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RESEARCH ARTICLE

Anemia predicts lower white matter volume and cognitive performance in sickle and non-sickle cell anemia syndrome

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Abstract

Severe chronic anemia is an independent predictor of overt stroke, white matter damage, and cognitive dysfunction in the elderly. Severe anemia also predisposes to white matter strokes in young children, independent of the anemia subtype. We previously demonstrated symmetrically decreased white matter (WM) volumes in patients with sickle cell disease (SCD). In the current study, we investigated whether patients with non-sickle anemia also have lower WM volumes and cognitive dysfunction. Magnetic Resonance Imaging was performed on 52 clinically asymptomatic SCD patients (age = 21.4 ± 7.7; F = 27, M = 25; hemoglobin = 9.6 ± 1.6 g/dL), 26 non-sickle anemic patients (age = 23.9 ± 7.9; F = 14, M = 12; hemoglobin = 10.8 ± 2.5 g/dL) and 40 control subjects (age = 27.7 ± 11.3; F = 28, M = 12; hemoglobin = 13.4 ± 1.3 g/dL). Voxel-wise changes in WM brain volumes were compared to hemoglobin levels to identify brain regions that are vulnerable to anemia. White matter volume was diffusely lower in deep, watershed areas proportionally to anemia severity. After controlling for age, sex, and hemoglobin level, brain volumes were independent of disease. WM volume loss was associated with lower Full Scale Intelligence Quotient (FSIQ; $P = .0048$; $r^2 = .18$) and an abnormal burden of silent cerebral infarctions ($P = .029$) in males, but not in females. Hemoglobin count and cognitive measures were similar between subjects with and without white-matter hyperintensities. The spatial distribution of volume loss suggests chronic hypoxic cerebrovascular injury, despite compensatory hyperemia. Neurocognitive consequences of WM volume changes and silent cerebral infarction were strongly sexually dimorphic. Understanding the possible neurological consequences of chronic anemia may help inform our current clinical practices.

1 | INTRODUCTION

Anemia is the most common blood disorder in the world affecting an estimated 5.6% of Americans and 24.6% of the global population.[1] Anemia is typically defined as a hemoglobin level less than 13.5 g/100 mL in men, and less than 12.0 g/100 mL in women. Some forms are easy to treat once recognized, but many patients with chronic anemia go untreated or partially treated because it is thought to generally be well tolerated. However, anemia has been associated with increased prevalence of stroke and cognitive morbidity.[2-7]

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Anemia increases risk of white matter strokes 1.8-fold in the elderly[5] and worsens the severity of ischemic strokes.[8-10] Since most reports concerning anemia and the brain have been performed in the elderly, there are many confounding factors that limit extrapolation to the general population. Inherited disorders of hemoglobin are among the most common genetic diseases in the world, and can serve as a model for anemia's cerebrovascular impact. Probing how anemia affects cerebrovascular and cognitive health in hemoglobinopathy patients could inform changes in current clinical practice that could have important economic and quality of life implications for many Americans.

In a prior investigation of asymptomatic sickle cell disease (SCD) patients, we demonstrated that the severity of anemia was the strongest predictor of whole-brain white matter volume loss.[11] Surprisingly, white matter volume was independent of well-known markers of SCD severity, including hemoglobin S, fetal hemoglobin, cell-free hemoglobin, lactate dehydrogenase, and the presence of silent cerebral infarctions.[11] Increased radial diffusivity in diffusion MRI, indicative of abnormal white matter microstructure, in the corpus callosum has correlated to hemoglobin level in SCD patients.[12] Additionally, in a longitudinal study of SCD patients, low baseline hemoglobin level was the only independent risk factor for silent cerebral infarcts.[13] Despite compensatory increases in cerebral blood flow,[14,15] deep white matter structures remain hypoxic[16] in SCD patients, proportionally to the severity of anemia.[17] Taken together this suggests that the severity of chronic anemia could be the strongest predictor of hypoxic-ischemia white matter injury in SCD patients.

However, anemia severity in SCD patients is confounded by many other factors. These patients have high circulating products of hemolysis that alter nitric oxide metabolism and produce vascular inflammation.[18,19] Sickle red blood cells are stiff and cause vascular occlusion. Additionally, SCD patients have high rates of lung disease, sleep apnea and abnormal hemoglobin dissociation curves that can cause resting hypoxemia, potentially exacerbating white matter ischemia risk.[20]

Thus, the goal of this study was to determine the relationship between white matter volume and anemia severity in a population with a wide range of hemoglobin values and genetic predisposition to anemia. This includes SCD patients and race-matched controls, as well as a cohort of patients having chronic anemia but normal red blood cell morphology, whom we refer to as anemic-controls. We further compared the severity of volume loss to the presence of silent cerebral infarcts. We also made comparisons to measures of specific cognitive processes, such as working memory, processing speed, and executive functions, as well as to a broad estimate of intellectual functioning. We hypothesize that group differences of WM volume loss could be explained by hemoglobin levels rather than disease state, and would correlate with cognitive performance.

