Cerebral Blood Flow Measurement in Children With Sickle Cell Disease Using Continuous Arterial Spin Labeling at 3.0-Tesla MRI

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Background and Purpose—Cerebral infarction is an important complication of sickle cell disease (SCD) and occurs in one third of the patients with SCD. The risk of infarction is commonly attributed to the hyperemia that is associated with anemia and reduces the cerebral vascular reserve. We measured regional cerebral blood flow (rCBF) by continuous arterial spin labeling MRI, which is a noninvasive method that does not require ionizing radiation. The purpose of this study was to examine rCBF in children with SCD and compare it with rCBF in healthy children.

Methods—rCBF was measured at 3-T continuous arterial spin labeling MRI in 24 neurological normal patients with SCD and in 12 healthy children matched for ethnicity and age (mean age in both groups 13 years). rCBF was calculated for 6 vascular territories (left and right anterior, middle and posterior cerebral artery). Asymmetry in rCBF was evaluated by measuring differences in flow between left and right hemispheres. The definition of asymmetry (>11.7 mL/100 g/min) was based on a repeatability study performed in 6 healthy adults.

Results—The rCBF was of similar magnitude in patients with SCD and control subjects in the frontal, middle, and posterior territories. The majority of patients with SCD (58%) demonstrated a left–right asymmetry of rCBF in one or more vascular territories, whereas none of the control subjects did.

Conclusion—In contrast to previous studies, we found no difference in cerebral blood flow between patients and control subjects. We did observe an asymmetry in rCBF in the majority of patients with SCD that was not present in healthy control subjects. (Stroke. 2009;40:795-800.)

Key Words: sickle cell anemia ■ cerebral infarction ■ regional blood flow

Sickle cell disease (SCD) is a hereditary anemia that is characterized by chronic hemolytic anemia and vascular occlusion, causing irreversible organ damage. Cerebral infarction is the most devastating complication of SCD. At the age of 18 years, cerebral infarcts are present on MRI scans in one third of patients with SCD,1–5 yet most of these infarcts are not accompanied by focal neurological deficits. These so-called silent infarcts appear to be associated with diminished neurocognitive functioning and an increased risk of new infarcts.5,7

Despite SCD being one of the most common causes of pediatric stroke, the pathophysiology of cerebral infarction in these patients is poorly understood. In patients with SCD, the blood flow to the brain may be reduced by stenosis of the large supplying arteries or by increased viscosity of the blood. Furthermore, the hemodynamics of the cerebral vasculature are compromised by chronic anemia and may be further challenged during acute medical events.8

In patients with anemia, adequate oxygenation of the brain tissue is presumably preserved by vasodilatation of the cerebral vasculature. When reductions in arterial pressure arise or metabolic demands increase, there is limited reserve for further vasodilatation to assure adequate oxygen supply to the brain. The ensuing ischemia predisposes to cerebral infarctions.

Silent infarcts in SCD are usually confined to the deep white matter. This pattern of infarction is presumably preserved by vasodilatation of the cerebral vasculature. When reductions in arterial pressure rise or metabolic demands increase, there is limited reserve for further vasodilatation to assure adequate oxygen supply to the brain. The ensuing ischemia predisposes to cerebral infarctions.

Silent infarcts in SCD are usually confined to the deep white matter. This pattern of infarction supports a hemodynamic mechanism rather than a thromboembolic pathophysiology, because the penetrating arterioles reaching into the deep regions have few anastomoses, thus limiting the hemodynamic reserve capacity in situations of increased demand.9

This hypothesis about the mechanism of deep (and often
silent) infarcts in SCD is supported by experimental studies measuring increased regional cerebral blood flow (rCBF) in patients with SCD. Cerebral blood flow was measured in patients with SCD using techniques such as \((^{15}\text{O}) \text{H}_2\text{O}\) positron emission tomography,\(^{10,11}\) dynamic susceptibility contrast MRI,\(^{12,13}\) xenon-133 inhalation MRI,\(^{14-17}\) or CT flow mapping.\(^{15}\) Disadvantages of these techniques are radiation exposure and/or injection or inhalation of exogenous contrast agents, making them less suitable for diagnostic testing in children. The more recently developed continuous arterial spin labeling (CASL) MRI allows noninvasive quantification of rCBF by using magnetically labeled arterial blood. Proximal to entry in the brain, protons in the arterial blood are labeled using a radiofrequency pulse and quantified in terms of tissue perfusion on distal images in the brain.\(^{18}\)

So far, data on rCBF obtained by CASL-MRI in children with SCD are sparse. Only one study compared rCBF in patients with SCD with control subjects. Using 1.5-T MRI, an increase of rCBF was measured in 14 patients in major cerebral arterial territories that appeared unaffected on conventional MRI.\(^{19}\) Presently, higher-resolution MRI (3-T) is available. We performed a study to evaluate whether CASL MRI could detect differences in rCBF when these regions appear unaffected on conventional 3-T MRI. The purpose of this study was to examine rCBF in children with SCD and compare it with rCBF in healthy children.

