Cerebral perfusion characteristics show differences in younger vs. older children with sickle cell anaemia: results from a multiple inflow time arterial spin labelling study

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Cerebral perfusion characteristics show differences in younger vs. older children with sickle cell anaemia: results from a multiple inflow time arterial spin labelling study

J.M. Kawadler* & P.W. Hales, F.J. Kirkham, S Barker, T.C.S. Cox and C.A. Clark

Sickle cell disease is associated with chronic anaemia and oxygen desaturation, which elevates cerebral blood flow (CBF) and increases risk of stroke. A multi-inflow time ASL acquisition in children with SCD and healthy controls. CBF was elevated globally in SCD patients; in younger children, CBF was negatively correlated with blood oxygen content. In older children, SCD patients had significantly shorter bolus arrival times. This may indicate increasing disparity between patients and controls during development, related to longer-standing burden of disease.
Cerebral perfusion characteristics show differences in younger vs. older children with sickle cell anaemia: results from a multiple inflow time arterial spin labelling study

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Keywords: sickle cell anemia; cerebral hemodynamics; cerebral circulation; cerebral blood flow; arterial spin labelling; posterior circulation

Abbreviations
SCA = sickle cell anaemia; HbSS = homozygous sickle cell anaemia, HbSC = haemoglobin SC disease; HbAS = sickle cell trait; Hct = haematocrit; SpO\textsubscript{2} = peripheral oxygen saturation; CBF = cerebral blood flow; ASL = arterial spin labelling; SCI\textsuperscript{-} = no evidence of silent cerebral infarction; SCI\textsuperscript{+} = evidence of silent cerebral infarction; ACA = anterior cerebral artery; MCA = middle cerebral artery; PCA = posterior cerebral artery; MRI = magnetic resonance imaging; DSC-MRI = dynamic susceptibility contract MRI; BAT = bolus arrival time; TI = inflow time; YC = younger children; OC = older children; TCD = transcranial Doppler ultrasound

Abstract
Sickle cell anaemia (SCA) is associated with chronic anaemia and oxygen desaturation, which elevate cerebral blood flow (CBF) and increase risk of neurocognitive complications. Arterial spin labelling (ASL) provides a methodology for measuring CBF non-invasively; however, ASL techniques using only a single inflow-time are not sufficient to fully characterise abnormal haemodynamic behaviour in SCA. This study investigated haemodynamic parameters from a multi-inflow-time ASL acquisition in younger (8-12 years) and older (13-18 years) children with SCA with and without silent cerebral infarction (SCI +/-) (n=20 and 19 respectively, 6 and 4 SCI+ respectively) and healthy controls (n=9 and 7 respectively). Compared with controls, CBF was elevated globally in both groups of patients. In the younger SCA patients, CBF was negatively correlated with blood oxygen content in the middle and posterior cerebral artery territories and significantly positively correlated with bolus arrival time (BAT) in the anterior and middle cerebral artery territories. In older children, SCA patients had significantly shorter BAT than healthy controls and there was a significant negative correlation between CBF and oxygen content only in the territory of the posterior cerebral artery, with a trend for a correlation in the anterior cerebral artery but no relationship for the middle cerebral artery territory. In the younger group, SCI+ patients had significantly higher CBF in the posterior cerebral artery territory (SCI+: mean=92.78 ml/100g/min, SCI-: mean=72.71 ml/100g/min; F=4.28, p=0.04) but this no longer reached significance when 2 children with abnormal transcranial Doppler and one with HbSC were excluded and there were no significant differences between patients with and without SCI in the older children. With age, there appears to be increasing disparity between patients and controls in terms of the relationship between CBF and oxygen content in the anterior circulation, potentially predicting the risk of acute and chronic compromise of brain tissue.
Introduction

Sickle cell anaemia (SCA) is the most common inherited genetic disorder in the UK, affecting approximately 1 in 2000 births. An abnormal destruction of red blood cells causes chronic anaemia and chronic oxygen desaturation, resulting in lifelong symptoms. There is a high rate of neurological complications, including in untreated children an incidence of clinical stroke of approximately 10% and silent cerebral infarction (SCI) in up to one-third. Low blood oxygen saturation is an identified risk factor for overt stroke, and has been associated with neuropsychological dysfunction and compromised white matter integrity.