2 | METHODS

2.1 | Participants

A group of 52 clinically asymptomatic SCD patients (age = 21.4 ± 7.7; F = 27, M = 25; hemoglobin = 9.6 ± 1.6), 26 non-sickle anemic patients (age = 23.9 ± 7.9; F = 14, M = 12; hemoglobin = 10.8 ± 2.5) and 40 control subjects (age = 27.7 ± 11.3; F = 28, M = 12; hemoglobin = 13.4 ± 1.3) were recruited. They were part of a larger study on SCD and neuropsychological outcomes at Children's Hospital Los Angeles.

The SCD subjects comprised 40 SS, 7 SC, 4 $S\beta^{0+}$ and 1 $S\beta^{0}$ hemoglobin patterns. Also, 43 patients self-reported as African-American non-Hispanic, and nine as White Hispanic. Since 2000, all pediatric SCD patients at Children's Hospital Los Angeles, have received universal access to transcranial Doppler screening and transfusions, when clinically indicated. In accordance with current NIH guidelines,[21] the Sickle Cell team at Children's Hospital Los Angeles recommends hydroxyurea treatment. That's for all children with SS and $S\beta_0$-thalasemia, after the age of nine months, unless they have been placed on chronic transfusion. The dose is advanced to the maximum tolerated dose according to standard protocol.[21]

Anemic-controls consisted of 12 subjects with β thalassemia major, three Eβ thalassemia, one thalassemia intermedia, three spherocytosis, two hemoglobin H, two hemoglobin H-Constant Spring, one aplastic anemia, one autoimmune hemolytic anemia and one congenital dyserythropoietic anemia type 1. Twelve of the anemic-controls were splenectomized. Anemic-controls were similar in age to SCD patients, but not matched for race and ethnicity: 14 reported as Asian, non-Hispanic, 11 as White non-Hispanic and one reported as mixed race. Groups of 17 anemic-controls and 19 SCD patients were on monthly transfusions, while the rest of the non-transfused SCD patients were prescribed hydroxyurea.

Control subjects were mostly recruited from family members of SCD patients and matched to SCD patients for race and ethnicity. A group of 43 subjects reported as African-American non-Hispanic, and 7 reported as White Hispanic. For 21 of the control subjects, they were identified with sickle cell trait.

The MRI scans, vital signs and blood samples were obtained on the same day for each subject. Exclusion criteria included pregnancy, previous overt stroke, or acute chest or pain crisis hospitalization, within one month. Patients were therefore deemed “clinically asymptomatic.” All subjects were recruited with informed consent or assent; the study was approved by the Institutional Review Board at Children's Hospital Los Angeles (CCI#11-00083). Demographics are reported in Table 1.

2.2 | Participant groups

Subgroups were identified as typical controls, controls with sickle cell trait, anemic-controls, SCD transfused and SCD non-transfused. Total (Section 3.2) and regional analysis (Section 3.4) of white matter volume showed no indications of group differences between typical controls, (HbAA) and sickle cell trait (HbAS). Therefore, subjects were grouped together as control subjects for reporting. The same was true for anemic patients undergoing monthly transfusion and non-transfused patients, so these subjects were also grouped together for reporting.

TABLE 1 Demographics

Description	ACTL	CTL	SCD	ACTL vs SCD	ACTL vs CTL	SCD vs CTL
N	26	40	52			
Sex (F:M)	14:12	28:12	27:25	0.87	0.20	0.083
Age	23.9 (7.9)	27.7 (11.3)	21.4 (7.7)	0.27	0.098	**0.0010**
Transfused	65.4%	0%	36.5%			
Abnormal Burden of SCI	24.0%	26.3%	47.8%	**0.044**	0.85	**0.039**
Hemoglobin (g/dL)	10.8 (2.5)	13.4 (1.3)	9.6 (1.6)	**0.005**	**<0.0001**	**<0.0001**
Hematocrit (%)	32.7 (5.9)	39.5 (3.7)	27.6 (4.2)	**<0.0001**	**<0.0001**	**<0.0001**
White blood cell count ($x10^3$)	7.3 (2.7)	5.6 (1.6)	9.8 (4.3)	**0.002**	**0.040**	**<0.0001**
Platelets	269 (122)	245 (58)	307 (119)	0.51	0.092	**0.006**
Mean platelet volume (fL)	10.6 (.9)	10.5 (0.9)	10.0 (.9)	**0.013**	0.65	**0.013**
Reticulocytes (%)	2.6 (2.8)	1.4 (0.6)	9.5 (5.5)	**<0.0001**	0.22	**<0.0001**
Cell-free hemoglobin	19.8 (20.4)	5.5 (3.7)	19.5 (16.7)	0.93	**0.0003**	**<0.0001**
Lactose dehydrogenase	536 (292)	537 (101)	1020 (532)	**<0.0001**	0.99	**<0.0001**
Absolute neutrophil count	4.2 (1.6)	3.2 (1.4)	5.5 (0.5)	**0.025**	0.11	**<0.0001**
Heart rate (min^{-1})	79.7 (10.8)	74.1 (18.7)	80.0 (12.3)	0.92	0.15	0.078
Systolic blood pressure (mmHg)	111.9 (9.3)	117.3 (12.0)	112.0 (10.8)	0.98	0.068	**0.038**
Diastolic blood pressure (mmHg)	61.8 (8.1)	67.7 (10.2)	60.1 (7.2)	0.40	0.010	0.0002
O_2 Saturation (%)	99.0 (0.9)	99.3 (0.9)	97.7 (2.0)	**0.0004**	0.47	**<0.0001**
Combined family income (20 K*)	2.7 (1.1)	2.9 (1.8)	2.3 (1.1)	0.12	0.73	0.31
Highest grade completed	14.6 (2.5)	13.3 (2.5)	13.7 (1.4)	0.80	0.13	0.13

Note: Mean (standard deviation) of demographic and selected complete blood count measurements. The last three columns report group comparisons using Student's *t*-test. Significant values in bold ($P \leq .05$).

