frontiers | Research Topics

Sickle cell disease

Edited by
Fenella Jane Kirkham, Andria Ford, IkeOluwa Lagunju
and Manoelle Kossorotoff

Published in
Frontiers in Neurology

ISSN 1664-8714
ISBN 978-2-8325-3600-1
DOI 10.3389/978-2-8325-3600-1

About Frontiers

Frontiers is more than just an open access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers journal series

The Frontiers journal series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the *Frontiers journal series* operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews. Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the *Frontiers journals series*: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area.

Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers editorial office: frontiersin.org/about/contact

Sickle cell disease

Topic editors

Fenella Jane Kirkham — University College London, United Kingdom
Andria Ford — Washington University in St. Louis, United States
IkeOluwa Lagunju — University of Ibadan, Nigeria
Manoelle Kossorotoff — Assistance Publique Hopitaux De Paris, France

Citation

Kirkham, F. J., Ford, A., Lagunju, I., Kossorotoff, M., eds. (2024). *Sickle cell disease*. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-8325-3600-1

Table of contents

frontiers in Neurology

SYSTEMATIC REVIEW
published: 13 December 2021
doi: 10.3389/fneur.2021.786065

Academic Performance of Children With Sickle Cell Disease in the United States: A Meta-Analysis

Andrew M. Heitzer[1], Latacha Hamilton[2], Claire Stafford[3], Jeffrey Gossett[4], Lara Ouellette[5], Ana Trpchevska[1], Allison A. King[6], Guolian Kang[4] and Jane S. Hankins[7]*

[1] Psychology, St. Jude Children's Research Hospital, Memphis, TN, United States, [2] School Program, St. Jude Children's Research Hospital, Memphis, TN, United States, [3] Psychology, Nova Southeastern College of Psychology, Fort Lauderdale, FL, United States, [4] Biostatistics, St. Jude Children's Research Hospital, Memphis, TN, United States, [5] Health Sciences Resource Center, Texas Medical Center Library, Houston, TX, United States, [6] Program in Occupational Therapy and Departments of Pediatrics and Medicine, Washington University, St. Louis, MO, United States, [7] Hematology, St. Jude Children's Research Hospital, Memphis, TN, United States

Background: Students with sickle cell disease are at risk for poor academic performance due to the combined and/or interactive effects of environmental, psychosocial, and disease-specific factors. Poor academic performance has significant social and health consequences.

Objective: To study academic achievement and attainment in children with sickle cell disease in the United States.

Design: Medline, Embase, SCOPUS, CINAHL, ERIC, and PsycINFO were searched for peer-reviewed articles. Studies of children (ages 5–18) diagnosed with sickle cell disease of any genotype reporting academic achievement (standardized tests of reading, math, and spelling) or attainment (grade retention or special education) outcomes were included. Outcomes were analyzed using a random effects model. Achievement scores were compared to within study controls or normative expectations. Prevalence of grade retention and special education services were compared to national (United States) estimates for Black students. Age at assessment and overall IQ were evaluated separately for association with reading and mathematics scores. Subgroup analyses of reading and math scores were analyzed by cerebral infarct status (no cerebrovascular accident, silent infarct, stroke).

Results: There were 44 eligible studies. Students with sickle cell disease scored 0.70, 0.87, and 0.80 ($p < 0.001$) SD below normative expectations on measures of reading, mathematics, and spelling, respectively. Compared to unaffected sibling and/or healthy controls ($k = 8$, $n = 508$), reading and math scores were 0.40 ($p = 0.017$) and 0.36 ($p = 0.033$) SD below expectations. Grade retention was approximately 10 times higher in students with sickle cell disease than Black students nationally. Intellectual functioning explained 97.3 and 85.8% of the variance in reading and mathematics performance, respectively ($p < 0.001$). Subgroup analyses revealed significant differences in reading ($p = 0.034$) and mathematics ($p < 0.001$) based on infarct status, with lower performance associated with presence of a silent infarct or stroke.

OPEN ACCESS

Edited by:
Fenella Jane Kirkham, University College London, United Kingdom

Reviewed by:
Alexandra Economou, National and Kapodistrian University of Athens, Greece
Lauren Allegra Beslow, Children's Hospital of Philadelphia, United States

****Correspondence:***
Andrew M. Heitzer
aheitzer@stjude.org

Specialty section:
This article was submitted to Stroke, a section of the journal Frontiers in Neurology

Received: *29 September 2021*
Accepted: *17 November 2021*
Published: *13 December 2021*

Citation:
Heitzer AM, Hamilton L, Stafford C, Gossett J, Ouellette L, Trpchevska A, King AA, Kang G and Hankins JS (2021) Academic Performance of Children With Sickle Cell Disease in the United States: A Meta-Analysis. Front. Neurol. 12:786065. doi: 10.3389/fneur.2021.786065

Conclusion: Students with sickle cell disease demonstrate notable academic difficulties and are at high risk for grade retainment. Development of academic interventions and increased access to school support services are needed for this vulnerable population.

Systematic Review Registration: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42020179062.

Keywords: sickle cell, anemia, academic performance, neurocognitive, education, stroke, silent infarct, grade retention

INTRODUCTION

Rationale

Students with sickle cell disease (SCD) are at risk for poor academic performance (1, 2). These academic difficulties have a substantial effect on quality of life and potential for future income. Approximately 30% of students with SCD do not graduate high school (3). These students with limited academic attainment are at much higher risk for unemployment (4) and have a significantly greater frequency of acute care hospitalizations (5). Thus, it is important to understand the extent of these academic difficulties in the SCD population to potentially intervene and prevent future academic failure.

SCD is one of the most common genetic hemoglobin disorders, occurring in approximately one in 400–500 African Americans in the United States (6). SCD is caused by a single point mutation that generates the abnormal hemoglobin S (HbS). The HbS altered behavior leads to red blood cell adhesion and vaso-occlusion, blocking oxygen and blood flow to vital organs and bones. This blockage results in episodic pain and may lead to cerebrovascular injuries, including silent cerebral infarcts (SCI) and overt strokes (7). Students with SCD often require frequent hospital admissions due to pain crises and other acute complications (8) and display high levels of fatigue (9).

An extensive literature has demonstrated the combined and/or interactive effects of environmental (10, 11), psychosocial (8, 12), and disease-specific (13, 14) factors on cognitive functioning in SCD, yet much less is known regarding academic performance. Academic and cognitive performance share many of the same risk factors (e.g., cerebrovascular injury, chronic anemia, socioeconomic status); however, they are unique constructs. In the general population, environmental factors account for a greater amount of variance in academic performance than cognitive ability (15, 16). This is particularly relevant for students with SCD, as they are more likely to be raised in single-parent families, live in low-income resource-poor neighborhoods, and attend financially disadvantaged school systems (17). Students with SCD miss an average of 20–40 school days per year due to acute pain, fatigue, and frequent outpatient visits (18), which are associated with poor academic performance and grade retention (19, 20). Social and behavioral difficulties (21, 22) observed in SCD (e.g., anxiety, depression, limited sleep) may further interfere with functioning in the school environment.

