White Matter Damage Relates to Oxygen Saturation in Children With Sickle Cell Anemia Without Silent Cerebral Infarcts

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Background and Purpose—Sickle cell anemia is associated with compromised oxygen-carrying capability of hemoglobin and a high incidence of overt and silent stroke. However, in children with no evidence of cerebral infarction, there are changes in brain morphometry relative to healthy controls, which may be related to chronic anemia and oxygen desaturation.

Methods—A whole-brain tract-based spatial statistics analysis was carried out in 25 children with sickle cell anemia with no evidence of abnormality on T2-weighted magnetic resonance imaging (13 male, age range: 8–18 years) and 14 age- and race-matched controls (7 male, age range: 10–19 years) to determine the extent of white matter injury. The hypotheses that white matter damage is related to daytime peripheral oxygen saturation and steady-state hemoglobin were tested.

Results—Fractional anisotropy was found to be significantly lower in patients in the subcortical white matter (corticospinal tract and cerebellum), whereas mean diffusivity and radial diffusivity were higher in patients in widespread areas. There was a significant negative relationship between radial diffusivity and oxygen saturation (P<0.05) in the anterior corpus callosum and a trend-level negative relationship between radial diffusivity and hemoglobin (P<0.1) in the midbody of the corpus callosum.

Conclusions—These data show widespread white matter abnormalities in a sample of asymptomatic children with sickle cell anemia, and provides for the first time direct evidence of a relationship between brain microstructure and markers of disease severity (eg, peripheral oxygen saturation and steady-state hemoglobin). This study suggests that diffusion tensor imaging metrics may serve as a biomarker for future trials of reducing hypoxic exposure. (Stroke. 2015;46:1793-1799. DOI: 10.1161/STROKEAHA.115.008721.)

Key Words: diffusion ■ pediatrics ■ sickle cell anemia

Sickle cell anemia (SCA) is a genetic condition affecting the oxygen-carrying capability of hemoglobin. Cerebral injury is common, with well-documented incidence of overt stroke in 10% of children,1 silent cerebral infarction (SCI) in ≤35% of patients,2 and tissue atrophy.3 Ischemic insults can have devastating effects on cognitive abilities, including intelligence4 and executive functioning.5 Children with SCA with no evidence of infarction on conventional magnetic resonance imaging (MRI) still show reduction in intelligence quotient (IQ) compared with healthy age- and race-matched controls6,7; therefore, one can assume there may be abnormalities in the brain beyond the detection of conventional MRI methods and that the presence of discrete lesions alone may not describe total brain injury.

The mechanisms for white matter injury in SCA are unclear. Cerebral hemodynamics are often abnormal, increasing the risk of ischemia in the territories of stenosed large vessels and the borderzones between them.8,9 The risk of tissue injury is also increased by chronic anemia1 and low daytime and nocturnal oxygen saturation.10,11 Low oxygen saturation has been linked not only to lower IQ12 and poorer performance on executive function tasks13 in SCA but also has been reported in children with obstructive sleep apnea experiencing similar nocturnal oxygen desaturation.14 The disruption to breathing, and subsequent oxygen desaturation, has been shown to interfere with synaptic plasticity and consequently various cognitive functions.15 However, in children with SCA,
there are no data on any direct link between brain structure, oxygen saturation, and hemoglobin.

Advanced neuroimaging techniques have shown structural differences between children with SCA and healthy controls. A voxel-based morphometry study showed decreased white matter density in the arterial borderzone distribution corresponding to disease severity, and smaller gray matter volume was found in children with no evidence of SCI but low IQ. There is evidence of cortical thinning in the frontal lobes and posterior medial surfaces of both hemispheres, and volumetric deficits of subcortical structures.