Abbreviations: ACTL, anemic-controls; CTL, controls; SCD, sickle cell disease; SCI, silent cerebral infarct; WMH, white matter hyperintensity.

*Combined family income was reported in step-size of 20 K (ie, 1 = less than $20 000; 2 = $20 000-39 999).

2.3 | MRI acquisition and cognitive assessment

The MRI data were acquired on a 3 T Philips Achieva (v.3.2.1), using an 8-channel head coil. The 3D T1-weighted images (TE = 3.8 ms TR = 8.3 ms; SENSE = 2; resolution = 1 mm^3), and T2-FLAIR images (TE = 2.5 ms; TR = 4.8 ms; resolution = 1.3 × 1.0 × 1.0 mm) were acquired for each subject.

White matter hyperintensities were documented on T2 images by the consensus of a neuroradiologist (BT) and neuroanatomist (SC). White matter hyperintensities were considered silent cerebral infarctions (SCI), if they were greater or equal to 3 mm in diameter in two orthogonal planes.[22] Subjects with more than one silent cerebral infarction per decade of life were considered to have an abnormal burden of SCI.[23] The T2 images from 10 subjects could not be evaluated due to excessive motion. All patients receiving chronic transfusions were studied within one week prior to their scheduled transfusion visit, when their hemoglobin levels were at a nadir.

Of the 118 participants, 104 completed a three- to four-hour battery of standardized psychometric measures. This study utilized results from measures of working memory, executive functions, processing speed, and general intellectual functioning. For patients on chronic blood transfusions, testing was performed within one week of transfusion to minimize fatigue effects. Testing was performed by the study neuropsychologist, or doctoral trainees under her supervision. A brief measure of cognitive ability was assessed with two verbal reasoning (Vocabulary and Similarities) and two nonverbal reasoning (Block Design and Matrix Reasoning) subtests. They yielded index scores for Verbal Comprehension (VCI), Perceptual Reasoning (PRI) and an overall Full Scale Intelligence Quotient (FSIQ) from the Wechsler Abbreviated Scale of Intelligence, Second Edition (WASI-II).[24] Working memory was assessed with Digit Span from the Wechsler Intelligence Scale for Children, Fourth Edition (WISC-IV)[25] or the Wechsler Adult Intelligence Scale, Fourth Edition (WAIS-IV).[26] Processing speed was assessed with Coding and Symbol Search from the WISC-IV or WAIS-IV. Executive functioning was assessed with the Trail Making test of the Delis-Kaplan Executive Function System (D-KEFS),[27] and with the copy portion of the Rey Complex Figure Test.[28] Current family income and highest grade completed were reported either by the subject or the subject's parent when the subject was younger than 18 years old. Highest grade completed was only reported for subjects over 18 years old (Table 1).

2.4 | MRI preprocessing and analysis

The T1-weighted images were processed and analyzed using BrainSuite (brainsuite.org, v17a). Brain extraction was performed by stripping away the skull, scalp and any non-brain tissue from the image. This was followed by tissue classification and surface

generation of the inner and pial cortices. Manual correction was performed on cortical and gray-white boundaries to minimize extraneous inclusion of meninges, or exclusion of cortex and to correct occipito-cerebellar boundaries. The brainstem was cut at the base of the cerebellum as seen on the axial slice. Extracted brain images were registered to the BCI-DNI Brain Atlas (http://brainsuite.org/svreg_atlas_description/) using BrainSuite's Surface-Volume Registration (SVReg17a).

We used tensor-based morphometry (TBM) to explore the brain for significant correlations between hemoglobin value and relative brain shrinkage. The TBM technique measures three dimensional differences in brain volume at the single voxel level, with respect to a reference brain.[29,30] The resulting shape change information can be collapsed in a single normalized Jacobian determinant, or "Deformation Index" that is greater than one. That is if the voxel expanded to fit the reference brain, and less than one if the voxel contracted to fit the reference brain. The TBM technique can be advantageous relative to region of interest statistics, because it is independent of predefined labels and provides spatially finer details. The TBM technique has been previously used to map structural atrophy in Alzheimer Dementia patients in 3D.[30]

Global WM volume were estimated two ways using BrainSuite: First, directly from T1-weight images, BrainSuite uses a partial volume tissue measurement model to calculate fractional measures of gray matter, white matter and cerebro-spinal fluid for each voxel.[31] The WM volume was calculated using this tissue map within a brain mask, that included the cerebrum, brainstem and cerebellum.[11] Second, using tensor-based morphometry (TBM), a mean Deformation Index was calculated across all WM voxels within a predefined binary mask within the cerebrum, cerebellum and brainstem. All voxels within the subcortical nuclei were additionally included. The ventricles, and the cortical ribbon of the cerebrum and cerebellum were excluded. Mathematically, these two indices should be similar, but we compare both as a consistency check (Section 3.2).