To assess academic performance, it is important to consider both academic knowledge (e.g., test achievement measures) and attainment (e.g., history of retention, graduation rates). Academic achievement skills are necessary for performing well in school but are not sufficient (23). Executive dysfunction (e.g., attention deficit) and hospital visits due to acute pain can interfere with school performance among students with age-appropriate achievement skills. For example, a student may possess the knowledge to perform well on a test or complete a homework assignment, yet they are unable to adequately display their knowledge as they cannot focus throughout the exam or cannot complete the assessment test in the allotted time. This distinction is highly relevant for intervention. Students with executive difficulties are likely to benefit from behavioral therapy or organizational support, whereas those with knowledge deficits require remediation and/or tutoring in subject area content.

There exists a range of federal, state, and local laws, regulations, and systems for special education and related services for children and adolescents with SCD. In brief, special education services are mandated through federal and state law under the Individuals with Disabilities Education Act (IDEA), typically taking the form of an Individualized Education Plan (IEP). If a student does not qualify for an IEP, related services are justified through Section 504 of the Rehabilitation Act of 1973, which prohibits discrimination based on disability within federal and federally assisted programs. The definition of a disability under Section 504 is much broader than in an IEP and often includes children with medical conditions such as SCD with no observed learning/cognitive deficits (24). In practice, the most important distinction between Section 504 and an IEP is that Section 504 is intended to eliminate barriers for students with disabilities whereas an IEP is remedial and often requiring the provision of programs and services (25).

To our knowledge, there have been five meta-analyses that have assessed cognitive deficits in individuals with sickle cell disease (26–30), however; none of these studies included academic achievement measures or academic attainment outcomes. The most recent, and comprehensive, meta-analysis conducted by Prussien et al. (26, 27), demonstrated deficits in cognitive functioning across several domains. They also reported a gradient of severity, with the most severe cognitive deficits observed in individuals with stroke, followed by SCI, and no cerebral vascular injury. Unlike previous meta-analyses, Prussien and colleagues compared SCD patients to normative expectations in addition to sibling or healthy controls. This comparison allowed for further consideration of sociodemographic factors effects on outcomes that are not fully captured when compared to individuals of similar socioeconomic status. Our objective is to quantitatively review academic outcomes in SCD, addressing both academic achievement and attainment, domains which

have yet to be reviewed in SCD. We hypothesized that students with SCD would perform below normative expectations and/or controls across measures of academic performance. We further hypothesized that worse academic achievement performance would be associated with increased age, reduced intelligence scores, and presence of a cerebral infarct.

Objectives

The primary focus of this meta-analytic review is to provide the first comprehensive quantitative analysis of academic achievement and attainment in SCD. Thus, our first objective was to assess reading, arithmetic, and spelling achievement skills as well as rates of grade retention and special education within the school environment in children with SCD. We compared reading, arithmetic and spelling academic outcomes to both normative expectations and sibling or healthy controls. Secondly, we aimed to evaluate if age at assessment and intellectual functioning moderated academic performance. Finally, we examined if academic achievement outcomes differed by cerebral infarct status.

METHODS

To increase transparency and reproducibility, our review complies with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) reporting guidelines.

Protocol Registration

Prior to starting the review, we developed our study protocol, which was published in PROSPERO, an international database of prospectively registered systematic reviews, on 5/7/2020, and assigned the identifier: CRD42020179062.

Eligibility Criteria

Inclusion criteria for study selection were (1) the study concerned individuals with SCD of any genotype; (2) age at academic assessment was between 5 and 18 years of age; (3) academic performance was assessed using standardized tests with reliability and validity statistics; (4) academic attainment was assessed via grade retention or special education services (e.g., Section 504 Plan, Individualized Education Plan); (5) publication was in a peer-reviewed journal as a full manuscript, (6) and publication was in English and concerned students in the United States. Studies were excluded if (1) the average age of the sample was $>$ 18.0 years; (2) only questionnaire-based assessments of academic achievement or grades were reported; (3) and publication involved intervention for cognitive or academic performance. If pre-intervention measures were reported, however, only these were included (not post-intervention) in the meta-analysis.

Information Sources and Search Strategy

We searched the following electronic bibliographic databases: Medline (Ovid) 1946–2020, Embase (embase.com) 1974–2020, SCOPUS (2004–2020), CINAHL (1937–2020), ERIC 1966–2020 (eric.ed.gov), and PsycINFO (APA) 1967-2020. A medical librarian (L.O.) developed the primary search in Medline, with three main concepts: (1) sickle cell, (2) academic performance and achievement, and (3) children. Each concept was developed using both controlled and natural languages. MeSH terms were identified, and keywords were gathered along with various synonyms. The keywords were searched using the title, abstract, and keyword fields within the Medline OVID database before being translated to other databases. The final Medline search strategy is found in our published protocol in PROSPERO (refer to **Supplementary Table 1**). Citation management and duplicate removal was accomplished with EndNote (Clarivate Analytics).

Study Selection

All titles and abstracts of studies were independently screened by two reviewers (A.H. and L.H.) to identify studies meeting the inclusion criteria. Discrepancies were resolved through discussion or through an arbitrator (J.H.). Covidence (www.Covidence.org) (31) was used to screen and review studies.

Data Extraction and Coding

Data from the final selection of eligible articles were independently extracted by two review authors (A.H. and L.H.) using a standardized template. Study data included: number and age of subjects, sex, disease genotype, cerebral infarct status (SCI, stroke, or none), and type of control subject (healthy, sibling, or none). Academic performance variables included: reading, arithmetic, and spelling performance. Reading performance incorporated measures of basic word reading and reading comprehension. Arithmetic performance was based on measures of arithmetic calculation or applied mathematics. Spelling performance was based on a single subtest in all studies. Composite scores were computed for studies that reported multiple arithmetic or reading subtests based on the weighted average of each subtest. A list of academic achievement measures used is provided in **Supplementary Table 1**. All performance data were converted to an age-standardized score with a mean of 100 and a standard deviation (SD) of 15.

For special education and grade retention, percentages of SCD and control children receiving any form of special assistance or requiring grade retention were collected. Descriptions of special education assistance by study are provided in the **Supplementary Material**. Due to extensive variability in how special education was defined and availability of normative data, only IEP status was examined in analyses. Normative data for IEP services (IDEA Part B) and grade retention were extracted from U.S. national datasets. Grade retention normative values were based on the U.S. Department of Commerce, Census Bureau, Current Population Survey average retention rates for Black students (K-12) from 1994 to 2016 (32). Normative values for IEP services (IDEA Part B) were based on U.S. Department of Education, Office of Special Education Programs, Individuals with Disabilities Education Act (IDEA) database for the average percentage of Black students (3–21 years) receiving services through IDEA Part B from 2000 to 2019 (33).

Study Quality Assessment

Two review authors (A.H. and L.H.) independently assessed study quality in chosen studies. A version of the National Institutes of Health Quality Assessment Tool for Observational

Cohort and Cross-Sectional Studies adapted by Prussien et al. (26) was utilized. Studies received one point for each criterion met, for a total score of 0–6 (higher values indicate higher study quality). Information about quality ratings for included studies are depicted in **Supplementary Table 2**. Study quality ranged 3–6 (Mean = 4.3, SD = 0.9).