In those patients with SCA without SCI, a complete picture of the extent of white matter injury is still unclear. Diffusion tensor imaging (DTI) provides information about white matter microstructure in normal-appearing white matter on conventional T2-weighted MRI. Fractional anisotropy (FA) represents directional dependence of water diffusion, reflecting coherence, and structural organization of underlying fibers. Other commonly used parameters from DTI are mean diffusivity (MD), axial diffusivity (AD), and radial diffusivity (RD). These parameters reflect the nature of water diffusion changes relating to when there is loss of structure, such as axonal damage or demyelination (ie, degeneration of microstructural cell barriers).

The technique known as tract-based spatial statistics (TBSS) is a whole-brain voxel-wise analysis that registers white matter matter, rather than the whole brain, between subjects resulting in improved registration, reduced partial volume effects, and no smoothing limitations. There are few diffusion data in SCA. TBSS was used to compare 2 groups of children with SCA, some of whom had mild gliosis although none had SCI. Increases in diffusivity and anisotropy reduction in the body of the corpus callosum (CC) were found only in patients with mild gliosis, whereas anisotropy reduction in the centrum semiovale was found in the no-SCI group; however, results were not significant at the 95% confidence level.

A recent study compared patients with SCA (age range: 11–45 years) and a matched control group. This study used various regions of interests across the brain, finding reduced FA in widespread areas. However, a significant proportion of the patient group (6 of 16 patients) had SCI present, a wide age range of patients was not controlled for, and there was no correction for multiple comparisons, potentially augmenting the results. Both studies did not include relationships between white matter and any physiological measures.

The objective of this study was to investigate differences in white matter structural integrity in children with SCA and controls. This study only compared those with SCA and no evidence of abnormalities on T2-weighted MRI to elucidate differences in only the normal-appearing white matter. We hypothesized first that there is structural damage, detectable by using a whole-brain voxel-wise white matter analysis, and second that the degree of damage is related to reduced daytime oxygen saturation and steady-state hemoglobin.

### Materials and Methods

**Participants**

Children and adolescents with SCA aged 8 to 18 years from 3 London sites (Whittington Hospital, North Middlesex Hospital, and Royal London Hospital) were recruited alongside sibling and familial, age- and race-matched controls. Ethical permission was granted by Southampton Research Ethics Committee and fully informed assent/consent was obtained from each participant and his or her parent/guardian.

**Physiological Measures**

From the medical records, peripheral oxygen saturation measurements were collected within 3 years of the date of the MRI scan at regular clinic visits when the patient was generally well, including a measurement taken on the day of MRI during a 5-minute period from a pulse oximeter (Masimo Pronto-7 Pulse CO-Oximeter). Patients had between 3 and 9 measurements (median 4), and the average oxygen saturation was used in subsequent analyses. Steady-state hemoglobin was recorded from the closest available full blood count to MRI date from patient’s medical records.

**Neuropsychological Variables**

A measure of full-scale IQ was obtained using the Wechsler Abbreviated Scale of Intelligence (WASI; 2-subtest), reporting scaled scores (mean of 100, SD of 15). For measures of executive function, 5 tests were acquired from the Delis–Kaplan Executive Functioning System test battery: Tower test (Tower) which measures strategic planning and rule learning, Trailmaking Test Visual Scanning condition (Scanning) which measures visuomotor processing speed/attention, Trailmaking Test number-letter switching condition (switching) which measures cognitive flexibility (ie, multitasking/simultaneous processing), Verbal Fluency letter condition which measures systematic retrieval of lexical items, and color-word interference which measures verbal inhibition. For all Delis–Kaplan Executive Functioning System tests, scaled scores are reported (mean of 10 and SD of 3). The Behavior Rating Inventory of Executive Function (BRIEF)—parent form was obtained for children <16 years, yielding a global executive composite that represents the child’s overall executive function behaviors. BRIEF-scaled scores are reported (mean of 50 and SD of 10), where higher scores represent more dysfunction. Demographics are reported in Table.