2.5 | Statistical analysis

To determine the predictors of global WM volume, simple linear regression and stepwise multivariate regression analysis were performed, using selected laboratory measurements against mean global WM Deformation Index.

Deformation index was used to localize WM volume changes voxel-wise in anemic subjects by performing correlation analysis across hemoglobin level, to determine which regions of the brain were larger or smaller in anemic subjects. Logarithmic transformation[32] was applied to Deformation Index maps, followed by Gaussian smoothing with a radius of 2 mm. After regressing out age (log transformed) and sex, 10 000 random permutations were run to determine a null distribution of correlations at each voxel, to determine the effect of hemoglobin level on Deformation Index. Approximately 2.2 million voxels were tested, and results were controlled for multiple comparisons using Benjamini and Hochberg False Discovery Rate ($\alpha = .1$). Significant voxels were retained, ($P < .05$), then a mean Deformation Index across all significant voxels was computed for each subject. Age, sex, hemoglobin, and mean Deformation Index were used to compute Mahalonobis Distances to detect outliers.[33] A group of 5 subjects with distances above an upper control limit (UCL) of 3.046 were excluded, and the TBM analysis was rerun.

All pairs of group means were compared using Student's-*t* test. Simple linear regressions were performed between mean Deformation Index (controlled for age and sex) with hemoglobin and Full Scale Intelligence Quotient. Linear regressions between mean Deformation Index and each of the cognitive measures were then performed for males and females separately. Multiple comparisons were corrected with FDR.

2.6 | Data sharing statement

For original data, please contact jwood@chla.usc.edu.

3 | RESULTS

3.1 | Demographics

Subject demographic information and clinical measurements are listed in Table 1. More females participated in the study in all groups. Most participants were in their teens and twenties.

Hemoglobin levels were highest in control subjects and lowest in SCD patients. While hemoglobin levels were higher in anemic-controls than in SCD patients, these two groups were well matched with the exclusion of 2 anemic-control subjects with spherocytosis, whose hemoglobin counts were both 17.5 g/dL. Including patients with normal-range and high hemoglobin levels was important in the scope of the study to test the hypothesis that hemoglobin level determined WM volume, therefore the two anemic-control subjects with spherocytosis were included in the analysis.

3.2 | Total white matter

To ensure the consistency of the Deformation Index, we compared mean global WM Deformation Index against global WM volume—region-based brain volumes across the entire cohort ($P < .0001$, $r^2 = .84$). (Section 2.4) After controlling for age and sex, there were no group-wise differences in global white matter volume or the corresponding mean Deformation Index.

To identify predictors of mean global WM Deformation Index, simple linear regression was performed between mean Deformation Index, demographic variables, laboratory values, treatment and presence of an abnormal burden of SCI. The full list of variables are listed in the Supplementary Information in Table S1. Male sex and higher levels of hemoglobin, mean platelet volume and hematocrit were significantly associated with higher mean Deformation Index. (Table S1) Stepwise multivariate regression analysis entered sex (Estimate = −0.052, SE = 0.0069, $P < .0001$) followed by hemoglobin (Estimate = 0.0069, SE = 0.00028, $P = .017$) and mean platelet volume (Estimate = 0.018, SE = 0.0074, $P = .18$) into the model yielding a combine r^2 of 0.42 ($P < .0001$). This analysis was replicated using global WM volume. Sex and hemoglobin were the only significant terms in the model with a combined r^2 of 0.27 ($P < .0001$).

Explorational sub analyses were performed with respect to patient group. Males had significantly higher mean global WM Deformation Index in each group. In anemic-controls, higher levels of hemoglobin, and hematocrit and chronic transfusion were significantly associated with higher mean Deformation Index. In stepwise multivariate regression analysis, sex and hematocrit remained in the model with a combined r^2 of 0.49 (P = .0005). In SCD patients, lower white blood cell count and reticulocyte count were associated with higher mean Deformation Index. Sex and reticulocytes remained in the multivariate model (P < .0001, r^2 = .47). In controls, higher hemoglobin and hematocrit, lower platelet counts, and higher mean platelet volume were associated with higher mean Deformation Index. Sex and mean platelet volume remained in the multivariate model (P < .0001, r^2 = .47).

3.3 | Spatial maps

Hemoglobin had a positive correlation to Deformation Index—lower volumes in proportion to the severity of anemia—diffusely throughout the bilateral frontal, parietal and temporal lobes, while the occipital lobe was relatively spared. Positive correlations were also found in the subcortical regions and, more conservatively, in the brainstem and cerebellum. Eight large clusters (>5000 voxels) were detected in 1) the anterior corpus callosum extending into the frontal lobes, 2) the bilateral putamen nuclei extending into the right internal capsule and thalamus, 3) the bilateral superior parietal gyrus clustered with the postcentral gyrus, paracentral lobule and precuneus, 4) the bilateral anterior temporal pole and 5) the brainstem where the cluster extends from the pons to the inferior cerebellar peduncles (Figure 1). Spatially, the results were generally bilateral and symmetrical. Four small clusters (<750 voxels) were found in the occipital lobe where hemoglobin had a negative correlation to Deformation Index—showing brain expansion in proportion to the severity of anemia.