Statistical Analyses

For studies based on the same sample or a subset of a sample, only the study with the largest available sample was included. Cochran's *Q*-value, τ^2 and/or the I^2 value were used to quantify heterogeneity. For example, the amount of heterogeneity (i.e., τ^2), was estimated using the restricted maximum-likelihood estimator. The Q-value was used to test whether differences in studies are due to systemic differences or to chance alone. The I^2 value assesses the percentage of inter-study variability that can be attributed to heterogeneity. Forest plots were used to display study results, effect sizes, measures of heterogeneity and statistical tests.

Reading, mathematics, and spelling outcomes were compared to normative expectations and controls. The normative comparisons were based on comparisons to a normal distribution with a mean of 100 and a standard deviation of 15. For each study and outcome, *z*-scores were constructed by subtracting 100 from each study mean and dividing by the resulting value by the standard deviation of 15. Study standard deviations were transformed by dividing by the normative standard deviation of 15. For studies with a sample of appropriate controls, patients with SCD were compared to controls. The Hedges' g was used as an estimate of the effect size. Standardized differences were calculated by multiplying Hedges' g by SD = 15.

Age at assessment and overall IQ were evaluated separately for association with reading and mathematics outcomes. Age and IQ were entered as linear predictors for reading and math composite scores. The pseudo *R*-squared value was computed as percent reduction in estimated τ^2 [amount of heterogeneity as estimated based on a random-effects model vs. the amount of (residual) heterogeneity as estimated based on the mixed-effects meta-regression model with predictors].

Pooled estimates of the proportions using services of Special education, 504, and IEP services and grade retention were estimated using random effects models. Heterogeneity of studies was also evaluated. For IEP's and grade retention, normative data were available, and comparisons were made via odds-ratios. Normative values for IEP services (IDEA Part B) were based on the average percentage of African-American students (3–21 years) receiving services through IDEA Part B from 2000 to 2019. Retention normative value of 3.8% based on average counts per year from 1994 to 2016: (285,749/752,462). IEP normative value of 15.6% based on average counts per year from 2000 to 2019: (1,261,827/8,068,662).

Subgroup analyses of assessments of reading and math composite scores were analyzed by cerebral infarct status. Within group heterogeneity and between group variation parameters were quantified using mixed-effects meta regression models.

Thus, for each outcome of interest either a random-effects model or mixed-effects (when moderator was present) was fitted to the data for each outcome. The amount of heterogeneity (i.e., τ^2), was estimated using the restricted maximum-likelihood estimator (34). The *Q*-test for heterogeneity (35) and the I^2 statistic (36) were evaluated. Studentized residuals and Cook's distances were used to identify possible outliers and/or influential studies (37). The regression test (38) was used to evaluate funnel plot asymmetry. The analysis was carried out using R (version 4.1.0) (R Core Team, 2020) and the metafor package (version 3.0.2) (39).

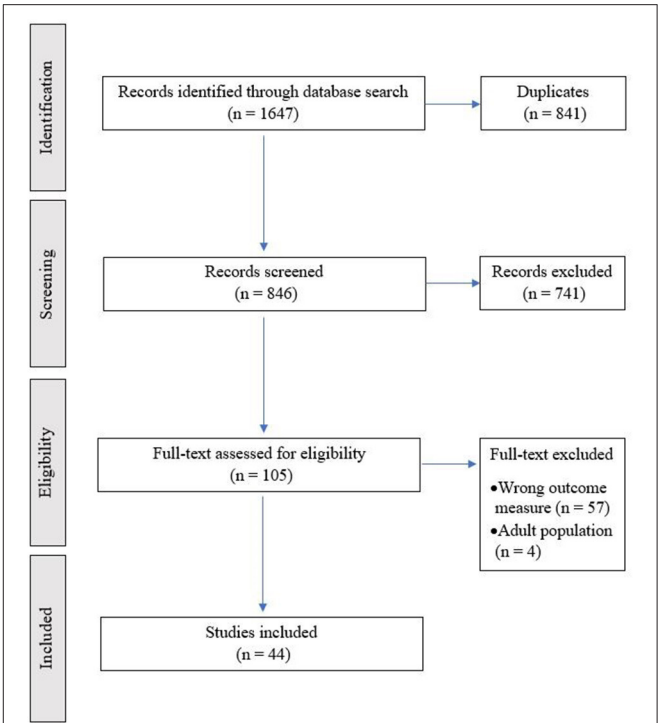


FIGURE 1 | PRISMA flow chart of study. All titles and abstracts of studies were independently screened by two reviewers (A.H. and L.H.) to identify studies meeting the inclusion criteria. Discrepancies were resolved through discussion or through an arbitrator (J.H.). Covidence (www.Covidence.org) was used to screen and review studies.

RESULTS

A total of 846 abstracts were screened for inclusion (see **Figure 1**). A total of 105 studies met initial screening criteria. Of those, 57 studies were removed for not reporting outcomes of interest (e.g., only measures of cognition or no academic attainment outcomes reported) and 4 studies were excluded for having an adult population (i.e., average age over 18). A final sample of 44 studies (total of 3,971 children with SCD) met criteria (**Figure 1**). Overlapping samples are displayed in **Supplementary Table 2**.

Reading, mathematics, and spelling outcomes were assessed via performance measures in 20, 18, and 7 unique studies, respectively (**Table 1**). Of these, 8 studies assessing reading and math performance included a control group of patients

TABLE 1 | Reading, mathematics, and spelling outcomes compared to normative expectations and controls.

	SCD vs. Normative expectations						SCD vs. Controls					
Outcome	***k***	***n***	**ΔSS**	***g***	**SE *g***	***p*-value**	***k***	***n***	**ΔSS**	***g***	**SE *g***	***p*-value**
Reading	20	1,520	−10.6	−0.70	0.08	**<0.001**	8	508	−6.0	−0.40	0.17	**0.017**
Math	18	1,369	−13.1	−0.87	0.10	**<0.001**	8	508	−5.4	−0.36	0.17	**0.033**
Spelling	7	279	−12.0	−0.80	0.17	**<0.001**	–	–	–	–	–	–

SCD, sickle cell disease; normative expectations, normative values based on sample used to create performance measures; controls, siblings or demographically matched controls (varies by study); k, number of unique studies included in analyses; n, total sample size from all included studies; ΔSS, difference in scores from normative expectations or controls (Mean = 100, SD = 15) calculated by multiplying Hedges' g by SD = 15; g = mean effect size Hedges' g). SE g = standard error for Hedges' g effect size.
Reading performance incorporated measures of basic word reading and reading comprehension. Math performance was based on measures of arithmetic calculation or applied mathematics. Spelling performance was based on a single subtest in all studies. Composite scores were computed for studies that reported multiple arithmetic or reading subtests based on the weighted average of each subtest.
p-value < 0.05 was in bold.

