Arterial spin labeling perfusion MRI: reproducibility & clinical applications
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Arterial Spin Labeling measurements of cerebral perfusion in children with Sickle Cell Disease

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Abstract

Purpose
The current study evaluates the applicability of arterial spin labeling (ASL) cerebral blood flow (CBF) measurements in children with sickle cell disease (SCD).

Materials and methods
We included 12 patients and 5 controls. Conventional MR imaging (T2, FLAIR and MR angiography) was performed to diagnose silent infarcts, vasculopathy or leukoencephalopathy. Pseudo-continuous ASL was performed to measure CBF, using two postlabeling delays to identify transit-time effects. Perfusion estimates were corrected for hematocrit and blood velocity in the labeling plane and compared to phase-contrast-MR. CBF asymmetries between the flow maps of the left and right internal carotid arteries were tested for significance using paired t-tests. Significant asymmetries were expressed in terms of an asymmetry ratio (AR=absolute difference/mean). An AR>10% was considered clinically relevant.

Results
Mean CBF was higher in patients than in controls. Agreement between CBF and flow improved after applying hematocrit and velocity corrections. At a 2100ms postlabeling delay one patient had a clinically relevant asymmetry. No association was observed between CBF asymmetries and silent infarcts.

Conclusion
Care must be taken in the interpretation of ASL-CBF measurements in SCD patients. A long postlabeling delay with blood velocity correction anticipates overestimation of CBF asymmetries.
Sickle cell disease (SCD) is a hereditary hemoglobinopathy, inducing a sickle shape of red blood cells in a deoxygenated state. The disease is characterized by chronic haemolytic anaemia and vascular occlusion. SCD is the most common cause of stroke in children. Overt infarcts, accompanied by focal neurological deficits, occur in approximately 11% of children with SCD before their 14th birthday (1). Patients at high risk for overt infarcts can be identified by an elevated blood velocity in the internal carotid and cerebral arteries, measured by Transcranial Doppler Ultrasonography (TCD) (2). High risk patients are currently treated with regular blood transfusions to prevent recurrent stroke.

Even more common are silent cerebral infarcts: areas of increased signal intensity on conventional MR imaging without focal neurological deficits. Silent infarcts are present in 22% of the children by the age of 14 years (3). Most silent lesions are located in the deep white matter (4). Since silent infarcts appear to be associated with neurocognitive decline and an increased risk of new infarcts, prevention of silent infarcts is important (5-8). Wang et al. demonstrated a lack of concordance between TCD findings and silent infarcts on conventional MRI (2). Currently, there is no screening method available to identify patients at risk of silent infarcts. Potentially, direct quantification of cerebral blood flow (CBF) could play a role to identify children at increased risk of developing silent infarcts.

Arterial spin labeling (ASL) is a non-invasive perfusion imaging technique, which uses arterial blood water protons as an endogenous tracer of perfusion (9). Different authors have used continuous and pulsed ASL sequences in patients with SCD and have reported conflicting results on increased CBF in SCD patients compared to healthy controls. All studies however, identified CBF asymmetries that could be an early indicator of subclinical pathological changes in microvasculature or hemodynamics. The described perfusion asymmetries however, could not always be associated with the presence of ischaemic lesions (10-13).

It is not clear whether the above mentioned CBF increase and CBF asymmetries, reflect pathological changes in cerebral perfusion or if they are related to technical issues encountered in ASL. The transit time of blood will influence the labeling efficiency and the time between initial labeling and imaging (postlabeling delay) will influence the observed perfusion signal. Chronic anaemia and compensatory increases in blood velocity and flow, reduce the arterial transit time, decreasing the labeling efficiency. Furthermore, as the efficiency of magnetic labeling of the arterial blood depends on the blood velocity at the level of the labeling plane, this should be carefully considered when comparing left-right symmetries. An assumed equal labeling efficiency in both internal carotid arteries (ICAs) may give rise to spurious CBF asymmetries, when these are in fact caused by differences in blood velocity at the labeling level. Finally, the longitudinal relaxation time of arterial blood is depending on individual hematocrit (Hct) level (14). Anaemia will lead to a longer longitudinal relaxation time of arterial blood, increasing the perfusion signal measured by ASL. It is important to consider the contribution of each of the above factors and their effect on the calculation of CBF in order to make robust estimates of this parameter.
The purpose of the current study was to evaluate the applicability of ASL for CBF measurements in children with SCD and address the technical issues that may affect the accuracy of ASL CBF measurements. Thereto a pseudo-continuous ASL (P-CASL) sequence was performed with two different delay times, to identify possible transit time effects on quantification. Perfusion estimates were corrected for labeling efficiency and for individual Hct level. We hypothesized that state-of-the-art ASL acquisition in combination with the appropriate correction methods, would provide in a better approximation of perfusion abnormalities in children with SCD.