As an adaptive response to chronic anaemia, abnormal cerebral blood velocity, as measured by transcranial Doppler of the middle cerebral artery, is associated with vasodilation of distal small vessels and reduction in cerebrovascular hemodynamic reserve, particularly if there is also proximal large vessel steno-occlusive disease. Haemodynamics may be further challenged during acute clinical events (e.g., fever or seizures) when metabolic rate is high, causing acute ischaemia especially in the border zones of arterial territories.

Perfusion MRI techniques, either after injection of a gadolinium-base paramagnetic contrast agent (e.g., dynamic susceptibility contrast (DSC) imaging) or non-invasive magnetic ‘tagging’ of water molecules in arterial blood (arterial spin labelling, ASL), have found abnormalities thought to be indicative of subclinical pathology or abnormal haemodynamics. In patients with chronic cerebrovascular pathology and stroke, DSC-MRI studies have shown focal areas of reduced CBF and prolonged mean transit time in the affected hemisphere corresponding to stroke-like lesions, as well as hemispheric asymmetry of the perfusion signal. However, there are concerns about the safety of contrast agents, including Gadolinium, in SCA and in children in particular although audits of practice appear to show no excess of adverse events.

The use of ASL for CBF quantification is preferable to DSC-MRI as there is no need for injection of a contrast agent or user-defined arterial input function. The majority of recent ASL studies in SCA confirm elevated global CBF, especially when correct quantification is used (e.g., considerations for blood T1, haematocrit, and labelling efficiency), although one did not find differences between patients and controls. Elevated CBF may be both a response to and a risk factor for cerebral hypoxia, and is related to low haematocrit as well as the proportion of haemoglobin S, A and F, determined from the host genetic characteristics and effect of treatment with hydroxyurea or blood transfusion.

Previous ASL studies, however, have differed in acquisition techniques. The majority of previous studies have measured CBF after a single inflow time (TI) or two TIs. An additional study has investigated the use of multiple post-labelling delay times in SCA using a pseudo-continuous ASL (pCASL) protocol. However, the necessary delay required for pCASL labelling may result in the arrival of the leading edge of the labelled bolus being missed, which could be particularly problematic in SCA patients with elevated blood velocities.

In healthy subjects, a single-TI ASL protocol is generally valid, as assumptions can be made about the delay time required to allow the entire bolus of labelled blood to traverse the feeding arteries before exchanging with the brain parenchyma (generally referred to as either the ‘arterial transit time’ or ‘bolus arrival time’), and CBF can be measured after this time. This eliminates apparent, erroneous regional variations in CBF due to so-called ‘transit-time effects’. However, in patients with SCA, the passage of labelled blood through the cerebral vasculature is likely to be abnormal. Furthermore, cerebral haemodynamics and related ASL fitted parameters change throughout childhood, which may be related to normal developmental processes, and will not be captured using a single-TI acquisition. A pulsed labelling (pulsed ASL; PASL) acquisition with multiple inflow times allows the passage of the labelled bolus of blood in a given imaging voxel to be fully captured, eliminating the need for prior assumptions about transit time effects, as the short TIs that can be achieved with PASL allow the capture of the arrival of the leading edge of the labelled bolus of blood. In addition, CBF quantification depends on the T1 relaxation time of blood; an assumed, fixed value is used in most
studies, but the accuracy of CBF quantification is improved if this is adjusted for the haematocrit of labelled blood.

By employing a pulsed labelling technique as part of the ASL acquisition, the passage of the labelled bolus of blood through the arterial vasculature and into the capillary bed can be measured by acquiring data over a wide range of TIs. This method allows the 'tracer kinetic' behaviour of the labelled water in the blood to be characterised, and by fitting a mathematical kinetic model to the data, fitted parameters which describe the delivery of blood throughout the brain can be derived. It should be noted that the upper limit on range of TIs will depend on the $T_1$ relaxation time of blood, as the SNR of the ASL difference signal will decrease as the inverted longitudinal magnetization of water in the blood recovers to its fully relaxed state.