TABLE 2 | Provision of special education services and rates of grade retention in sickle cell disease compared to rates for African Americans nationally.

Outcome	***k***	***n***	**Percentage (95% CI)**	**OR (95% CI)**	***p*-value**
Special education	19	1,669	38% (29, 47%)	–	–
504 plan	5	808	10% (6, 15%)	–	–
Individualized Education Plan (IEP)	10	1,210	32% (22, 41%)	2.4 (1.5, 3.7)	**<0.001**
Retention	16	1,803	27% (22, 33%)	9.7 (7.4, 12.7)	**<0.001**

k, number of unique studies included in analyses; n, total sample size from all included studies; CI, confidence interval; OR, odds ratio for comparison to normative data.
Grade retention normative values were based on the average retention rates for Black students (K-12) from 1994 to 2016. Normative values for IEP services (IDEA Part B) were based on the average percentage of Black students (3–21 years) receiving services through IDEA Part B from 2000 to 2019. Retention normative value of 3.8% based on average counts per year from 1994 to 2016: (285,749/7,524,621). IEP normative value of 15.6% based on average counts per year from 2000 to 2019: (1,261,827/8,068,662).
p-value < 0.05 was in bold.

without SCD, and only 4 studies of spelling performance recruited controls.

Ten studies reported IEP status among 1,210 students with SCD (**Table 2**). Grade retention was assessed in 16 studies, including 1,803 students with SCD. Only 3 studies reporting IEP status or grade retention included a control group. Participants' ages ranged from 5 to 18 years across all studies.

Reading, Math, and Spelling Performance

Results revealed significant differences in math, reading, and spelling performance compared to age-normative expectations (**Table 1**). Reading, math, and spelling scores were 10.6 ($g = -0.70$), 13.1 ($g = -0.87$), and 12.0 ($g = -0.80$) points below normative expectations ($p < 0.001$), respectively, consistent with medium to large effect sizes. Results for SCD vs. normative reading, math, and spelling scores were all highly heterogeneous across studies ($Tau^2 = 0.11–0.18$, $p < 0.01$). Therefore, pooled results should be interpreted with caution.

Reading scores of students with SCD fell 6.0 ($g = -0.40$) points below demographically matched and/or sibling controls without SCD (**Figure 2**). Performance on measures of mathematics were 5.4 ($g = -0.36$) points below matched controls (**Figure 3**). These studies also displayed a great amount of heterogeneity in findings ($Tau^2 = 0.15–0.16$, $p < 0.01$). Only 4 studies assessing spelling performance recruited a control group, therefore we did not calculate pooled group differences.

IEP Support and Grade Retention

Based on the 10 studies with IEP data (**Table 2**), the pooled estimate for percentage of students with SCD having an IEP was 32% (95% CI: 22, 41%). When comparing the portion of students with SCD who have an IEP to a normative value of 15.6%, the estimated pooled odds ratio based on the random-effects model was 2.4 (95% CI: 1.5–3.7, $p < 0.001$). Thus, children with SCD were more than 2 times more likely to receive an IEP than African American students nationally.

Based on 16 studies including rates of grade retention, the pooled estimate was 27% (22, 33%). The estimated average odds ratio based on the random-effects model was 9.7 (95% CI: 7.4–12.7), $p < 0.001$ (**Table 2** and **Figure 4**). Thus, children with SCD were almost 10 times more likely to be grade-retained compared with African American children nationally.

Age and IQ Moderation of Reading and Math Performance

As displayed in **Table 3**, across 19 studies that measured reading performance, older age was marginally associated with poorer reading performance (Estimate = −0.99, Standard Error = 0.52, $p = 0.056$). Consistently, age was negatively associated with math performance across 17 studies (Estimate = −1.72, Standard Error = 0.83, $p = 0.037$).

Both reading and mathematics were highly associated with overall IQ across studies (**Table 3**). After inclusion of IQ as a moderator in analyses, 11.2 and 51.8% of the variability in reading and mathematics outcomes, respectively, can be attributed to the remaining between-study heterogeneity.

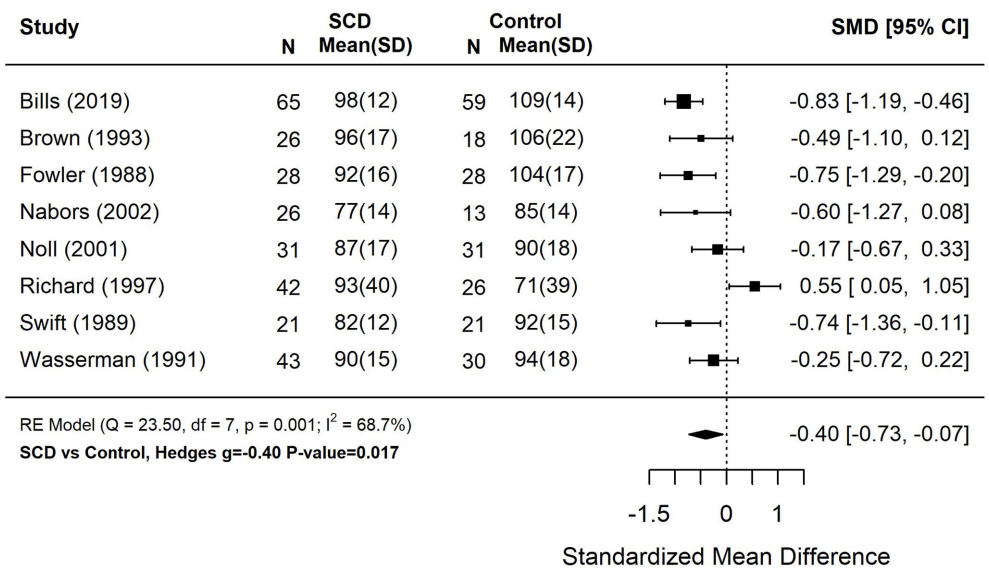


FIGURE 2 | Forest plot for composite reading score among patients with sickle cell disease compared to controls. Reading performance incorporated measures of basic word reading and reading comprehension. Composite scores were computed for studies that reported multiple reading subtests based on the weighted average of each subtest. *N*, the sample size per study subgroup. SD, standard deviation. SMD, standardized mean difference. 95% CI, 95% confidence interval.