Materials & Methods

Study Population
Patients with SCD (HbSS or HbS-β-thalassemia since both genotypes result in absence of normal haemoglobin), aged between 8 and 22 years, were recruited for this study at the Emma Children’s Hospital, Amsterdam, The Netherlands. Patients with normal velocity on TCD screening and no history of neurological events were eligible for participation. Patients with abnormal TCD (>200 cm/s) or a history of neurological events were excluded because they are treated with regular blood transfusions in our hospital (given monthly during one or more years, which may influence CBF. Single blood transfusions were only an exclusion criterion if donor blood was received shorter than three months prior to inclusion. Family members and children without known brain disease, matched for ethnicity and age, were recruited as controls. For ethical reasons (blood withdrawal in healthy control children) the presence of the carrier status for sickle cell disease was not tested in controls for this study. However, in all controls the genotype was known because of postnatal screening for sickle cell disease. MR imaging of the patients was performed in a stable clinical situation without fever or vaso-occlusive crisis in the previous three months.

Study protocol
The study protocol was approved by the local ethics committee. Informed consent was obtained from all parents as well as from children aged older than 12 years. Patients underwent neurological examination, performed by an experienced pediatric neurologist (M.E.), blinded for clinical data and MRI results. A blood sample for measurement of Hct was taken as part of standard clinical care. Both neurological examination and measurement of Hct took place within 1 month of the MR examination. All children underwent MR imaging without sedation. MR imaging was performed on a Philips 3T MR system using a SENSE-8-channel head coil and body coil transmission.

Imaging protocol
The imaging protocol consisted of: conventional MR imaging including axial T2 and FLAIR weighted imaging, high-resolution MOTSA 3D-time of flight MR angiography (MOTSA 3D-TOF MRA), two pseudo-continuous ASL (p-CASL) acquisitions with different delay times for estimation of CBF, selective ASL to visualize the actual flow territory of the right and left ICA and the basilar artery (BA) and time resolved 2-dimensional phase contrast MRI (PC-MRI) for flow measurements in the ICAs and BA. Total scan duration was 45 minutes.

For the MOTSA 3D-TOF sequence, imaging parameters were as follows: 3D fast-field echo T1-weighted sequence; TR/TE, 25/4 ms; flip angle, 17°; FOV 200×200 mm²;
ASL measurement of cerebral perfusion in children with SCD

512 x 324 matrix (reconstructed to 512 x 512); 1.0 mm thick sections, interpolated to 0.5 mm; and 184 sections acquired in 8 slabs; SENSE 2.5.

P-CASL with additional background suppression pulses was employed with a 1525 or 2100 ms postlabeling delay (15-17). Imaging parameters were: TR/TE 4000/14 ms for a 1525 ms delay and TR/TE 4500/14 ms for a 2100 ms delay; flip angle 90°; FOV 240×240 mm²; matrix size 80×79; 17 slices; thickness 7 mm; no gap; gradient echo single shot echo planar imaging; SENSE 2.5; labeling duration 1650 ms; number of 40 repeated measurements. For selective ASL, we used vessel-encoded pseudo-continuous ASL as developed by Wong (18). Selective labeling was achieved by spatial manipulation of the labeling efficiency in sets of five dynamics (75 dynamics were obtained). Further imaging parameters for selective ASL were the same as for nonselective p-CASL. Selective ASL was used to define the flow territories of the brain feeding arteries. Those flow territories were used to mask the perfusion weighted images obtained by non-selective ASL and calculate CBF in the individual flow territories of the internal carotid and basilar arteries.