Mean Deformation Index was larger in males (P < .0001; r^2 = .25), reflecting larger WM volumes. After correcting for sex, mean Deformation Index increased with patient age (P = .045; r^2 = .03). Age and sex corrected mean Deformation Index had similar positive relationship with hemoglobin in both males and females (Figure 2).

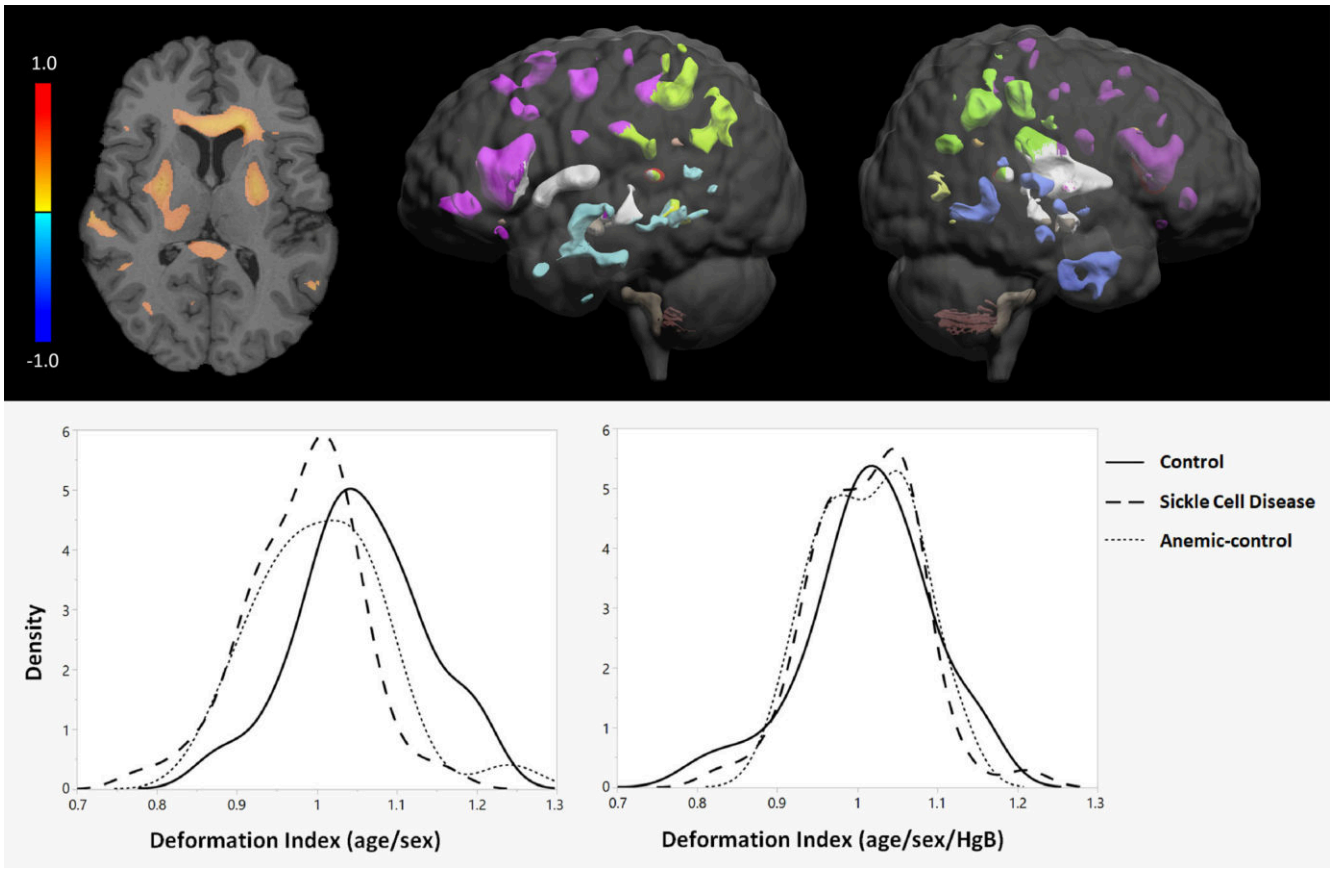

FIGURE 1 Top Row: Anemia is correlated with brain volume in specific WM regions. (Left) R-values for the correlation between Deformation Index and hemoglobin are shown superimposed on the brain atlas template. Red indicates a positive correlation (lower WM with anemia) and blue indicate a negative correlation (higher WM with anemia) as indicated by the colorbar. Only voxels having a P-value ≤.05 (after false discovery rate correction) are shown. (Middle and right) 3D-rendering of left and right hemispheres demonstrating significant voxels colored by region (purple: frontal lobe; green: parietal lobe; blue: temporal lobe; yellow: occipital lobe; white: deep WM and subcortex; red: corpus callosum; light brown: brainstem; dark brown: cerebellum). Bottom Row: Group comparisons of Deformation Index. Histograms of Deformation Indices from regions in top row. (Left) Anemic-controls and SCD patients have lower Deformation Indices than controls. (Right) After controlling for hemoglobin differences, there is complete overlap across the three subject groups [Color figure can be viewed at wileyonlinelibrary.com]

3.4 | Regional group comparisons

Main group analysis (Section 2.2) showed SCD ($P < .0001$) and anemic-controls ($P = .012$) with significantly lower mean Deformation Indices, after controlling for age and sex, in comparison to control subjects. However, SCD and anemic-control patients were not statistically different ($P = .19$). After correcting for hemoglobin level, mean Deformation Index was similar between the three groups (Figure 1).