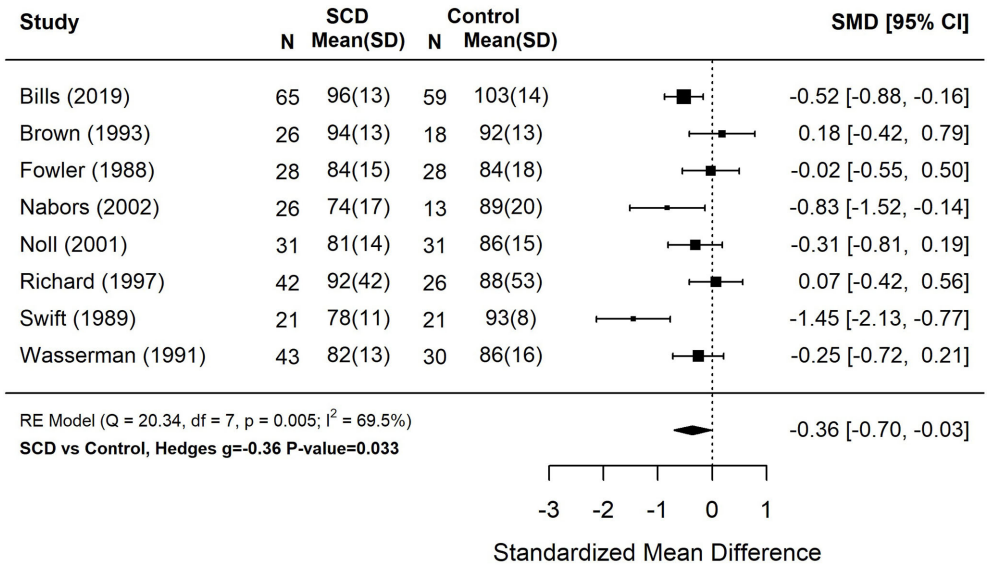


FIGURE 3 | Forest plot for composite math score among patients with sickle cell disease compared to controls. Math performance based on measures of arithmetic calculation or applied mathematics. Composite scores were computed for studies that reported multiple arithmetic subtests based on the weighted average of each subtest. *N*, the sample size per study subgroup. SD, standard deviation. SMD, standardized mean difference. 95% CI, 95% confidence interval.

Academic Performance by Infarct Status

Figures 5, **6** display reading and math performance for SCD patients with stroke, silent cerebral infarct, or no cerebrovascular accident (CVA).

The test for subgroup differences in composite reading scores by cerebral infarct status suggests that there was a statistically significant subgroup effect ($p = 0.034$), meaning that cerebral infarct status significantly affects reading performance. However, there was substantial unexplained heterogeneity between the studies within each of the subgroups (no CVA: $I^2 = 50.5\%$, silent cerebral infarct: $I^2 = 31.5\%$, and stroke: $I^2 = 71.4\%$). The mean reading score was 80.2 (95% CI: 66.7–86.2) in the stroke group, 86.3 (95% CI: 81.7–90.9) in the silent infarct group, and 89.2 (95% CI: 85.1, 93.3) in the group without history of a CVA.

The test for subgroup differences in composite math scores by cerebral infarct status suggests that there was a statistically significant subgroup effect ($p < 0.001$), meaning

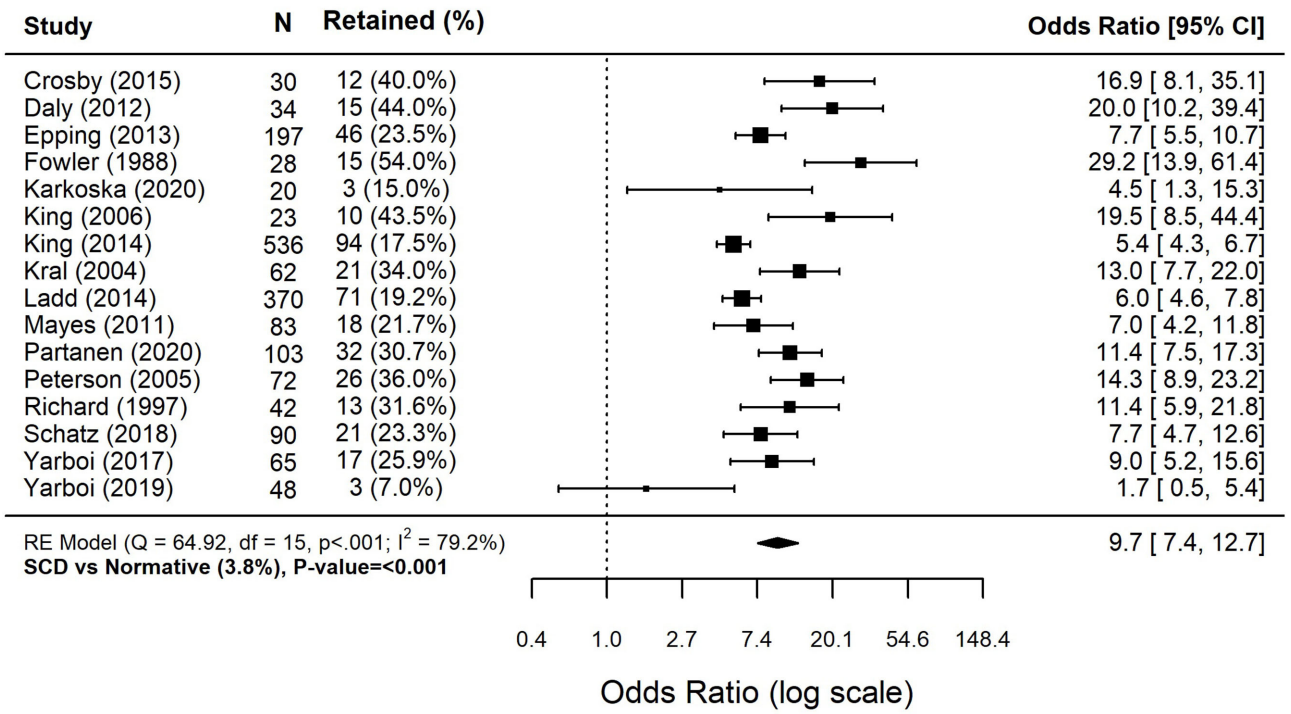


FIGURE 4 | Forest plot of grade retainment among patients with sickle cell disease compared to national estimates for black students. Normative data for grade retention were extracted from the U.S. Department of Commerce, Census Bureau, Current Population Survey (2017). Grade retention normative values were based on the average retention rates for Black students (K-12) from 1994 to 2016. N is the sample size of SCD subjects per study. 95% CI, 95% confidence interval.

TABLE 3 | Moderator analyses of reading and mathematics by intellectual functioning and age at assessment.

	IQ					Age				
Outcome	***k***	**Beta**	***p*-value**	I^2	R^2	***k***	**Beta**	***p*-value**	I^2	R^2
Reading	13	0.76	**<0.001**	11.22	97.3	19	−0.99	0.056	84.60	18.0
Math	11	0.84	**<0.001**	51.82	85.8	17	−1.72	**0.037**	88.02	21.0

IQ, intelligence quotient captured from a validated intelligence measure (mean = 100, standard deviation = 15); Age, average age (in years) of the study sample; k, number of unique studies included in the analyses. Reading performance incorporated measures of basic word reading and reading comprehension. Math performance was based on measures of arithmetic calculation or applied mathematics. Spelling performance was based on a single subtest in all studies. Composite scores were computed for studies that reported multiple arithmetic or reading subtests based on the weighted average of each subtest.

I^2 estimates the amount of heterogeneity relative to the total variance. For example, in the model with reading as the outcome and IQ as the moderator, we estimate that 11% of the total variance is due to heterogeneity.