Using the model described by Buxton et al., the key fitted parameters are CBF, bolus arrival time (BAT), which represents the earliest arrival time of labelled blood throughout the brain, and $\tau$. The latter represents the temporal width of the labelled bolus, and is influenced by both the spatial coverage of the transmit RF coil (which is often curtailed by so-called QUIPSS-II saturation pulses), and the level of dispersion of the labelled bolus during its passage through the vasculature. Not only do these additional fitted parameters provided by multi-TI ASL help to characterize tracer kinetics in a subject, but they can serve as clinically useful hemodynamic measurements in themselves, in addition to CBF.

Using previously published acquisition and quantification techniques, the purpose of this study was to compare the distribution of CBF in the territories of the anterior, middle and posterior cerebral arteries (ACA, MCA, PCA) in relation to arterial oxygen content of paediatric patients with SCA and healthy controls. This is the first ASL-based study which uses a wide range of inflow times to measure the hemodynamic behaviour in children with SCA. We hypothesize an association between ASL fitted parameters and oxygen content of the blood in children with SCA, which has yet to be demonstrated for separate arterial territories.

Methods

Study Participants

Children and adolescents with SCA and healthy sibling controls aged 8-18 years (split into younger children [8-12 years] and older children [13-18 years]) were recruited between 2012-2013 from three London sites (Whittington Hospital, North Middlesex Hospital, and Royal London Hospital). Exclusion criteria included history of clinical stroke. Ethical permission was granted by Southampton Research and Ethics Committee (REC reference 11/H0502/5) and fully informed assent/consent was obtained from each participant and his or her parent/guardian.

Clinical data

Genotype (HbSS=homozygous sickle cell anaemia; HbSC=haemoglobin SC disease; HbAS=sickle cell trait; HbAA=normal haemoglobin) was collected from medical notes of patients or from the parent of sibling controls. In patients, haematocrit (Hct) and haemoglobin were derived from a standard full blood count taken an average of 96 days from date of MRI during routine clinic appointments. Peripheral oxygen saturation ($SpO_2$) was measured by a finger probe pulse oximeter on the day of the scan. Oxygen content was estimated using:

$$\text{Oxygen content} = (1.34 \times \text{Haemoglobin} \times SpO_2) + (0.003 \times pO_2)$$

$pO_2$ = partial pressure of oxygen, which is assumed to be 100 torr on room air.
MRI acquisition

MR data were acquired in all subjects on a 1.5T Siemens Avanto scanner (Erlangen, Germany) with 40 mT/m gradients and 32-channel head-coil. The imaging protocol consisted of an axial T2-weighted turbo spin echo sequence (TR=4920ms, TE=101ms, voxel size=0.7x0.7x4.0mm) for diagnosis of SCI by two experienced neuroradiologists (SB, TC). A flow-sensitive alternating inversion recovery pulsed-ASL sequence was acquired, with background suppression and 3D single shot GRASE readout, using 6 inflow times ranging 0.2-2.2 seconds in 0.4-second intervals. In addition, an inversion-recovery sequence was used to calculate voxel-wise values of T₁ relaxation time and M₀ (equilibrium longitudinal relaxation) in the tissue. Total scan time was 8 minutes. Details of the imaging protocol and arterial territory segmentation (Figure 1) have been described previously.

As the T₁ relaxation time of blood (T₁bl) is dependent on Hct and SpO₂, and a value of T₁bl is required during the ASL model fitting, estimated values of T₁bl were calculated for each patient based on their measured Hct and SpO₂, using the model described by Hales. Further details of the model fitting method are described in Hales et al. For healthy controls, in whom blood specimens were unavailable, Hct was estimated from the literature values, based on the subject’s age and gender.

Magnetic resonance angiography was not undertaken as it would have extended the time required for the protocol to be completed in these young children, but we obtained the results of clinically indicated screening transcranial Doppler, categorised as normal (<170 cm/sec), conditional (170-199cm/sec) and abnormal (≥200 cm/sec).

Statistical Analysis

Statistical analyses were performed in R (www.r-project.org). An analysis of covariance model was performed to control for age and gender in patients vs. controls separately in younger children and older children. Pearson’s product moment correlations (r) were carried out for correlations between oxygen content and ASL fitted parameters, correcting for age and gender.