**MRI Acquisition**

MR data were acquired in all subjects on a 1.5T Siemens Magnetom Avanto (Siemens, Erlangen, Germany) with 40 mT/m gradients and a 32-channel receive headcoil. For lesion diagnosis, we acquired a T2-weighted turbo spin echo sequence (repetition time=4920 ms; echo time=101 ms, voxel size=0.7x0.6x0.4 mm). Two independent experienced neuroradiologists (S.B. and T.C.S.C.) read all subjects’ T2-weighted MRI blinded to disease status. The DTI protocol consisted of a diffusion-weighted echo planar imaging sequence (repetition time=7300 ms; echo time=81 ms; voxel size=2.5 mm isotropic) with 60 unique gradient directions (b=1000 s/mm²) and 3 interleaved b=0 images.

**DTI Preprocessing**

All scans were visually inspected for abnormalities because of motion, or other artifacts. The DTI data were preprocessed using TractoR version 2.3 and FMRIB Software Library version 5.0. Within each subject, a reference b=0 volume was brain extracted, and diffusion-weighted volumes were registered to this volume to correct for eddy current distortions. A diffusion tensor was derived at each voxel using a standard least-squares process to provide a voxel-wise calculation of FA, MD, AD and RD.

**TBSS Whole-Brain Analysis**

Voxel-wise statistical analysis of FA data was carried out using TBSS. Each subject’s FA image was aligned to every other one and the most representative image was identified as the target image, which was then affine-aligned to Montreal Neurological Institute standard space. All subjects’ FA data were transformed to standard space by combining nonlinear registration to the representative target FA image and affine transform to standard space. A mean FA skeleton (threshold at FA=0.2) was created by restricting to voxels with the highest
Table. Demographics

<table>
<thead>
<tr>
<th></th>
<th>SCA (n=25)</th>
<th>Control (n=14)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HbS genotype</strong></td>
<td>25 HbSS</td>
<td>8 HbAS, 6 HbAA</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td>13.07±2.82</td>
<td>13.71±2.93</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td>13 M/12 F</td>
<td>7 M/7 F</td>
</tr>
<tr>
<td><strong>FSIQ</strong></td>
<td>103.12±11.95</td>
<td>108.29±11.69</td>
</tr>
<tr>
<td><strong>Tower†</strong></td>
<td>9.25±2.71</td>
<td>9.86±2.11</td>
</tr>
<tr>
<td><strong>Scanning</strong></td>
<td>9.60±3.63</td>
<td>10.00±2.88</td>
</tr>
<tr>
<td><strong>Switching†</strong></td>
<td>8.29±3.37</td>
<td>7.93±3.45</td>
</tr>
<tr>
<td><strong>Verbal fluency†</strong></td>
<td>10.29±2.71</td>
<td>10.43±2.90</td>
</tr>
<tr>
<td><strong>CWI</strong></td>
<td>8.76±3.22</td>
<td>8.79±3.07</td>
</tr>
<tr>
<td><strong>BRIEF global executive composite‡</strong></td>
<td>52.18±10.03</td>
<td>51.17±10.73</td>
</tr>
<tr>
<td><strong>Oxygen saturation§</strong></td>
<td>Mean: 96.70%</td>
<td>Mean: 99%</td>
</tr>
<tr>
<td>**Hemoglobin</td>
<td></td>
<td>**</td>
</tr>
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</table>

BRIEF indicates Behavior Rating Inventory of Executive Function (parent form); CWI, D-KEFS color word interference—inhibition/switching; F, female; FSIQ, WASI 2-subtest full-scale intelligence quotient; HbAA, normal hemoglobin; HbAS, sickle cell trait; HbSS, sickle cell anemia; M, male; SCA, sickle cell anemia; Scanning, D-KEFS trailmaking test–visual scanning; Switching, D-KEFS trailmaking test–switching; Tower, Delis–Kaplan Executive Function System (D-KEFS) Tower test; and Verbal fluency, D-KEFS verbal fluency–letter.

†Scores available for 24 of 25 SCA subjects.
‡Scores available for 12 controls and 22 patients.
§Scores available for all 25 SCA subjects.
||Hemoglobin available in all SCA subjects.