R^2, value is computed as percent reduction in estimated Tau^2 which is computed using the difference in Tau^2 estimates from the random effects model vs. the mixed-effects meta-regression model (i.e., with predictor).

p-value < 0.05 was in bold.

that cerebral infarct status significantly affects composite math scores. However, there was substantial unexplained heterogeneity between the studies within each of the subgroups (no CVA: $I^2 =$ 33.4%, silent infarct: $I^2 = 34\%$, and stroke $I^2 = 75.2\%$). The mean math score was 76.2 (95% CI: 70.7–81.7) in the stroke group, 85.5 (95% CI: 80.8–90.2) in the silent infarct group, and 90 (95% CI: 85.9, 94.2) in the group without history of a CVA.

Publication Bias

The regression test (38) was used to test for publication bias via funnel plot asymmetry. Egger's Tests using two-tailed criterion at *p*-values <0.05 were flagged for potential funnel plot bias and include: percentage retained in the SCD population ($p < 0.01$) and the composite reading scores among controls ($p = 0.03$).

As a sensitivity analysis, we used a trim and fill data augmentation technique (40–42) to estimate how many studies would need to be included above or below the meta-analytic mean to make the funnel plot symmetrical, and to estimate how the hypothetical missing studies might affect the estimated grade retention estimate. The trim and fill yielded an estimate of the need to add 4 (SE = 2.7) studies on the left side (**Supplementary Figure 1**), and the resulting estimated rate of grade retention was 23% (95% CI: 17–30%).

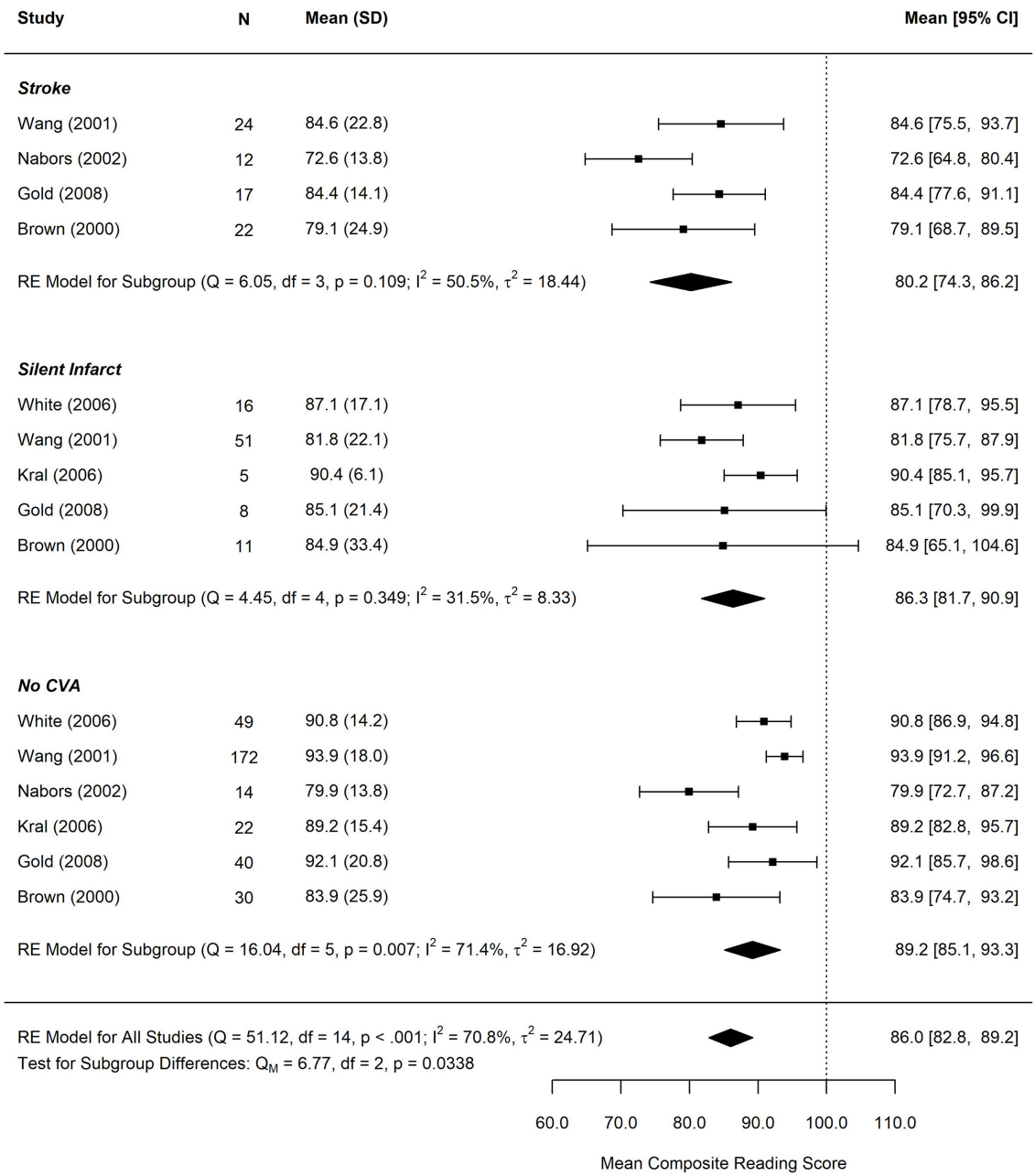


FIGURE 5 | Forest plot for Mean Composite Reading score by infarct status. CVA, cerebrovascular accident. Reading performance incorporated measures of basic word reading and reading comprehension. Composite scores were computed for studies that reported multiple reading subtests based on the weighted average of each subtest. *N*, study subgroup sample size. SD, standard deviation. 95% CI, 95% confidence interval.

DISCUSSION

Students with SCD demonstrate deficits in academic achievement across domains compared to normative expectations and healthy/sibling controls. This achievement gap appears to worsen as students with SCD age and demonstrate slowed academic growth. Students with SCI or stroke displayed greater achievement deficits than their peers without history of CVA. Despite frequently receiving formal academic supports, students with SCD are retained at an alarming rate.

This is the first meta-analysis examining academic performance in children with SCD in the United States. Compared to normative expectations, students with SCD demonstrated substantial deficits in reading, arithmetic, and