Results

A total of 39 patients with SCA (38 HbSS, 1 HbSC) and 16 sibling controls (5 HbAS, 11 HbAA) were scanned, split into Younger Children (YC; n=20 patients, n=9 controls) and Older Children (OC; n=19 patients, n=7 controls). In the YC group, 6/20 had evidence of SCI on T2-weighted MRI, and clinical history included 2 on hydroxyurea, 3 with conditional velocities, 2 on chronic transfusions for abnormal transcranial Doppler, both of whom also had SCI, and 1 who received a transfusion within 3 months of MRI. In the OC group, 4/19 had evidence of SCI, and clinical history included 4 on hydroxyurea, none with conditional velocities, 1 on chronic transfusions for abnormal transcranial Doppler without SCI and 1 who received a transfusion within 3 months of MRI.

Figure 2 shows an illustration of the raw multi-TI ASL data acquired in a representative subject.

CBF in all three arterial territories was significantly higher in SCA patients than controls (Table 1; Figure 3). Within the YC group, SCI+ patients had significantly higher CBF in the PCA territory (SCI+: mean=92.78 ml/100g/min, SCI-: mean=72.71ml/100g/min; F=4.28, p=0.04). In the OC group, BAT in all three territories was significantly shorter in patients, and there was a trend for shorter τ in patients in the PCA territory (p=0.09). There were no significant differences in cerebral haemodynamics between patients with and without SCI in the OC group. TCD category had no effect on cerebral haemodynamics, neither in the whole group nor in the older or younger children.

In an additional analysis, one patient with HbSC genotype and the 3 patients on chronic transfusions for abnormal transcranial Doppler were excluded. In the YC group, 4/17 patients had evidence of SCI
on T2-weighted MRI, and treatment history included 2 on hydroxyurea and 1 who received a blood transfusion within 3 months of MRI. In the OC group, 4/18 had evidence of SCI, and treatment history included 4 on hydroxyurea and 1 who received a transfusion within 3 months of MRI. In both YC and OC groups, CBF in all three arterial territories was significantly higher in SCA patients than controls (Table 2). In the OC group, BAT in all three territories and $\tau$ in the MCA territory was shorter in patients. With the 4 patients excluded, there were no significant differences between patients with and without SCI in either the YC or OC group.

Blood oxygen content was only available for patients as it was not considered ethical to obtain a blood specimen in controls (Table 1). In YC, oxygen content was available for all patients; there was a significant negative correlation between oxygen content and CBF in the MCA territory, with a trend for significance in the ACA and PCA territories, a significant positive correlation with BAT in the ACA and MCA territories, and a significant negative correlation with $\tau$ in the MCA territory (Figure 4). In OC, oxygen content was available for 16/19 patients; there was a significant negative correlation between oxygen content and CBF in the PCA territory with a trend for significance in the ACA territory, and a significant positive correlation with BAT in the MCA territory (Figure 5).

Discussion

This is the first study to use multiple inflow time ASL to examine regional cerebral haemodynamics in children with SCA (total scan time: 8 minutes). Our study demonstrates the expected elevation in CBF in SCA patients. We have recently discussed a methodological issue relating to this, which is that when calculating CBF using ASL data, a value for $T_{1bl}$ is required noting that $T_{1bl}$ is highly sensitive to Hct and weakly sensitive to blood oxygenation\(^3\). For instance, low Hct will significantly increase the value of $T_{1bl}$, and if this is not accounted for when calculating CBF (i.e. a normal value of $T_{1bl}$ is assumed), CBF will be overestimated\(^3\). Accordingly, the CBF values reported here were based on $T_{1bl}$ values which were adjusted for the Hct and oxygen saturation values measured in each patient. Our results confirm elevated CBF in patients after this adjustment, in the ACA, MCA and PCA territories respectively.

Our control data suggest that in normal development, BAT generally increases in later childhood (there was a consistent increase in BAT between the YC and OC controls in all arterial territories, Figure 3). However, the SCA patients did not follow the same pattern. The younger SCA patients demonstrated similar BAT values to the younger controls, in all arterial territories. However, the BAT values in the older SCA patients remained similar to the values seen in the younger SCA patients, rather than demonstrating the consistent increase in BAT as seen in the controls. This suggests that in SCA, the consistently elevated CBF values seem to outweigh the expected delay in the arrival of labelled blood in older patients. This leads to a stratification in haemodynamic fitted parameters, beyond CBF, between SCA patients and healthy controls in the older age group. This is most pronounced in the PCA territory, which generally has a longer BAT than the ACA and MCA territories – here, the difference in BAT between SCA and healthy controls is an order of magnitude more significant than in the other two territories.