FA at the center of the major white matter tracts. Each participant’s FA data were projected onto the mean skeleton to allow for voxel-wise cross-subject statistics. In a similar manner, MD, AD, and RD data were projected onto the skeleton, and these data were used for voxel-wise permutation-based analysis. Age and sex were included as covariates in the TBSS analyses, which included structural comparisons between patient and control groups, and correlations with oxygen saturation and hemoglobin in the patient group only. Results were corrected for multiple comparisons using threshold-free cluster enhancement.

Results

Forty-three patients with homozygous SCA (HbSS) and 18 controls, including 12 siblings, were initially scanned. Thirty-one patients with SCA had no abnormality on T2-weighted MRI, whereas 12 patients with SCA were excluded because of presence of SCI. Ten subjects (6 patients and 4 controls) were excluded because of poor quality DTI data (ie, dental brace artifact, head motion, and poor quality data). The final data set used for DTI analysis included 25 children and adolescents with SCA without SCI and 14 age- and race-matched controls. Figure 1 shows a graphical representation of excluded subjects and Table describes demographics of the final data set.

In the patient group, 2 children were undergoing regular transfusion treatment (abnormal transcranial Doppler ultrasound, top-up for painful crises), 2 additional children had transfusions within 3 months before scan, 3 children were on hydroxy-carbamide treatment, and 17 children were receiving no treatment.

Physiological Measures

Oxygen saturation was available for all patients and 5 controls, and hemoglobin was available for all patients; patients had lower hemoglobin oxygen saturation and hemoglobin than published norms of healthy non-SCA children (Table).25

Neuropsychological Variables

There were no significant differences between the patient and control groups about age, sex, IQ, or any executive function measures (Table 1). Mean IQ and Delis–Kaplan Executive Functioning System scores (except switching) were lower in patients, and BRIEF scores were higher in patients, but these differences were not statistically significant (Figure 2).

In the control group, 8 had sickle cell trait (HbAS) and 6 had normal hemoglobin genotype (HbAA). Between HbAS and HbAA, there was no significant difference in mean IQ score (HbAS=109.6, HbAA=106.5, t=−0.804, P=0.413), mean scanning score (HbAS=9.8, HbAA=10.3, t=−0.318, P=0.725), and mean color-word interference score (HbAS=8.38, HbAA=9.33, t=−0.318, P=0.725). Children with HbAS had significantly better Tower interference score (HbAS=8.38, HbAA=9.33, t=−0.318, P=0.725), and mean color-word interference score (HbAS=8.38, HbAA=9.33, t=−0.318, P=0.725).

Whole-Brain TBSS Comparison of Lesion Negative SCA and Controls

Results of the TBSS analysis comparing patients and controls are shown in Figure 3. After controlling for the effects of age and sex, FA was significantly lower in patients than in controls (P<0.05) in the cerebral peduncles and cerebellum. Widespread increases in MD were found. Across frontal and parietal lobes, genu of the CC, and subcortical structures and cerebellum, patients had significantly higher MD than controls (P<0.05). RD was significantly higher in patients than controls in the frontal and parietal lobes, and bilaterally in the internal capsule, anterior thalamic radiations, corticospinal tract and cerebellum (P<0.05). No significant structural
differences were found between patients and controls for AD (Figure 3). There were no differences between controls with HbAA and those with HbAS. In exploratory regressions comparing FA and diffusivity measures with age, there were no obvious differences in correlations between patients with SCA and controls with age (data not shown).

Whole-Brain TBSS Correlation With Oxygen Saturation and Hemoglobin

Results of the TBSS analysis testing correlations between DTI parameters and oxygen saturation and hemoglobin are shown in Figure 4. In the patient group, a significant negative correlation was found between RD and daytime oxygen saturation in
a cluster in the genu of the CC ($P<0.05$). A trend-level negative correlation was found between RD and hemoglobin in the midbody and posterior parts of the CC ($P<0.1$; Figure 4).