Non-selective and selective labeling was applied in the same labeling plane placed perpendicularly to the internal carotid arteries and the basilar artery, just above the level of the pontomedullary junction. Planning of the imaging volume and the labeling plane for non-selective and selective ASL is illustrated in Figure 1.

Figure 1. A shows the imaging volume and labeling plane for non-selective and selective ASL, illustrated on a sagittal 3DT1 weighted image. B shows an axial slice at the level of the labeling plane. In case of selective labeling, labeling is spatially manipulated during the acquisition resulting in independent labeling of the right and left internal carotid artery and the basilar artery.
Chapter 7

Retrospectively gated spoiled gradient echo PC-MRI measurement was performed at the position of the labeling plane at a resolution of 0.65x0.65x3 mm³; TE/TR 5.2/8.0 ms; flip angle: 10°. Velocity encoding was performed in three directions simultaneously by using a four point method (100 cm/s in each direction). 30 cardiac phases were acquired.

Data analysis

Conventional MR series

Conventional MRI series (T2 and FLAIR and MRA) were interpreted by an experienced neuroradiologist (C. M.), blinded to the clinical and CBF data. A standardized evaluation form was used to assess vasculopathy, circle of Willis’ anatomy, ischaemic injury and leukoencephalopathy. Vasculopathy was classified according to severity of vascular stenosis (as a percentage of the vessel diameter, < 25%, 25-50%, 50-75% or 75-99%) or vascular occlusion. In case of stenosis or occlusion the exact location of the lesion had to be specified (artery and segment of the artery). The T2-weighted and FLAIR images were used to identify focal areas of high signal intensity, consistent with ischemic lesions, or leukoencephalopathy, defined as degeneration or demyelination of white matter which is visible as diffuse areas of high signal intensity. Infarcts were classified according to number and size (< 0.5 cm, 0.5-1.5 cm and >1.5 cm) and anatomic location of hyperintense lesions on T2-weighted images (hemisphere, cortex or white matter, frontal, parietal, temporal or occipital region, basal ganglia, thalamus or cerebellum). In case of diffuse leukoencephalopathy the anatomic region was specified (frontal, parietal, temporal or occipital region, basal ganglia, thalamus or cerebellum).

Arterial spin labeling

FSL (FMRIB-Software-Library, Oxford, UK, http://www.fmrib.ox.ac.uk) and Matlab (The MathWorks Inc., Natick, USA; http://www.mathworks.com) were used for offline processing of ASL data. Subtraction of labeled and control ASL images yielded whole brain perfusion weighted images. All control and labeled images were visually inspected and excluded in case of gross movement.

Flow territories of the ICAs and the BA, were defined according to the methods previously described by Wong (19). After co registration of flow territories and globally perfusion weighted images, perfusion weighted images were segmented into flow territories.

Quantification

Quantification of CBF was performed using the following equation, which was based on the model developed by Wang et al (20) and incorporates the influence of the different T1 of arterial blood and brain tissue on quantification of CBF.

$$ CBF = \frac{\Delta M \exp(\delta/T1_a) \exp(TE/T2*)}{\rho M_0 \alpha 2a T1_a (\exp((\delta - w)/T1_a) - \exp((\delta - \tau - w)/T1_a))} $$

where CBF is the flow in milliliters per gram per second, $\Delta M$ is the difference between the control and labeled image intensities, $w$ is the post labeling delay, $T1_a$ is the T1 of arterial blood (1.70 s for Hct = 0.4), $TE$ is the echo time (14 ms), $T2^*$ is the T2* of arterial blood (50 ms) (21), $\rho$ is the density of brain tissue (1.05 g/ml) (22), $M_0$ is the equilibrium magnetization of arterial blood for which an average scanner value was used that was calculated according to Chalela et al (23), $\alpha$ the labeling efficiency (0.85 for p-CASL), $T1_a$
is the T1 of brain tissue (1.24 s), δ is the transit time from the labeling region to the tissue compartment (tissue arrival time) (assumed to be 1500 ms) and τ is the tagging duration (1650 ms) (15, 24, 25).