A trend for lower values of $\tau$ was measured in the PCA territory in SCA patients, indicating a shorter temporal width of the labelled bolus of blood delivered to these regions. Similar to the pattern seen in BAT, this is likely to be due to increased CBF in SCA patients – faster delivery of the bolus of labelled blood allows less time for bolus dispersion en route to the perfused tissue. The effect on $\tau$ appears to be more subtle however, and as such stratification between SCA patients and controls is only apparent in the PCA territory, in which labelled blood generally takes longer to arrive.

We have demonstrated that CBF is significantly negatively correlated with oxygen content in MCA and PCA territories in the YC group, and in the PCA territory in the OC group. Overall, this reflects the compensatory increase in blood flow in SCA to counteract the reduced oxygenation of the blood itself. This confirms previous studies of increased CBF in paediatric SCA patients\(^17,18\), even with Hct...
correction. In older children, the expected relationship between oxygen content and CBF does not appear to be maintained in the anterior circulation and specifically not in the territory of the MCA. This is contrary to data from CBF measured using phase contrast MRI of the carotid and vertebral arteries, which suggests the relationship with global CBF is similar to that seen in people with normal haemoglobin. It will be important for future studies to investigate this discrepancy and to determine whether and breakdown in the coupling between CBF and oxygen content is of clinical importance, either at rest or if the ability to further increase CBF is exceeded.

The degree of anaemia and haemolysis and haemoglobin F levels may also affect haemodynamics in the anterior and posterior circulation differently. As mentioned earlier, BAT is generally longer in the PCA territory, in both SCA patients and controls. It is in this region that the correlation between blood oxygen content remains strongest in the older SCA patients, suggesting that the delayed arrival time of blood in this region necessitates a greater compensatory increase in CBF in blood with lower blood oxygenation, which may be achieved in children with SCA through adaptations including basilar artery dilation. Differences in the adaptive mechanisms, and comparative success in maintaining CBF in the face of increased metabolic demand for oxygen may account for the variety in the distribution of neuroradiological abnormality associated with acute central nervous system events in response to challenges such as acute anaemia or hypoxia. Extension of the simultaneous measurement of oxygen extraction to children may allow prediction of the risk of neurological compromise.

A limitation of our study was that a temporal resolution of 400ms was used in the multi-TI ASL acquisition, which may have rendered it insensitive to more subtle changes in haemodynamic fitted parameters such as BAT. However, increased sensitivity would have necessitated a longer acquisition, and it was our aim to apply an ASL protocol which would be clinically feasible in paediatric patients. Additionally, although we accounted for the effect of Hct on T1 relaxation time of blood, which has been shown to have a strong influence, an elevation in methaemoglobin may also influence T1 values in these patients, which was not accounted for in this study.

This study is also limited by small numbers of controls and mixed healthy control genotypes (sickle cell trait, normal haemoglobin); although there were no significant differences in any ASL fitted parameters between HbAA and HbAS genotypes. In addition, there was some heterogeneity in genotype and treatment for the patients with SCA; although perhaps accounting in part for the lack of association between CBF and oxygen content in the older age group, this increases the generalisability of the results. Haemoglobin was not available for the healthy children on ethical grounds and in the children with SCA, it was not considered ethical to add an additional blood draw but we were careful to use an appropriate steady state value and there is relatively little change over a year in most patients with SCA. The age groups were chosen to include those who were mainly prepubertal in the younger age group and those going through puberty in the older age group. However, we did not assess puberty in individuals as they often find this embarrassing and it was not considered critical to the study.