**Discussion**

This is the first diffusion MRI study in children with SCA with no evidence of MRI abnormalities to link brain microstructure with physiological markers of disease severity. Oxygen saturation was found to correlate significantly with RD in the anterior CC and a trend-level correlation between steady-state hemoglobin and RD in the midbody of the CC. This study also confirmed the presence of widespread white matter abnormalities. FA was found to be significantly lower in patients, mainly in cerebellar white matter, and MD and RD significantly higher in patients in widespread regions.

The sample of children with SCA with no SCI showed no differences compared with the control group in terms of IQ and 6 measures of executive function. We can conclude that differences in microstructural white matter were not because of underlying differences in cognitive ability in these domains.

This study is in line with findings of decreased white matter density,\textsuperscript{16} anisotropy reductions and increases in diffusivity in patients with SCA.\textsuperscript{22} This study, however, found more widespread and statistically significant differences between SCA patients with no SCI compared with controls in FA and diffusivity measures.

There were statistically significant differences in RD, but not AD. AD is thought to reflect structural coherence of axons, whereas RD is thought to reflect axon density and membrane permeability.\textsuperscript{26} Reduced myelination can be represented by elevated RD, without a change in AD.\textsuperscript{27} The observed diffusion parameter changes could be because of larger axon diameter, lower packing density, or increased membrane permeability,\textsuperscript{28} although in view of the pathology literature suggesting that SCA is associated with acute demyelination\textsuperscript{29} as well as ischemic stroke, we suggest that axonal loss and demyelination are more likely explanations.

**Link With Oxygen Saturation and Hemoglobin**

Acute silent cerebral ischemic events occur relatively frequently,\textsuperscript{30} particularly in relation to acute, severe anemia,\textsuperscript{31} and may or may not be visible later on clinical sequences. Our data confirm that oxygen desaturation also renders the brain at continual risk for hypoxic-ischemic injury.\textsuperscript{32} In diffusion data, high RD could be because of myelin loss and axonal damage, and is likely to be related to the cycle of hypoxia, endothelial inflammation, and tissue injury in children with SCA without SCI.

In this study, there was a significant negative correlation between oxygen saturation and RD in the anterior CC and a negative trend-level correlation between hemoglobin and RD in the midbody of the CC. Although oxygen saturation and hemoglobin are correlated,\textsuperscript{33} 1 study found that only a small proportion of variance in steady-state oxygen desaturation was explained by chronic anemia.\textsuperscript{34} It is probable that oxygen desaturation has a more vital role than hemoglobin in white matter viability of frontal lobe white matter.

**Neuropsychology**

The results from this sample indicate no significant differences in IQ between children with SCA without SCI compared with controls, or that IQ of patients was outside the normal range, in contrast to previous reports.\textsuperscript{7,35} A meta-analysis\textsuperscript{36} of cognitive studies in SCA showed a small effect size in IQ measures, with patients ≤4 to 5 points lower than controls. Performance IQ, made up from perceptual organization and processing speed and not given by the 2-subtest WASI, has been associated with
cerebral blood flow and basal ganglia volume and may also be associated with white matter integrity. No information about socioeconomic status was available for these subjects, although 10 of 14 controls (71%) were siblings of patients, and all were of the same ethnic background. It is unlikely these factors are so different between patient and control groups to mediate a statistically significant effect on white matter microstructure. White matter microstructure may be indicative of future cognitive difficulties in general measures such as academic achievement, as 1 study reported 27% of children with SCA without SCI experienced poor academic achievement compared with only 6% of healthy siblings. With this sample, future work is needed to investigate academic performance longitudinally to elucidate any mediating effects of oxygen desaturation and white matter microstructure.

Implications for Treatment
This study establishes a link between daytime oxygen saturation and white matter microstructural abnormality, which may be potentially ameliorated with interventions to reduce hypoxic exposure, most practically overnight. These DTI metrics may serve as biomarkers for future randomized controlled trials in SCA to investigate white matter microstructure before and after a period of overnight respiratory support.

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Disclosures
None.

References


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