3.5 | Cognitive correlations

Full scale intelligence quotient (FSIQ) showed significant positive correlations to age and sex corrected mean Deformation Index ($P = .015$; $r^2 = .056$) and hemoglobin ($P = .0093$; $r^2 = .064$). However, these associations demonstrated a strong interaction with sex. In male subjects (N = 42), FSIQ positively correlated to mean Deformation Index and hemoglobin. This association was driven primarily by one of FSIQ's four subtests, called Matrix Reasoning. In female subjects (N = 62), neither mean Deformation Index nor hemoglobin correlated with FSIQ or its subtests (Table 2).

3.6 | Silent cerebral infarcts

The SCD patients had a higher proportion of subjects identified with an abnormal burden of SCI (47.8%) than anemic-controls (24.0%, $P = .044$ and control subjects (26.3%, $P = .039$) (Table 1). For 24 of 61 total female subjects (39.3%), and 14 of 48 total male subjects (29.2%), had an abnormal burden of SCI ($P = .27$). The presence of an abnormal burden of SCI predicted low mean Deformation Index (controlled for age) in male subjects ($P = .029$), but not female subjects ($P = .78$). These group differences could not be detected using mean global WM Deformation Index. Mean age, hemoglobin, FSIQ and Matrix Reasoning were not different between those with normal and an abnormal burden of SCI, regardless of sex.

4 | DISCUSSION

Lower hemoglobin level was associated with regional decreases in white matter volume throughout the brain, in patients with sickle cell disease, anemic-control and typical control subjects. Our study is novel in showing that the severity of anemia, rather than disease state, predicted lower WM volumes independent of red blood cell morphology. Decreased nonverbal intellectual functioning was associated with lower white matter volumes and hemoglobin levels, but only in males, suggesting sex differences in the response to brain injury. Additionally, lower WM volumes were found in males with an abnormal burden of silent cerebral infarctions.

4.1 | Spatial pattern of WM susceptibility to anemia

Deformation Index was positively correlated with hemoglobin in the frontal, parietal and temporal lobes but not in the occipital lobe. This finding aligned with reported regional WM volume loss,[11] silent stroke distribution,[34-37] and decreased white matter integrity in SCD

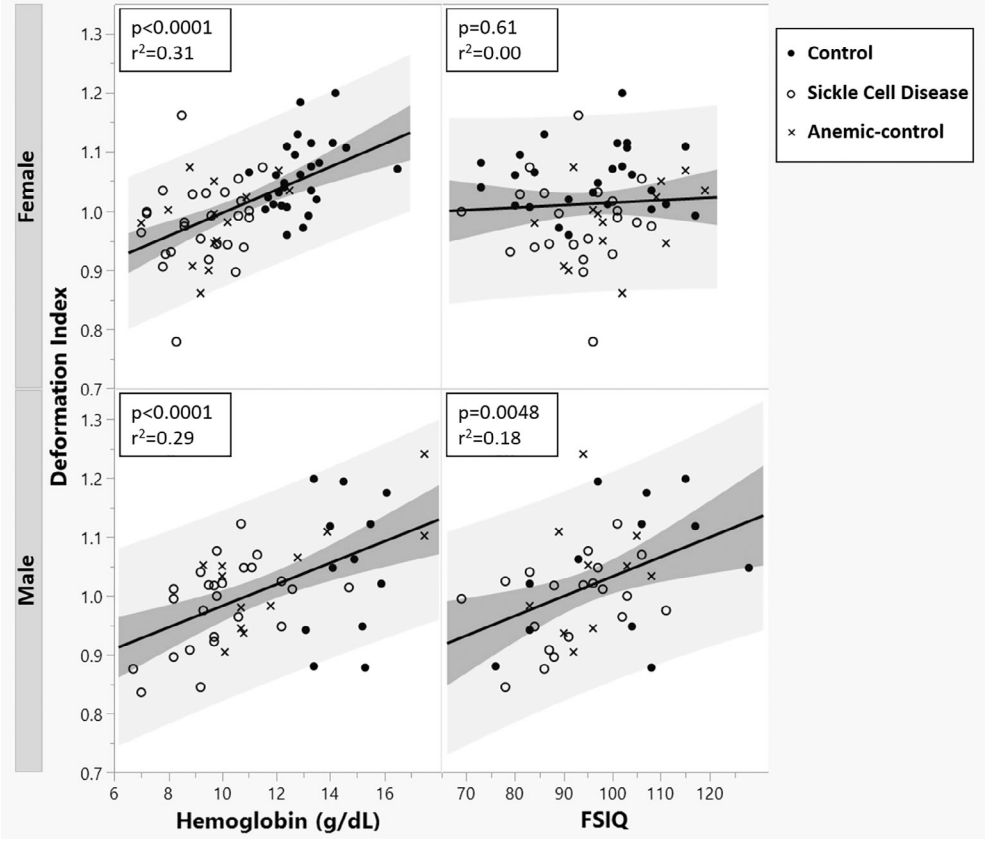

FIGURE 2 Associations between Deformation Index, hemoglobin level and FSIQ. Linear regression between mean Deformation Index, from the regions in Figure 1, and hemoglobin (left column) and Full-Scale Intelligence Quotient (FSIQ, right column). Females are shown in the top row and males in the bottom row. Solid lines represent the best linear fit, dark and light shaded area delimits the 95% confidence intervals for the regression line and for individual values respectively. Data points are labeled by disease state