Corrections of perfusion estimates

T1a was corrected for the individual Hct level of all patients, according to the equation developed by Lu et al. (14):

\[
\text{T1a} = \frac{1}{(0.52\text{Htc} + 0.38)}
\]

For healthy controls an Hct level of 0.40 was assumed.

Labeling efficiency was corrected for mean blood velocity in the right and left ICA (26). The labeling efficiency correction for mean velocity was based on simulations of the p-CASL labeling process, comparable to the simulations of Wu et al. (16). Figure 2 shows the behaviour of labeling efficiency according to blood velocity at the level of the labeling plane.

\textbf{Figure 2.} Labeling efficiency according to blood velocity at the level of the labeling plane.
Chapter 7

Phase-contrast MR
GT Flow (Gyrotools, Zurich, Switzerland) was used to assess blood flow (ml/s) and velocity (cm/s) in semi-automatically defined ROIs in the internal carotid arteries and the basilar artery. If at the level of PC-MRI, the vertebral arteries had not yet joined to form the basilar artery, a mean value of flow in the vertebral arteries was used. Velocities were used for velocity correction of the labeling efficiency.

To be able to compare PC-MRI flow measured in millilitres per second with ASL CBF in millilitres per 100 grams of brain tissue per second, flow in millilitres per second was converted into flow in millilitres per minute and corrected for the volume of the supplied brain tissue. The latter was converted from millilitres into grams, assuming a density of brain tissue of 1.05 g/l (22).

To compare whole brain flow measured by PC-MRI with CBF measured by ASL, the sum of flow in the ICAs and BA artery was divided by the total volume of grey and white matter. This volume was calculated by calculation of the volume of all nonzero voxels in the perfusion weighted image. To compare ICA flow measured by PC-MRI with CBF measured by ASL, ICA flow was divided by the volume of the ICA flow territory defined by selective ASL.

We further refer to flow corrected for brain volume, as PC-MRI CBF.

Statistical analysis
CBF values and agreement between PC-MRI and ASL
Continuous variables are expressed as the mean ± the standard deviation (SD). Mean CBF was compared between hemispheres and between different postlabeling delays, using paired t-tests. Mean whole brain CBF and mean ICA flow and velocity were compared between patients and controls using unpaired t-tests. All analyses were performed using SPSS statistics 16.02 (SPSS, Inc., Chicago, Illinois), regarding p-values <0.05 significant.

To assess the agreement between PC-MRI CBF and ASL CBF and the influence of Hct and velocity corrections, we calculated the mean of the paired differences (Σ) and the corresponding standard deviation of the paired differences (SD_{paired}) between PC-MRI and ASL with and without Hct and velocity corrections. Values were averaged across all individuals. Data analysis was performed whole brain for Hct correction and in the ICAs for velocity correction. The agreement between PC-MRI and ASL with and without Hct corrections was visualized using Bland-Altman plots (27, 28).

Asymmetries
For both delay times, CBF asymmetries were evaluated in three steps:
1. We compared mean CBF in the left and right ICA flow territory for all paired acquisitions, using a paired t-test.
2. If significance was reached in step 1 (P<0.05), we calculated the asymmetry ratio (AR), defined as the absolute CBF difference between the right and left ICA flow territory divided by the mean CBF in both flow territories.
3. Asymmetries were regarded clinically relevant if significance was reached in step 1 and if AR>10% (step 2). This 10% threshold was based on the current knowledge on ASL reproducibility and on previous findings on perfusion asymmetries in children with SCD (10, 11, 13, 29).
Results

Study population

We enrolled 15 patients and 6 controls. The data of 3 patients and 1 control subject could not be used, because of severe motion artefacts. Mean age did not differ significantly between groups and was 14.7 (range 9.3-20.8) years for patients and 17.0 (range 11.0-19.2) for controls. Sexes were equally represented in both groups (6 female and 6 male patients, 2 female and 3 male controls). Three controls were known to be a carrier of the sickle cell gene (HbAS) and two of the controls were known to have a normal haemoglobin (HbAA) genotype. In our patient population mean Hct level was 0.24 (range 0.18-0.33). In control subjects a mean Hct level of 0.4 was assumed for the estimation of arterial blood T1. Neurological examination was performed in 10 of 12 patients. No neurological symptoms were observed. All patients were in the steady state at the time of investigation. Five patients frequently suffered from vaso-occlusive crises (mostly abdominal or bone crises).