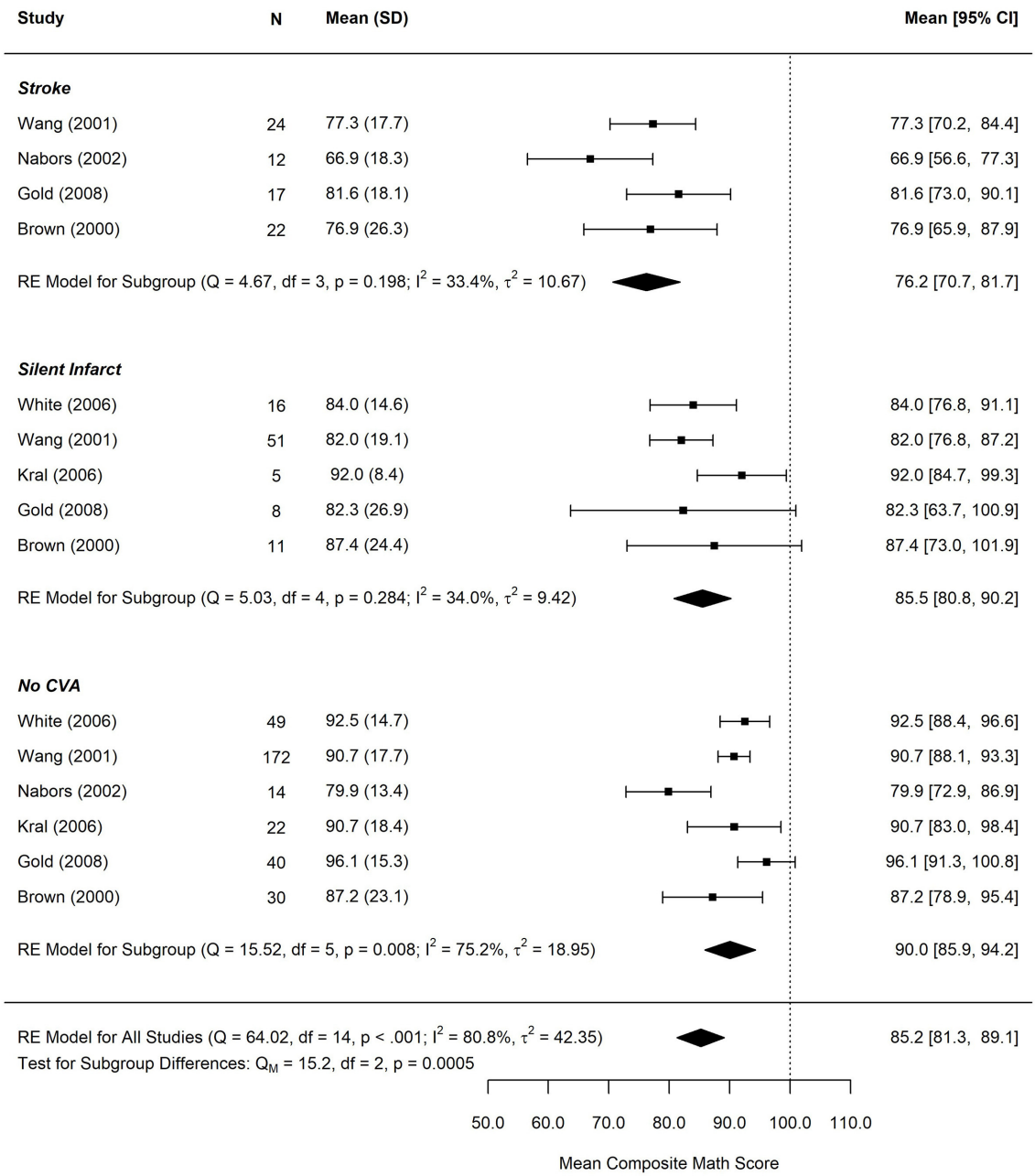


FIGURE 6 | Forest plot for composite math score by infarct status. CVA, cerebrovascular accident. Math performance based on measures of arithmetic calculation or applied mathematics. Composite scores were computed for studies that reported multiple arithmetic subtests based on the weighted average of each subtest. SD, standard deviation. *N*, study subgroup sample size. 95% CI, 95% confidence interval.

spelling. These deficits generally did not vary across academic domains, with consistent medium to large effects. A large portion of the variance in academic achievement was accounted for by intellectual functioning, particularly reading skills. This is consistent with findings in the general population, showing a strong correlation between intellectual functioning and academic knowledge and attainment (43, 44). Slowed academic development is likely due in part to accumulated micro-infarcts, chronic hypoxemia, and repeated tissue ischemia as well as the cumulative impact of school absences. Academic deficits were reduced when compared to healthy and/or sibling controls, likely due to the contribution of sociodemographic factors. Most studies assessing academic achievement in SCD only reported a single subtest for each academic domain or a composite index, precluding more granular analysis of academic weaknesses. Only two of the included studies (11, 45) reported measures

of academic fluency. In both studies, fluency measures were a relative academic weakness, consistent with processing speed deficits observed in SCD (46).

The implementation of school services differed notably across studies. Provision of IEPs and 504 Plans ranged from 14 to 74% and 4 to 16%, respectively. As expected, students with SCD were over twice as likely to receive an IEP than a national sample of African American students. These findings are consistent with other chronic medical conditions of childhood, such as congenital heart disease (47). Due to a limited number of studies, we were unable to examine if cognitive and/or academic performance were associated with provision of school services. Among a national sample of students diagnosed with attention deficit/hyperactivity disorder, provision of an IEP was associated with disease severity, diagnosis of a developmental delay and/or neurodevelopmental disorder, academic, and cognitive performance (48). In contrast, implementation of a Section 504 Plan was primarily associated with sociodemographic factors, such as primary language used in the home and type of health care coverage (48). Ghafuri et al. (49) observed that patients with SCD were more likely to receive school services following a neuropsychological evaluation. Yet, it is unclear if the results of the assessment resulted in improved access rather than advocacy by the providers. Conclusions drawn regarding school services are limited due to differing descriptions of special education services, and many studies did not differentiate between receiving a Section 504 Plan vs. an IEP (see **Supplementary Table 3**). Surprisingly, rates of services through Section 504 were much lower than those reported for an IEP. The definition of a disability under Section 504 is much broader than in an IEP and often includes children with no observed learning/cognitive deficits. Many families with limited resources may be unaware of accommodations provided through Section 504 (48) and would benefit from school advocacy. At a minimum, most children with SCD, regardless of cognitive/academic deficits, should qualify for accommodations under Section 504 to address medical concerns (e.g., bathroom breaks, excused absences for medical visits). For students with learning difficulties, accommodations and services should be informed through a more comprehensive evaluation done through the school and/or pediatric neuropsychologist.

The odds of grade retention for students with SCD were approximately 10 times greater than African American students nationally. On average, 27% of students with SCD were retained in at least one grade. These findings are concerning given the research on the long-term effects of grade retention demonstrating no benefits on academic attainment but negative effects on psychosocial outcomes (50). Students who are retained in grade are 50% more likely to drop out of high school (51) and their odds of attending college are cut in half, even after accounting for academic achievement, race, and socioeconomic status (52). King et al. (1) conducted the largest ($n = 536$) study assessing correlates of grade retention in students with SCD. They found that older age, male sex, and lower household income significantly increased risk of grade retention. Medical factors such as silent cerebral infarcts or frequency of pain episodes were not associated with grade retention. Because most studies did not report correlates of grade retention, we were unable conduct a meta-regression evaluating predictors of grade retention. It is recommended that medical and behavioral health providers advocate *against* retention for most students with SCD, instead recommending school services through an IEP or 504 Plan based on need.

Pooled effect sizes were smaller across academic achievement and attainment outcomes when compared to healthy controls relative to national normative data. These differences are likely accounted for by sociodemographic factors, as students with SCD tend to live in lower socioeconomic neighborhoods (3). Lower SES is associated with reduced academic achievement and attainment independent of disease status (53). A few of the included studies reported relationships between SES and academic achievement (19, 23, 54) or grade retention (1), but we were unable to calculate any pooled values due to the lack of standardization in the SES indices.