Methods

Study Population

Patients with SCD (HbSS or HbS/β-thalassemia) aged between 8 and 19 years were recruited for the study at the Emma Children’s Hospital, Amsterdam, The Netherlands. Patients with normal flow on transcranial Doppler ultrasonography and no history of neurological events were eligible for participation. Patients with abnormal transcranial Doppler ultrasonography (<40 cm/s or >200 cm/s) or a history of neurological events were excluded because they are treated in our study center with regular blood transfusions, which may influence cerebral blood flow (CBF). Healthy family members (HbAA) of patients and healthy children, matched for ethnicity and age, were recruited as control subjects. MRI examination in patients was performed in a stable clinical situation without fever or vaso-occlusive crisis.

Study Protocol

The study protocol was approved by the Institutional Review Board of the study center and informed consent was obtained from all parents and from children aged 12 years or older. All patients and control subjects underwent an extensive standardized neurological examination performed by a pediatric neurologist (L.W.-S.) who was blinded for clinical data and MRI results. The neurological examination and the blood test for hematocrit were performed within 3 months of the MRI/MR angiographic examination of the brain. All children underwent MRI and MR angiography (MRA) at 3-T without sedation.

Magnetic Resonance Imaging Protocol

All MR examinations were performed on a 3-T system (Philips Intera; Philips Medical Systems, Best, The Netherlands) between August 2006 and June 2007 in unsedated children using a 6-channel phased array head coil. All patients underwent the same MRI protocol, including axial T2-weighted fast spin echo, axial fluid-attenuated inversion recovery, and multiple overlapping thin slab acquisition (MOTSA) 3-dimensional time of flight (TOF) MRA sequences.

Imaging parameters for the fluid-attenuated inversion recovery sequence were 11 000/2600/100 TR/TE/TI, 224×224 matrix (reconstructed to 512×512), 230-mm field of view, 90% rectangular field of view, and 3-mm thick sections with a 1-mm gap. Parameters for the T2-weighted fast-spin echo sequence were 3000/80 (TR/TE), 400×400 matrix (reconstructed to 512×512), 230-mm field of view, 90% rectangular field of view, and 3-mm thick sections with a 1-mm gap. The volume of the MOTSA 3-dimensional TOF MRA was localized on a sagittal 2-dimensional phase contrast scout image. A presaturation band was applied above the imaging volume to saturate incoming venous blood. For the MOTSA 3-dimensional TOF MR sequence, the parameters were as follows: 3-dimensional fast field echo T1-weighted sequence, 21/4.1 (TR/TE), flip angle 20°, 512×512 matrix (reconstructed to 1024×1024), 200-mm field of view, 85% rectangular field of view, 1.0-mm thick sections, interpolated to 0.5 mm, and 160 slices acquired in 8 chunks. The measured voxel size of the MOTSA 3-dimensional TOF MR sequence was 0.39×0.61×1 mm and the reconstructed voxel size 0.2×0.2×0.5 mm. Imaging time of the high-resolution MOTSA 3-dimensional TOF sequence was reduced by parallel imaging.

CASL imaging was performed by using the amplitude modulated CASL approach originally described by Alsop and Detre using a postlabeling delay of 1.2 seconds. This method is implemented at 3-T using a transmit–receive head coil without compromising clinical specific absorption rate levels. The position of the labeling plane was planned using a MRA scan perpendicular to the posterior ascending portion of the internal carotid artery. Single-shot spin-echo EPI images (TR/TE=4500/32 ms) were acquired of 11 slices of 7 mm with 1-mm slice gap (imaging matrix of 64×64, field of view 210×210 mm). Acquisition of 50 pairs of labeled and control volumes took approximately 8 minutes. CASL sequence parameters were chosen identical to Oguz et al.\(^{19}\) For calculating absolute CBF values, the model as presented by Alsop and Detre was used. The following model parameters were used: T1 of tissue: 1.33 seconds, T1 in the presence of off-resonance radiation 0.994 seconds, transit time: 1.2 seconds, labeling efficiency: 0.68, tissue to blood partition coefficient: 0.98. Subtraction and 2-dimensional motion correction was performed offline using the FMRIB software library.\(^{21}\)