TABLE 2 Simple linear regression of cognitive assessment against average Deformation Index, age regressed, by sex

	Female			Male		
	P value	adjusted	r^2	*P* value	adjusted	r^2
FSIQ	.61	0.78	0.004	**.005**	**0.038**	**0.18**
VCI	.69	0.77	0.003	.052	0.25	0.093
PRI	.65	0.78	0.003	**.005**	**0.038**	**0.19**
Vocabulary	.46	0.70	0.009	**.023**	0.14	0.12
Similarities	.93	0.93	<0.001	.18	0.42	0.045
Block design	.49	0.70	0.008	.11	0.39	0.062
Matrix Reasoning	.92	0.93	<0.001	**.001**	**0.029**	**0.24**
Digit Span	.19	0.42	0.028	.16	0.42	0.049
Coding	.35	0.60	0.014	.57	0.76	0.007
Symbol Search	.50	0.70	0.007	.30	0.56	0.030
RCFT Copy	.10	0.39	0.045	.330	0.60	0.024
Trail-Making	.90	0.93	<0.001	.14	0.41	0.055

Note: Pearson's correlation unadjusted and adjusted (FDR corrected) *P*-values are reported. Significant results are emphasized in bold text ($P \leq .05$).
Abbreviations: FSIQ, Full Scale Intelligence Quotient; PRI, Perceptual reasoning Index; RCFT, Rey Complex Figure Test; VCI, Verbal Comprehension Index.

patients.[12,38-40] More specifically, regions susceptible to anemia were generally restricted to the bilateral deep white matter of watershed regions, and the territory of the middle cerebral artery.

Bilateral white matter susceptibility follows a vascular distribution that more closely aligns with patterns of chronic, global cerebral hypoxia, rather than acute, localized hypoxia. Chronic hypoperfusion caused by the narrowing of the carotid arteries, in both human patients and animal models, shows selective injury to oligodendrocytes in white matter tissue in-between the distal ends of penetrating arteries.[41-43] The spatial patterns of injury following global hypoxia occurs diffusely within watershed regions, and has a relatively symmetrical appearance across brain hemispheres.[44] In contrast, patients identified with ischemic damage in the cortical border zone will more frequently be observed with localized cortical infarcts, as in embolic stroke.[45]

The brain tightly regulates oxygen delivery, and over 98% of oxygen is transported by hemoglobin. Lower hemoglobin levels in anemic patients effectively decrease their oxygen carrying capacity, which is exacerbated by dyshemoglobins in SCD and anemic-control groups. The brain compensates by elevating baseline cerebral blood flow, in attempt to maintain normal global oxygen delivery at rest.[14,46] However, while oxygen delivery to gray matter appears to be preserved, deep white matter structures are relatively hypoperfused[17] and ischemic.[16] In addition, elevated baseline cerebral blood flow consequently lowers cerebral vascular reserve,[9,14,47] and leaves patients more susceptible to ischemic brain injury under metabolic stress.[48] Lower cerebral vascular reserve predicts white matter hyperintensities cross-sectionally and longitudinally, presumably through acute-on-chronic interruptions in oxygen delivery.[49] Additionally, low extraction reserve has been found in patients with high cerebral blood flow.[50] This is possibly attributed to decreased diffusion time,[51] capillary transit time heterogeneity,[52] and/or artereo-vascular shunting in capillary beds.[50] All of this lowers sufficient time for normal gas and nutrient exchange. Furthermore, elevated brain iron deposition correlates with low hemoglobin level, and presence of SCI. But, this is independent to transfusional iron overload in adults with SCD suggestive of chronic cerebral hypoxic exposure.[53] The model of lower cerebrovascular reserve and/or elevated cerebral blood flow, may help explain the pathophysiology of chronic anemia's effect on the brain.

4.2 | Anemia and WM injury

Lower WM volume was detected in proportion to the severity of anemia, independent of the type of anemia. Patients with non-sickle anemia are not commonly thought to be at risk for neurological injury, however, our analysis showed complete overlap between SCD and non-sickle anemic patients. Much like patients with SCD,[54,55] thalassemia patients are at risk for silent strokes,[56] cerebrovascular disease, and cognitive decline.[56-58] Studies on sickle cell mice have reported increased hypoxic injury thought to be specific to the unique biology of sickle red blood cells.[59] In this current analysis, however, we observe that the severity of anemia is an equally important determinant of WM volume in patients with and without HbS, and regardless of treatment type (Section 3.2).

Based on our current findings, we suggest that a patient's ability to maintain hemoglobin level serves as a good biomarker of WM risk. We speculate that our findings may be extended to chronically anemic patients without genetic hematologic disorders. We found the effects of anemia, despite varying disease states and treatment courses, was a strong predictor of structural outcome. We recognize that various other factors could have contributed to a patient's decline, and further exploration will be required to tease out the nuances of specific disease pathology.