Conventional MR imaging

In six patients (46%), abnormalities were seen on MR angiography or T2 and FLAIR weighted MRI. Five patients had multiple small infarcts in the deep white matter. One patient had three small infarcts (< 5 mm) in the frontal-parietal regions of both hemispheres. One patient had one small infarct (< 5 mm) in the right frontal region. One patient had multiple infarcts in the frontal-parietal regions of both hemispheres; two of those infarcts were larger than 10 but smaller than 15 mm in diameter. In this patient, diffuse leukoencephalopathy in the frontal-parietal regions of both hemispheres was also observed. Another patient had one small infarct (< 5 mm) in the left cerebellar hemisphere. The fifth patient had one small (< 5 mm) infarct in the right frontal region. In one patient a possible stenosis of the M2 segment of the middle cerebral artery was observed. However, no clear distinction could be made with local flow artefacts and therefore this patient was scheduled for follow-up by MRA within two years and routine TCD screening of maximum blood velocity within the cerebral arteries every six months. The patient who had been treated by transfusion previous to inclusion in the current study did not have any abnormalities on conventional MRI. In one of the control subjects a small infarct (smaller than 5 mm in diameter) was observed.

Cerebral blood flow

Arterial Spin Labeling MRI

Mean whole brain CBF without individual Hct correction of arterial blood T1, was higher in patients than in controls for both delay times (67.5 ± 13.7 mL/100g/min in patients and 38.7 ± 5.6 mL/100g/min in controls (P<0.001) using a 1525 ms postlabeling delay and 68.7 ± 11.9 mL/100g/min in patients and 44.9 ± 5.4 mL/100g/min in controls (p=0.006) using a 2100 ms postlabeling delay). Initially, the longitudinal relaxation time of arterial blood was based on an Hct level of 0.4. Hct correction in SCD patients with lower Hct values resulted in higher longitudinal relaxation times of arterial blood, thereby reducing CBF values in SCD patients. Mean whole brain CBF corrected for Hct, remained significantly higher in patients than in controls for both delay times (59.3 ± 11.3 mL/100g/min using a 1525 ms
postlabeling delay (p=0.001) and 60.4 ± 9.9 mL/100g/min using a 2100 ms postlabeling delay (P=0.02) in SCD patients). In both groups, CBF did not differ significantly between delay times.

Phase Contrast MRI
Flow and velocity were successfully acquired in 9 patients and 3 controls. Mean ICA velocity was significantly higher in patients (R-ICA 52.8 ± 12.1 and L-ICA 55.6 ± 14.8 cm/s) than in controls (R-ICA 35.1 ± 7.4 and L-ICA 37.5 ± 11.9 cm/s) (P=0.01 and P=0.05), leading to a lower labeling efficiency in patients (about 0.8) than in controls (about 0.9) as can be appreciated from Figure 2.

Mean ICA flow was also higher in patients (R-ICA 6.0 ± 1.9 and L-ICA 5.9 ± 1.5 mL/s) than in controls (R-ICA 3.1 ± 1.3 and L-ICA 2.8 ± 1.8 mL/s) (P=0.007 and P=0.001). This difference remained when flow was corrected for the volume of the supplied brain tissue (PC-MRI CBF: R-ICA 83.0 ± 22.5 and L-ICA 75.5 ± 18.4 mL/100g/min in patients and R-ICA 45.2 ± 20.2 and L-ICA 38.4 ± 25.2 mL/100g/min in controls (P=0.01 and P=0.007). Whole brain PC-MRI CBF was 51.1 ± 13.1 in patients and 32.9 ± 5.0 mL/100g/min in controls (P=0.04).