For studying interscan reproducibility, we obtained CASL data from 6 healthy adult volunteers who were scanned on 3 different occasions within a period of 3 weeks. Reproducibility was expressed in terms of the coefficient of repeatability, defined as 1.96 SD of the difference between repeated measurements. Whole brain repeatability is 11.7 mL/100 g/min,\(^{22}\) which is comparable to previously published data.\(^{20,23,24}\) Mean whole brain CBF in the group of volunteers was 47.1±8.1 mL/100 g/min.

Magnetic Resonance Image Analysis

Conventional MRI (T2-weighted and fluid-attenuated inversion recovery) and MRA (MOTSA 3-dimensional TOF) images were assessed by a standardized evaluation protocol by 2 independent observers (M.A.A.v.W. and C.B.I.M.M.) who were blinded to the clinical data. Cerebral infarcts, leukoaraiosis, and vasculopathy were scored. An infarct was defined as an area of hyperintensity on T2-weighted pulse sequences of the MRI and classified by size and anatomic location (cerebrum, cerebellum, thalamus, or basal ganglia). Vasculopathy was classified according to the severity of intracranial vascular stenoses or vascular occlusion. In case of disagreement between the 2 observers, consensus was reached by discussion. For calculation of the CBF, the vascular territories in the cortical gray matter of the anterior cerebral artery, the middle cerebral artery, and the posterior cerebral artery were manually drawn as defined by Tatu et al.\(^{25}\) using dedicated delineation software (Volumetool; UMC Utrecht, Utrecht, The Netherlands).

Statistical Analysis

The Statistical Package for Social Sciences (SPSS), Windows version 12.0, was used for the analysis. Mean differences and 95% CIs were calculated between patients and control subjects. Differences between the groups were considered significant if the proba-
Results

We enrolled 24 patients with SCD and 12 control subjects. The mean age was 13.4 years (SD, 3.0) for patients and 13.4 years (SD, 3.5) for the control subjects. In both groups, sexes were represented equally. Mean hematocrit was lower in patients (0.25 l/L; SD, 0.03) compared with control subjects (0.37 l/L; SD, 0.03). Three patients had subtle pyramidal deficits by neurological examination and one control subject demonstrated mild coordination abnormalities by neurological examination.

Magnetic Resonance Imaging and Magnetic Resonance Angiography

In 16 patients (67%), abnormalities were seen on T2-weighted MRI, MRA, or both. Seven patients (29%) had infarcts in the deep white matter and a normal MRA; 4 patients (17%) had stenosis (<25% in 3; 25% to 50% in one) of one or more cerebral arteries and a normal MRI and 5 (21%) had both infarcts and stenosis (<25% in 3; 25% to 50% in 2). In 3 of 9 patients with cerebral arterial stenosis, the infarcts were located in the territory supplied by the stenotic artery.

Of the 3 patients with subtle pyramidal tract deficits, all located on the left side, one had 2 small frontal infarcts and leukoaraiosis in both parietal lobes. The second patient had mild stenosis (<25%) of both middle cerebral arteries and the left anterior cerebral artery and the third patient had no abnormalities on MRI and MRA. No infarcts were detected in the control group. Two children in the control group, including the one with mild coordination abnormalities, had a mild stenosis (<25%) of one or more cerebral arteries.

Regional Cerebral Blood Flow by Continuous Arterial Spin Labeling Magnetic Resonance Imaging

The rCBF of the patients and control subjects, as calculated by CASL-MRI, is given in Table 1 for the 6 major arterial territories corresponding to left and right anterior, middle, and posterior cerebral artery. There was no significant difference in CBF between patients and control subjects.

In addition, we evaluated left–right asymmetry in rCBF for the anterior, middle, and posterior vascular territories by measuring differences in flow between both hemispheres. Asymmetry was defined as a difference in CBF >11.7 mL/100 g/min. Proportions and 95% CI of patients and controls with rCBF asymmetry in each territory were calculated (using the computer program Confidence Interval Analysis Version 2.0.0 according to the method described by Altman et al.26) Fisher’s exact test was performed to test whether the differences in these proportions were statistically significant.

