 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 study¹.

*T₂*-prepared tissue relaxation with inversion recovery MRI

For the venous oxygenation measurements in adults, *T₂*-prepared tissue Relaxation with an Inversion Recovery MRI (*T₂*-TRIR) sequence² 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 mm², voxel size = 2 x 2 mm², 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 *T₁* 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 mm², voxel size = 0.45 x 0.45 mm², 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 model³ with subject-specific arterial transit time (ATT), labeling efficiency and blood *T₁* measured in the sagittal sinus using *T₂*-TRIR. In pediatric patients, an average blood *T₁* of 1818 ms was used as previously measured in these patients⁴, 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\textsubscript{2} measurements

For the OEF measurements, T\textsubscript{2} values were computed from T\textsubscript{2}-TRIR and converted to venous oxygen saturation (SvO\textsubscript{2}) using an SCD-specific model\textsuperscript{5} (HbS model) for the patients and a healthy subject model for controls\textsuperscript{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\textsubscript{2}) was assumed to be 98%. OEF was calculated using the following equation:

\[
\text{OEF} (\%) = \frac{(\text{SaO}_2 - \text{SvO}_2)}{\text{SaO}_2} \cdot 100\%.
\] [1]

The oxygen carrying capacity (CaO\textsubscript{2}) was calculated using the following equation:

\[
\text{CaO}_2 (\text{μmol O}_2 / 100 \text{ mL blood}) = \frac{[(\text{Hb} \cdot 1.34 \cdot \text{SaO}_2) + (0.0031 \cdot \text{pO}_2)]}{22.4 \cdot 1000}
\]

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\textsubscript{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\textsubscript{2} was calculated according to Fick’s principle using the previously quantified parameters:

\[
\text{CMRO}_2 (\text{μmol O}_2/100\text{g/min}) = \text{CBF} \cdot \text{OEF} \cdot \text{CaO}_2
\] [2]

Calibration model

Multiple calibration models for the calculation of venous oxygenation from T\textsubscript{2} values in SCD and controls have been proposed over the years\textsuperscript{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$_2$ ($\beta=-0.58$ F(1,82)=12.1, p<0.001) (Fig. 1), Hb ($\beta=-10.4$ F(1,66)=6.6, p = 0.012), and participant group ($\beta=-56.7$, 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 ($\beta=-17.2$ p<0.001) and LDH having a positive association ($\beta=0.15$, 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 ($\beta=-1.44$, F(1,62)=17.6, p<0.001), and higher LDH was associated with higher CMRO$_2$-HbA ($\beta=0.08$, 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

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Pediatric SCD (N)</th>
<th>Adult SCD (N)</th>
<th>Controls (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>28</td>
<td>38</td>
<td>10</td>
</tr>
<tr>
<td>Sex</td>
<td>28</td>
<td>38</td>
<td>10</td>
</tr>
<tr>
<td><strong>Clinical parameters</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>28</td>
<td>37</td>
<td>10</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>28</td>
<td>38</td>
<td>10</td>
</tr>
<tr>
<td>Reticulocytes (10e9/L)</td>
<td>28</td>
<td>37</td>
<td>8</td>
</tr>
<tr>
<td>LDH (U/L 37 °C)</td>
<td>28</td>
<td>36</td>
<td>7</td>
</tr>
<tr>
<td>Total Bilirubin (mg/dL)</td>
<td>27</td>
<td>37</td>
<td>7</td>
</tr>
<tr>
<td>HbF (%)</td>
<td>25</td>
<td>37</td>
<td>10</td>
</tr>
<tr>
<td>HbS (%)</td>
<td>25</td>
<td>38</td>
<td>2</td>
</tr>
<tr>
<td>Hydroxyurea (N[%])</td>
<td>9</td>
<td>15</td>
<td>-</td>
</tr>
<tr>
<td>Exchange transfusion (N[%])</td>
<td>-</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td><strong>Imaging parameters</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GM CBF (mL/100g/min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-ACZ</td>
<td>28</td>
<td>37</td>
<td>10</td>
</tr>
<tr>
<td>Post-ACZ</td>
<td>-</td>
<td>38</td>
<td>10</td>
</tr>
<tr>
<td>VGR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-ACZ</td>
<td>28</td>
<td>37</td>
<td>10</td>
</tr>
<tr>
<td>Post-ACZ</td>
<td>-</td>
<td>38</td>
<td>10</td>
</tr>
<tr>
<td>Weighted velocity (cm/s)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-ACZ</td>
<td>28</td>
<td>36</td>
<td>10</td>
</tr>
<tr>
<td>Post-ACZ</td>
<td>34</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>OEF (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-ACZ</td>
<td>-</td>
<td>36</td>
<td>8</td>
</tr>
<tr>
<td>Post-ACZ</td>
<td>-</td>
<td>36</td>
<td>7</td>
</tr>
<tr>
<td>CMRO$_2$ (µmol O$_2$/100g/min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-ACZ</td>
<td>-</td>
<td>35</td>
<td>8</td>
</tr>
<tr>
<td>Post-ACZ</td>
<td>-</td>
<td>35</td>
<td>6</td>
</tr>
<tr>
<td>Lesion volume (mL)</td>
<td>28</td>
<td>38</td>
<td>10</td>
</tr>
</tbody>
</table>
References