While white matter volumes were decreased in proportion to anemia severity, there was no progressive decrease in Deformation Index with age. This observation is in stark contrast to the published rise in SCI prevalence with age,[55,60,61] as well as progressive volume loss described in school age children with SCD, from the silent infarct transfusion (SIT) trial.[62] We hypothesize that the underlying white matter volume changes we observed may have primarily occurred during the first decade of life, where cerebrovascular demand is highest.[63,64] Our study population was biased toward young adults and might be underpowered to detect an age effect, particularly if volume loss was greatest in the first decade of life. On average, the prevalence of overt stroke and silent cerebral infarct peak at 6-10 years of age in SCD patients.[34,65,66] Additionally, acute anemic events has been identified as risk factors for children with, and without, SCD.[67] Colocalized tissue atrophy,[37,68] and elevated oxygen extraction,[16] in deep-white matter watershed regions, have been reported in children with SCD, even in the absence of white matter hyperintensities. We hypothesize that maintaining a high hemoglobin count in patients with hemoglobinopathies during school age years will help preserve white matter volumes, although a longitudinal study is necessary to confirm this hypothesis.

4.3 | Cognitive outcomes

Lower WM volumes were associated with FSIQ and Perceptual Reasoning Index which was driven by Matrix Reasoning, a measure of fluid reasoning, which involves an extensive bilateral frontoparietal reasoning network.[69,70] Both the splenium and genu of the corpus callosum shrank in anemic subjects. Parall findings by Schatz et al demonstrated that SCD patients exhibit smaller corpus callosum size, related to lesion volume and decline in a number of cognitive test scores.[71] Our analysis revealed that lower white matter related to both lower fluid reasoning and the presence of SCI, although only in males. While the current analysis did not show a direct relationship between SCI and lower Matrix Reasoning scores, this may indicate that white matter shrinkage may be an earlier or more sensitive marker for predicting than SCI. The SCD patients frequently present with cognitive deficits in comparison to healthy controls, even without indication of neurological damage by current clinical standards.[36,72,73] Therefore, exploring alternative markers of cognitive deficit may become a useful diagnostic tool for patient care.

4.4 | Sex differences

We found similar SCI frequency and correlation between WM volume and hemoglobin in men and women, however SCI only correlated to cognitive performance in men. For men, WM volumes were able to account for roughly 18% of the variability in FSIQ, and 24% of Matrix Reasoning. Our laboratory has previously shown that females with SCD had more profound changes in their resting state connectivity than males in the presence of anemia and SCI.[74] Taken together with our current findings, we suggest that female patients may exhibit favorable remodeling on a microstructural level in response to injury that ameliorates cognitive impairment.

Studies in rodent models with early, prepubescent traumatic brain injuries have revealed sexually dimorphic remodeling that persists into adulthood,[75,76] possibly due to differences in brain maturation trajectories.[77] The onset of WM volume changes may be occurring outside of the critical period in female subjects allowing appropriate compensation for early damage.[78,79] In addition, estrogen and progestogen in females play a critical neuroprotective function in response to stroke, chronic hypoxia and traumatic brain injury in adulthood.[80,81] Stroke epidemiology indicates that first stroke occur earlier and are more common in men worldwide.[82] Men with SCD have lower life expectancies and higher rates of silent strokes than women with SCD.[83] Female anemic patients may have an innate ability to compensate for hypoxic WM damage across the lifespan. Understanding the sexually dimorphic responses to hypoxic white matter damage may be valuable for future clinical applications.

4.5 | Limitations

The Deformation Index represents a cross-sectional morphological analysis required to map a study subject onto a reference template. Although we controlled for the known differences introduced by age and sex, we cannot conclude that lower Deformation Indices in anemic subjects reflect brain shrinkage; longitudinal studies would be required to make that conclusion. However, the systematic relationship of Deformation Index with hemoglobin, the spatial co-localization with known watershed areas and concordance with other neuroimaging markedly increase the plausibility of causality.

Our study had a limited number of anemic-controls, all of whom did not have the same diagnosis. While we did test for and did not find evidence that their diagnosis had an effect within the scope of our study, we do recognize that we cannot discount other effects of specific disease pathology due to our lack of power. Including anemic-controls, however, was valuable to give us insights on how anemia alone may commonly affect the brain across disease states. Hopefully, that will stimulate future studies on the topic of the neurological consequences of hemoglobinopathies.

4.6 | Conclusion

White matter volume was proportional to anemia severity diffusely across the brain in chronically anemic subjects. Lower white matter volume with anemia correlated with cognitive performance and white-matter hyperintensities in males, but not females, indicating a sexually dimorphic response to chronic anemia. The overlap in findings between SCD and anemic-control groups, suggest that the neurological injury patterns commonly found in SCD patients are primarily due to their low hemoglobin levels, rather than abnormal red blood cell morphology. Understanding the possible neurological consequences of chronic anemia may help inform our clinical practices.

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AUTHOR CONTRIBUTIONS

S.C., J.W. and R.M.L. designed this study. S.C., A.A.J. and A.J.L. contributed analytical tools, performed statistical analysis and analyzed data. A.M.B., J.C.W. and S.H.O. collected the data. S.C., J.C.W. and S.H.O. interpreted the data. S.C., S.H.O., A.A.J., A.M.B., T.D.C., R.M.L. and J.C.W. prepared the manuscript.

CONFLICT OF INTEREST

No competing financial interests.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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