Agreement between PC-MRI and ASL and the influence of correction methods
Hematocrit correction
The mean of the paired differences, $\Delta$, between whole brain CBF measured by PC-MRI and ASL with and without Hct correction and corresponding SDpaired, are depicted in Table 1. The lower $\Delta$s and SDs between both measurements after Hct correction, indicate a better agreement between both measurements after Hct correction (see Figure 3).

Velocity correction
The mean of the paired differences, $\Delta$, between CBF in ICA flow maps measured by PC-MRI and ASL with and without velocity correction and the corresponding SDpaired, are presented in Table 2. Lower mean values after velocity correction of the labeling efficiency in ASL, indicate a slightly better agreement between both measures after velocity correction.

Asymmetries
After applying Hct and velocity corrections, asymmetry results can be summarized as follows.

Firstly, significant asymmetries were observed in 11 patients (92%) and in 3 controls (60%) at a short postlabeling delay. Prolonging the delay time reduced the number of patients displaying significant asymmetries to 5 (42%) and only one control subject (20%) remained to have an asymmetry at a 2100 ms postlabeling delay. Figure 4 shows the ASL CBF maps of a patient in whom the perfusion asymmetry is no longer significant with a long postlabeling delay.

Secondly, calculation of asymmetry ratios yielded a mean asymmetry ratio of 9% in patients and 5% in controls at a short postlabeling delay. The mean asymmetry ratio decreased to 7% in patients at a long postlabeling delay. In the remaining control subject, the AR increased to 6% at a long postlabeling delay.

Thirdly, asymmetries that we considered clinically relevant (significant and AR>10%) were only observed in SCD patients. At a short postlabeling delay, 2 patients
ASL measurement of cerebral perfusion in children with SCD

had a significant asymmetry with an asymmetry ratio above 10%. At a long postlabeling
delay, 1 patient had a significant asymmetry with an asymmetry ratio above 10%.

**CBF asymmetries and Conventional MRI findings**

Of five children with significant asymmetries remaining at the long delay time ASL scans,
two children had ischaemic lesions (smaller than 5 mm in one child and smaller than 15
mm in the other child). Two children had ischaemic lesions without significant CBF
asymmetries.

The child with a significant asymmetry and an asymmetry ratio >10% at a long
delay time, had multiple small ischaemic lesions located in the frontal-parietal region of
both hemispheres (smaller than 15 mm). Based on these findings we could not draw any
conclusions on the association between the presence of CBF asymmetries and the
presence of cerebral pathology.

**Table 1.** Mean difference (Δ) and SDpaired between PC-MRI and ASL measurements of
whole brain cerebral blood flow (CBF). Values are shown with and without hematocrit
(Hct) correction.

<table>
<thead>
<tr>
<th>Correction method</th>
<th>Delay (ms)</th>
<th>Δ (mL/100g/min)</th>
<th>SDpaired (mL/100g/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>1525</td>
<td>11.2</td>
<td>12.9</td>
</tr>
<tr>
<td></td>
<td>2100</td>
<td>14.7</td>
<td>12.8</td>
</tr>
<tr>
<td>Hct</td>
<td>1525</td>
<td>5.6</td>
<td>11.6</td>
</tr>
<tr>
<td></td>
<td>2100</td>
<td>8.9</td>
<td>12.3</td>
</tr>
</tbody>
</table>

**Table 2.** Mean difference (Δ) and SDpaired between PC-MRI and ASL measurements
cerebral blood flow (CBF) in the flow maps of the internal carotid arteries (ICAs).
Values are shown with and without velocity correction. Hct = hematocrit.

<table>
<thead>
<tr>
<th>Correction method</th>
<th>Delay (ms)</th>
<th>Δ (mL/100g/min)</th>
<th>SDpaired (mL/100g/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hct</td>
<td>1525</td>
<td>-16.0</td>
<td>24.2</td>
</tr>
<tr>
<td></td>
<td>2100</td>
<td>-22.8</td>
<td>24.5</td>
</tr>
<tr>
<td>Hct &amp; Velocity</td>
<td>1525</td>
<td>-13.7</td>
<td>23.8</td>
</tr>
<tr>
<td></td>
<td>2100</td>
<td>-20.2</td>
<td>23.9</td>
</tr>
</tbody>
</table>
Figure 3. Bland Altman plots of the difference between PC-MRI and ASL measurements of cerebral blood flow, without and with hematocrit (Hct) corrections, plotted against the mean of both measurements. Bold dotted lines indicate the mean difference. Dotted lines represent the limits of agreement defined as the mean difference plus or minus 1.96*SD. Hct correction reduces the mean difference between both techniques and improves the limits of agreement.
The purpose of this study was to evaluate the applicability of ASL CBF measurements in children with SCD, using pseudo-continuous ASL with two different delay times and applying Hct and labeling efficiency corrections. The main findings of this study are threefold.