We did not include MRA of the intracranial and extracranial vasculature in this protocol because of the already long scanning duration in these relatively young patients but there is evidence of focal perfusion abnormality distal to vascular stenosis. We did not find an effect of TCD category but this would have been confounded by the regular transfusions in those with abnormal TCD as well as the fact that high velocities represent increased global CBF as well as vascular narrowing leading to focal hypoperfusion. It would be of interest to examine the effect of variations in the cerebral vasculature, for example, the possibility that the size of the basilar artery or posterior communicating arteries affects the CBF/oxygen content relationship in the older patients or the risk of subsequent infarction. Differences in cerebral vasculature, whether congenital, adaptive in response to hypoxic exposure or pathological, may account for some of the variability in the haemodynamic fitted parameters illustrated in Figure 3 and perhaps for difference in clinical outcomes. It will be particularly important to extend studies into the adult age range as the risk of progressive silent infarction continues to increase with age.
In the clinic, single-TI ASL protocols are generally used. However, in patients with abnormal hemodynamic behaviour, as in SCA, it is important to understand how changes in the blood itself (such as low haemoglobin, haematocrit and oxygenation) lead to deviations in the dynamic ASL signal, which can only be captured with acquisitions at multiple inflow times. Although the relationship between CBF and \( \text{SpO}_2 \) has been suggested previously\(^8\), in contrast to previous reports\(^{18,21}\), these are the first data to explicitly show a significant correlation between ASL-based CBF and blood oxygen content in children and adolescents with SCA. Our data also suggest that stratification in bolus arrival times, between SCA and healthy controls, occurs predominantly in older children. Furthermore, due to the delayed arrival of blood in the PCA territory, it is here that differences in haemodynamic behaviour in SCA are most pronounced. Having acquired these data, we can use this to guide both the design of single-TI ASL protocols aimed specifically for monitoring these patients, and the clinical interpretation of data acquired in these patients. It will be particularly important to establish whether abnormalities in CBF documented with ASL predict clinically important outcomes, including cognitive performance as well as neurological events, as treatments to improve CBF, such as revascularisation, are feasible.
References


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<th>Older Children (n=26)</th>
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<td>Patients (n=19) Controls (n=7)</td>
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<td>Age</td>
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<td>Gender</td>
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<td>10F, 9M 2F, 5M</td>
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<td>Genotype</td>
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<td>19 HbSS 2 HbAS 5 HbAA</td>
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<tr>
<td>SCI</td>
<td>14 SCI- 6 SCI+</td>
<td>15 SCI- 4 SCI+</td>
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<tr>
<td>Hct</td>
<td>0.234 ± 0.04</td>
<td>0.238 ± 0.05</td>
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<tr>
<td>SpO₂</td>
<td>96.3 ± 2.1</td>
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<td>11.27 ± 1.87</td>
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<td>ACA CBF (ml/100g/min)</td>
<td>F=14.96 p=0.0007***</td>
<td>F=20.13 p=0.0002***</td>
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<td>MCA CBF (ml/100g/min)</td>
<td>F=24.32 p=0.0005***</td>
<td>F=29.34 p=0.0002***</td>
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<td>PCA CBF (ml/100g/min)</td>
<td>F=22.44 p=0.0008***</td>
<td>F=14.61 p=0.0009***</td>
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<tr>
<td>ACA BAT (s)</td>
<td>0.37 ± 0.08 0.38 ± 0.05</td>
<td>0.36 ± 0.07 0.46 ±0.10</td>
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<tr>
<td>MCA BAT (s)</td>
<td>0.37±0.08 0.36±0.06</td>
<td>0.36 ± 0.07 0.48 ± 0.12</td>
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<tr>
<td>PCA BAT (s)</td>
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<td>0.46 ± 0.06 0.62 ± 0.14</td>
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<td>ACA τ (s)</td>
<td>1.18 ± 0.24 1.11 ± 0.13</td>
<td>1.13 ± 0.28 1.18 ± 0.24</td>
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<td>MCA τ (s)</td>
<td>1.11 ± 0.16 1.15 ± 0.13</td>
<td>1.03 ± 0.16 1.14 ± 0.05</td>
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<tr>
<td>PCA τ (s)</td>
<td>1.21 ± 0.97 1.29 ± 0.19</td>
<td>1.18 ± 0.12 1.26 ± 0.09</td>
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HbSS= homozygous sickle cell anaemia; HbSC= SC disease; HbAS= sickle cell trait; HbAA= normal haemoglobin; SCI= absence of silent cerebral infarct; SCI+= presence of silent cerebral infarct

*ANCOVA F statistic covarying for the effects of age, gender and the interaction between age and gender.