In 6 patients, asymmetry was found in more than one arterial territory; in 2 of them asymmetry was present in 3 vascular territories. In these 6 patients with asymmetry in 2 or 3 territories, the decreased CBF was consistently on the same side. There was no association between ipsilateral CBF and flow measured by transcranial Doppler ultrasonography.

Asymmetry in rCBF was not associated with stenoses in the corresponding supplying arteries because 6 of 9 patients with stenosis did not have asymmetry in the corresponding vascular territory. There was no association between asymmetrical rCBF and infarcts on MRI either. Twelve of the 14 patients with asymmetrical rCBF did not have deep infarcts in the corresponding territory.

Because CBF did not differ between patients and control subjects, both groups were taken together to evaluate the association of CBF with age. There was a weak correlation between age and CBF (r = −0.378, P < 0.05; Figure 2). The correlation between CBF and hematocrit in patients was r = −0.594 (P = 0.057) and for healthy control subjects r = −0.178 (P = 0.581).

Table 1. rCBF (mL/100 g/min) Values of Patients and Healthy Control Subjects

<table>
<thead>
<tr>
<th>Territories</th>
<th>Patients (n=24)</th>
<th>Healthy Control Subjects (n=12)</th>
<th>Mean Difference</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anterior cerebral artery</td>
<td>73.2 ± 17.4</td>
<td>71.5 ± 14.4</td>
<td>−1.7</td>
<td>−13.6 to 10.1</td>
</tr>
<tr>
<td>Middle cerebral artery</td>
<td>77.1 ± 19.9</td>
<td>76.1 ± 16.4</td>
<td>−1.0</td>
<td>−14.5 to 12.5</td>
</tr>
<tr>
<td>Posterior cerebral artery</td>
<td>89.6 ± 16.4</td>
<td>84.5 ± 16.6</td>
<td>−5.1</td>
<td>−17.0 to 6.7</td>
</tr>
<tr>
<td>Right hemisphere</td>
<td>77.6 ± 19.2</td>
<td>76.3 ± 15.3</td>
<td>−1.3</td>
<td>−14.3 to 11.5</td>
</tr>
<tr>
<td>Left hemisphere</td>
<td>77.5 ± 17.7</td>
<td>76.6 ± 16.1</td>
<td>−0.9</td>
<td>−13.3 to 11.4</td>
</tr>
<tr>
<td>Total</td>
<td>77.6 ± 17.4</td>
<td>76.4 ± 15.6</td>
<td>−1.2</td>
<td>−13.4 to 11.0</td>
</tr>
</tbody>
</table>

Table 2. Asymmetry in rCBF (ie, rCBF Difference >11.7 mL/100 g/min) for the ACA, MCA, and PCA Territories Between the 2 Hemispheres

<table>
<thead>
<tr>
<th>Territories</th>
<th>Patients (n=24)</th>
<th>Control Subjects (n=12)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACA</td>
<td>4</td>
<td>7</td>
<td>0–24%</td>
</tr>
<tr>
<td>MCA</td>
<td>11</td>
<td>15–65%</td>
<td>0–24%</td>
</tr>
<tr>
<td>PCA</td>
<td>7</td>
<td>15–49%</td>
<td>0–24%</td>
</tr>
</tbody>
</table>

ACA indicates anterior cerebral artery; MCA, middle cerebral artery; PCA, posterior cerebral artery.
Discussion

The main finding of this study was that we could not confirm an increased rCBF in pediatric patients with SCD compared with healthy control subjects reported in an earlier study.\textsuperscript{19}

The CBF measured in the present study is relatively low in comparison to previous studies that measured cerebral blood flow in patients with SCD using different techniques, reporting CBF varying from 65 to 153 mL/100 g/min.\textsuperscript{11,14,15,19,27} Variation in measured CBF may be caused by differences in perfusion imaging techniques. Because estimation of CBF is not standardized to an absolute measure, values obtained by different techniques cannot be compared. Three studies compared CBF in patients with SCD with control subjects.\textsuperscript{11,15,19} Oguz measured CBF by CASL-MRI at 1.5-T in 14 asymptomatic pediatric patients with SCD and 7 control subjects and found an increased CBF in patients compared with control subjects (CBF of 153±43 mL/100 g/min in patients and 98±10 mL/100 g/min in control subjects).\textsuperscript{19} The other 2 studies included adult patients. An increased CBF was measured in 27 asymptomatic patients (123±27 mL/100 g/min) in comparison to 31 healthy control subjects (73±12 mL/100 g/min) using inhalation of a mixture of xenon gas.\textsuperscript{15} Using positron emission tomography–CT, Herold quantified a CBF of 65±12 mL/100 g/min in 6 asymptomatic patients with SCD, which was higher than CBF of the control group of 14 subjects (44±5 mL/100 g/min).\textsuperscript{11}