Firstly, we confirmed that CBF was higher in SCD patients than in controls, both without and with Hct correction in the patient group. This is in line with previously documented data on cerebral hyperaemia in children with SCD that were obtained by ASL as well as other techniques such as the 133Xe inhalation method or PET (10-12, 30-32). Also, mean flow and velocity assessed by PC-MRI were higher in patients than in controls, supporting the finding of hyperaemia in children with SCD, which is likely to be anaemia related (32). Mean whole brain CBF in our control population (38.7 ± 5.6 mL/100g/min), was in agreement with but within the lower range of the spectrum of CBF values that have been reported in ASL literature (29, 33, 34).

Secondly, the agreement between ASL and PC-MRI CBF measurements slightly increased after Hct correction, indicating that correction for blood Hct adds to the accuracy of the ASL perfusion measurement. Still ASL CBF and PC-MRI CBF values are different most probably caused by measurement noise. Acquisition of PC-MRI and ASL took place in a randomized sequence within a forty minute timeframe inducing the possibility of physiological fluctuations in resting state CBF. The agreement between ICA CBF measured by ASL and PC-MRI, did not improve substantially after velocity correction. This can be attributed to the small modifications in labeling efficiency that do not lead to marked differences measurable in this relatively small population.
Thirdly, we found less and smaller perfusion asymmetries than in previous studies, in which continuous and pulsed ASL sequences were employed with 1200 to 1400 ms postlabeling delays (10-13). In the current study, significant perfusion asymmetries were also encountered in controls using a short postlabeling delay. Most of those asymmetries disappeared by using a 2100 ms postlabeling delay. The number of patients with significant asymmetries as well as the asymmetry ratios decreased using a long postlabeling delay. Only in one of twelve patients, a clinically relevant asymmetry (significant and AR>10%) was observed with a long postlabeling delay. This illustrates that the perfusion asymmetries in children with SCD as they have been described before, can at least partly be attributed to transit time effects. Recently, Liu et al. studied compartment localization of labeled spins in ASL and concluded that a postlabeling delay of 2000 ms is sufficient to allow the spins to completely enter the gray matter (35). Their data also suggested that all labeled spins exchange to tissue and that only very few remain in the vasculature to enter the vein. An insufficient postlabeling delay could thus lead to incorrect estimation of CBF and to the observation of asymmetries that are in fact resulting from regional differences in arterial transit time. Although at the expense of a lower signal to noise ratio due to loss of label from longitudinal relaxation, a longer postlabeling delay will thus not lead to loss of signal via the venous compartment, while it does allow for all labeled spins to enter the brain parenchyma.

Also, since the labeling efficiency in p-CASL depends on blood velocity, velocity differences between both ICAs at the level of the labeling plane could induce asymmetries in the perfusion signal (26). This might especially hold true for children with SCD, in whom stenosis of major cerebral vessels has been amply mentioned as one of the pathogenetic mechanisms underlying cerebral infarcts. On the individual level, the maximum difference between mean velocity in the left and the right ICA in our patient population was 16 cm/s. This difference resulted in a labeling efficiency difference of approximately 1% between both sides (0.84 vs 0.83 in the right and left ICA). So, although differences in velocity can be taken into account, they will not be solely responsible for perfusion asymmetries of more than 10% that have been encountered in children with SCD. It is unlikely for all asymmetries to be related to technical issues encountered in ASL, since regional perfusion differences between hemispheres have also been measured by SPECT (36).