†p<0.1, *p<0.05, **p<0.01, ***p<0.001

Table 1. Demographics. Means and standard deviations reported for age and all ASL parameters in 16 controls and 39 patients with sickle cell anaemia.
### Table 2. Demographics. Means and standard deviations reported for age and all ASL parameters in 16 controls and 35 patients with homozygous sickle cell anaemia not on chronic transfusion.

<table>
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<th>Younger Children (n=26)</th>
<th>Older Children (n=25)</th>
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<tr>
<td></td>
<td>Patients (n=17)</td>
<td>Controls (n=9)</td>
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<tr>
<td><strong>Age</strong></td>
<td>11.2 ± 1.4</td>
<td>10.6 ± 1.6</td>
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<td><strong>Gender</strong></td>
<td>6F, 1aM</td>
<td>7F, 2M</td>
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<td><strong>Genotype</strong></td>
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<td>3 HbAS</td>
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<td><strong>SCI</strong></td>
<td>13 SCI-</td>
<td>4 SCI+</td>
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<tr>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Hct</strong></td>
<td>0.241 ± 0.03</td>
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<tr>
<td><strong>SpO₂</strong></td>
<td>96.1 ± 2.1</td>
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<td><strong>T₁blood</strong></td>
<td>1.873 ± 0.054</td>
<td>(range: 1.736-1.962)</td>
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<tr>
<td><strong>Oxygen Content</strong></td>
<td>10.96 ± 1.73</td>
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<td>(ml O₂/100ml blood)</td>
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</table>

**ACA CBF** (ml/100g/min): 88.0 ± 21.8 (77.0 ± 13.1 | 57.5 ± 5.8)

**MCA CBF** (ml/100g/min): 100.2 ± 18.3 (88.2 ± 10.9 | 64.6 ± 6.1)

**PCA CBF** (ml/100g/min): 89.5 ± 19.8 (81.9 ± 16.0 | 55.6 ± 7.9)

**ACA BAT (s)**: 0.37 ± 0.08 (0.36 ± 0.07 | 0.46 ± 0.10)

**MCA BAT (s)**: 0.36 ± 0.08 (0.37 ± 0.07 | 0.48 ± 0.12)

**PCA BAT (s)**: 0.47 ± 0.06 (0.46 ± 0.06 | 0.62 ± 0.14)

**ACA τ (s)**: 1.17 ± 0.24 (1.13 ± 0.29 | 1.18 ± 0.24)

**MCA τ (s)**: 1.10 ± 0.17 (1.03 ± 0.16 | 1.14 ± 0.05)

**PCA τ (s)**: 1.22 ± 0.11 (1.18 ± 0.12 | 1.26 ± 0.09)

HbSS=homozygous sickle cell anaemia; HbAS=sickle cell trait; HbAA=normal haemoglobin; SCI-=absence of silent cerebral infarct; SCI+=presence of silent cerebral infarct, Hct=haematocrit, SpO₂=peripheral oxygen saturation

³ANCOVA F statistic covarying for the effects of age and gender.

*p<0.05, **p<0.01, ***p<0.001

The table shows the comparison of demographics between younger (n=26) and older (n=25) children, as well as between patients (n=17) and controls (n=9) for younger children and patients (n=18) and controls (n=7) for older children. The table includes age, gender, genotype, SCI, Hct, SpO₂, T₁, oxygen content, ACA CBF, MCA CBF, PCA CBF, ACA BAT, MCA BAT, PCA BAT, ACA τ, MCA τ, and PCA τ. The table also includes ANCOVA F statistic covarying for the effects of age and gender.

**Figure legends**
Figure 1. Example of ASL image segmentation based on vascular flow territories: ACA, MCA, PCA = anterior-, middle- and posterior-cerebral arterial flow territories.

Figure 2. Difference maps of multiple inflow time ASL acquisition in native space of one SCA patient.

Figure 3. Differences in ASL parameters between patients and controls in younger children and older children, separately. CBF=cerebral blood flow, BAT=bolus arrival time, τ (tau), †p<0.1, *p<0.05, **p<0.01, ***p<0.001.

Figure 4. Correlations with oxygen content in younger patients (n=20). These graphs show residual data from partial correlations correcting for age, gender and the interaction between age and gender.

Figure 5. Correlations with oxygen content in older patients (n=16). These graphs show residual data from partial correlations correcting for age, gender and the interaction between age and gender.
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