The different results of our study may be explained by characteristics of the patient group, eg, disease severity or age, matching of the control group, and parameters used for the calculation model.

In our study, 50% of the patients has silent infarcts, whereas in the other pediatric study performed by Oguz et al, only one of 12 patients had hyperintensities on conventional MRI. The advanced stage of disease in our patient group could provide disturbances in cerebral autoregulation that are not big enough to cause necrotic tissue but might reduce CBF.

![Figure 1. rCBF maps for control subject and patient with SCD. A, CBF maps of a control subject with no asymmetry; (B) the CBF maps of a patient with asymmetry between the 2 hemispheres in anterior cerebral artery and middle cerebral artery territories.](image)

![Figure 2. Correlation between total CBF and age.](image)
However, in the subpopulation of 8 patients without MRI and MRA abnormalities, the mean CBF was 76.2 mL/100 g/min, which is almost the same as the CBF in the complete patient group. Therefore, the fact that our patient group had a more advanced disease stage in comparison to the patients in the study of Oguz et al cannot explain the lower rCBF values we found in comparison to the study of Oguz et al.

On the other hand, the higher proportion of patients with silent infarcts in our study could contribute to a lower CBF in our patient group, because perfusion deficits have been detected at the site of silent infarcts. However, this effect will not be very prominent, because the infarcts detected in this study were smaller than 5 mm in most patients.

In the Oguz et al study, patients were 2 years younger than control subjects (8.7 years versus 11.0 years, respectively). This may partly explain the higher CBF that was found in these patients, because age is negatively correlated with CBF as we confirmed in our study. Differences that have been reported between patients and control subjects in the adult studies may be attributed to the further progressed vascular pathology in adult patients.

Differences in CBF between patients and control subjects may also be influenced by parameters used for the calculation model, e.g., hematocrit and labeling efficiency. Lower hematocrit levels result in higher T1 values for arterial blood, thereby increasing the labeling efficiency at measurement time. When CBF values are corrected for hematocrit, CBF in patients with SCD decreases. This is illustrated by the study of Strouse et al, who evaluated 24 children with SCD, including patients from the previous study by Oguz et al. After correction of CBF for hematocrit, a lower CBF was found in comparison to values earlier reported by Oguz et al (110±40 mL/100 g/min and 152.8±42.5 mL/100 g/min, respectively).

We did find asymmetry in rCBF between the left and right hemisphere in the majority of patients (58%), whereas asymmetry was not present in healthy control subjects. Asymmetry in rCBF was not associated with the presence of infarcts, stenoses, or asymmetries in blood flow measured by transcranial Doppler ultrasonography. This asymmetry in rCBF is an intriguing observation. In principle, the pathophysiological model of infarcts in SCD is symmetrical. However, infarcts do not occur in a symmetrical pattern. Lack of symmetry in rCBF may be an early indication of subclinical pathological changes in the microvasculature or hemodynamics. A longitudinal study would be required to investigate this and to establish a relation with subsequent ipsilateral infarctions.

The resolution in our study is limited by the large size of the territories in which rCBF is measured. Decreased rCBF in smaller territories (voxels), which might be an early indicator of cerebral ischemia, may be missed due to averaging rCBF over a larger volume. Voxel-by-voxel-based analysis could overcome this problem but is hampered by the sensitivity of the arterial spin-labeling technique and the complexity of accurate alignment of low-resolution CBF maps to pediatric standard brains for different age groups.

Because this is a cross-sectional study, we could not examine whether changes in rCBF predict the development of infarcts. This will be addressed in a longitudinal study in the future.

Summary
In our series, we found no difference in rCBF between patients with SCD and control subjects. We did find left–right asymmetry in rCBF in the majority of patients. The latter may be a risk factor for development of cerebral infarcts and should be studied further in longitudinal studies.

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Disclosures
None.

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