Although we found less and smaller perfusion asymmetries than previously described in literature (10, 11, 13), some asymmetries above 10% were observed. In 5 children significant asymmetries remained with a longer delay time (mean AR 7%). If one would assume a vaso-occlusive origin for ischaemic injury in children with SCD, one would expect a CBF decrease in regions with cerebral infarcts. In all children with significant CBF asymmetries at a long postlabeling delay, no association could be observed between decreased CBF and the localization of ischaemic injury. If on the other hand, one would assume the anaemia and vasodilatation resulting in impairment of cerebrovascular reactivity as the underlying pathogenetic mechanism for cerebral infarcts, as has been suggested by Prohovnik et al., one would expect a CBF increase in regions with cerebral infarcts (30). No consistent observations on CBF increase or decrease and the presence of cerebral pathology could be made in our population. Part of this problem could reside in
ASL measurement of cerebral perfusion in children with SCD

the fact that we did not measure CBF at the time ischaemic lesions arose and CBF might have changed thereafter. Also, due to its low spatial resolution, ASL perfusion imaging is not sensitive to the hemodynamic changes that occur in the smallest branches of the vascular tree and appear to underlie the small ischaemic lesions seen in our patient population. Furthermore, the use of relatively large templates for calculation of perfusion asymmetries could obscure smaller regions of hemodynamic imbalance. Finally, the lack of concordance between localization of pathology and perfusion asymmetries might be attributed to the fact that most pathology occurs in white matter, whereas perfusion signal in ASL is mainly originating from grey matter.

Some limitations should be mentioned, including the small control population and the fact that we included both children with a normal haemoglobin genotype and children who were carriers of the sickle cell gene as controls. However, from literature it can be appreciated that being carrier of the sickle cell gene does not increase the risk for anaemia (37, 38). Although neurological sequelae of the sickle cell trait have been described incidentally, a large study comparing the incidence of neurological abnormalities between carriers and controls revealed no group difference (39, 40). In this study, we did not perform an ASL sequence with multiple inversion times. The latter could be used to visualize regional differences in arterial arrival time for each individual. We do believe however, that the use of two different delay times in this study clearly revealed the transit time effects on perfusion asymmetries, which were reduced by using a longer postlabeling delay. An issue in quantification of mean whole brain CBF might be the assumed equal tissue arrival time \((i)\) for patients and controls, whereas higher mean blood velocity in SCD patients might result in a shorter tissue arrival time. Furthermore, scanning in this relatively young population was performed without sedation and consequently, we had to exclude some datasets because of gross motion artefacts. A different set-up in which children can watch videos during scanning might have been a simple and surely a recommendable solution to this problem. Finally, it might have been interesting to analyze white matter perfusion since most ischemic lesions are located in the deep white matter. However, the scanning times we used were too short for this purpose as longer scanning times (approximately 10 min) are necessary to measure significant white matter perfusion in single voxels in the deep white matter (41). In an additional analysis we observed that asymmetries were more prominent in the white matter perfusion in patients, but similar to the grey matter asymmetries no association with pathology could be observed.

In conclusion, perfusion asymmetries in our population were found less frequently and were smaller than in previous studies. By using a 2100 ms postlabeling delay allowing for all labeled spins to reach the tissue compartment and by applying a labeling efficiency correction for blood velocity, few asymmetries were observed. Whether these asymmetries are related to the occurrence of cerebral infarcts is not clear at present and the ability of ASL to identify patients at risk of silent infarcts remains to be assessed in a prospective longitudinal study design. Care must be taken in the interpretation of ASL perfusion imaging in children with SCD, since both physiological characteristics of this patient population (increased blood flow and velocity) as well as technical pitfalls (transit time, Hct level and blood velocity at the level of the labeling plane) will influence perfusion maps. A
long postlabeling delay with a correction for blood velocity could anticipate overestimation of CBF asymmetries.
ASL measurement of cerebral perfusion in children with SCD

Reference List


37. Bowers AS, Pepple DJ, Reid HL. Optimal haematocrit in subjects with normal haemoglobin genotype (HbAA), sickle cell trait (HbAS), and homozygous sickle cell disease (HbSS). Clin Hemorheol Microcirc 2011;47:253-60