Poor academic attainment and grade retention have significant consequences. In the general population, reduced academic attainment is associated with poorer health literacy (55) and increased mortality (56), while grade retention is related to poorer psychosocial adjustment (57), delays in entering the workforce (58), and reduced salaries (59). Among patients with SCD, those without a high school education visit the emergency department three times as frequently as patients with post-secondary education (5). This association between educational attainment and hospital visits persists even after controlling for sociodemographic and disease factors (5). The academic attainment of patients with SCD has social and health consequences that go beyond the classroom environment. Further research is needed to evaluate the breadth of these academic consequences and potential intervention targets.

Individualized academic intervention and broader policy changes are needed to address the significant academic needs of students with SCD. Several institutions utilize academic liaisons to support patients with SCD from early childhood through adolescence. These liaisons typically work closely with neuropsychologists to screen patients for learning difficulties (60). If academic deficits and/or cognitive delays are identified, the academic liaisons can advocate for patients within the school environment and attend school meetings (e.g., IEP meetings). Further, liaisons can provide psychoeducation to families about their rights within the school environment and availability of academic resources, such as Section 504. Beyond services provided within the school environment, targeted academic interventions are needed. A majority of SCD patients lack academic readiness skills in preschool (61), and these early skills are the strongest predictor of later academic attainment (62, 63). Patients with SCD would benefit from increased access to early intervention, preschool services, and targeted academic readiness interventions.

Several study limitations exist. Due to variability in academic achievement measures across studies, we could not analyze specific areas of academic weakness beyond broad categories of mathematics, reading, and spelling. Future research should examine more specific academic outcomes (e.g., calculation,

phonological processing, fluency). Moderation analyses were limited to age and intellectual functioning. Additional factors known to influence academic performance, such as socioeconomic status, could not be analyzed due to inconsistent reporting across studies. Factors which may affect academic performance also include missed school days due to outpatient appointments, illness and hospitalizations, as well as accrual of strokes or silent infarcts. Only a small number of studies reported academic achievement outcomes based on cerebral infarct status. From the available studies, it appears that there is a clear gradient of performance based on infarct severity. Further research is needed to understand the functional consequences of these cerebral vascular injuries and implications for academic intervention. There is also a need to understand the influence of infarct location and size on academic performance.

To conclude, the present meta-analysis clearly demonstrates the negative effects of SCD on academic achievement and attainment. Consistent with cognitive deficits, the academic achievement gap in math and reading appears to worsen as students with SCD age. A substantial portion of academic achievement is determined by levels of cognitive performance. Further research is needed to understand this relationship, and the specific cognitive domains that influence academic functioning in SCD. Despite well-documented academic deficits, most patients with SCD do not receive any form of academic services. Students with SCD would benefit from increased school advocacy and access to academic intervention services.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

AH, JH, and LO contributed to the conception and design of the study. AH, LH, CS, and LO collected the data. JG and GK performed the statistical analyses. AH wrote the first draft of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

FUNDING

JH received funding from U01HL133996 during the conduct of this study. AK received funding from R01HL129241, K24HL148305, K12HL137942, U01HL143477, and 5U01HL133994 during the time of his study. This research was supported by the American Lebanese Syrian Associated Charities (ALSAC).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2021.786065/full#supplementary-material

REFERENCES

1. King AA, Rodeghier MJ, Panepinto JA, Strouse JJ, Casella JF, Quinn CT, et al. Silent cerebral infarction, income, and grade retention among students with sickle cell anemia. *Am J Hematol.* (2014) 89:E188–92. doi: 10.1002/ajh.23805
2. Schatz J, Brown R, Pascual J, Hsu L, DeBaun M. Poor school and cognitive functioning with silent cerebral infarcts and sickle cell disease. *Neurology.* (2001) 56:1109–11. doi: 10.1212/WNL.56.8.1109
3. Farber MD, Koshy M, Kinney TR. Cooperative study of sickle Cell D. Cooperative study of sickle cell disease: demographic and socioeconomic characteristics of patients and families with sickle cell disease. *J Chronic Dis.* (1985) 38:495–505. doi: 10.1016/0021-9681(85)90033-5
4. Sanger M, Jordan L, Pruthi S, Day M, Covert B, Merriweather B, et al. Cognitive deficits are associated with unemployment in adults with sickle cell anemia. *J Clin Exp Neuropsychol.* (2016) 38:661–71. doi: 10.1080/13803395.2016.1149153
5. Jonassaint C, Beach MC, Haythornthwaite J, Bediako S, Diener-West M, Strouse J, et al. The association between educational attainment and patterns of emergency department utilization among adults with sickle cell disease. *Int J Behav Med.* (2016) 23:300–9. doi: 10.1007/s12529-016-9538-y
6. Piel FB, Steinberg MH, Rees DC. Sickle cell disease. *N Engl J Med.* (2017) 376:1561–73.
7. DeBaun MR, Armstrong FD, McKinstry RC, Ware RE, Vichinsky E, Kirkham FJ. Silent cerebral infarcts: a review on a prevalent and progressive cause of neurologic injury in sickle cell anemia. *Blood.* (2012) 119:4587–96. doi: 10.1182/blood-2011-02-272682
8. Sil S, Cohen LL, Dampier C. Psychosocial and functional outcomes in youth with chronic sickle cell pain. *Clin J Pain.* (2016) 32:527–33. doi: 10.1097/AJP.0000000000000289
9. Ameringer S, Smith WR. Emerging biobehavioral factors of fatigue in sickle cell disease. *J Nurs Scholarsh.* (2011) 43:22–9. doi: 10.1111/j.1547-5069.2010.01376.x
10. Schatz J, Finke R, Roberts CW. Interactions of biomedical and environmental risk factors for cognitive development: a preliminary study of sickle cell disease. *J Dev Behav Pediatr.* (2004) 25:303–10. doi: 10.1097/00004703-200410000-00001
11. Yarboi J, Compas BE, Brody GH, White D, Rees Patterson J, Ziara K, et al. Association of social-environmental factors with cognitive function in children with sickle cell disease. *Child Neuropsychol.* (2017) 23:343–60. doi: 10.1080/09297049.2015.1111318
12. Tarazi RA, Grant ML, Ely E, Barakat LP. Neuropsychological functioning in preschool-age children with sickle cell disease: the role of illness-related and psychosocial factors. *Child Neuropsychol.* (2007) 13:155–72. doi: 10.1080/09297040600611312
13. Craft JSS, Koby M, Siegel MJ, Resar L, Lee RR, Chu J-Y, et al. Neuropsychologic deficits in children with sickle cell disease and cerebral infarction: role of lesion site and volume. *Child Neuropsychol.* (1999) 5:92–103. doi: 10.1076/chin.5.2.92.3170
14. King AA, DeBaun MR, White DA. Need for cognitive rehabilitation for children with sickle cell disease and strokes. *Expert Rev Neurother.* (2008) 8:291–6. doi: 10.1586/14737175.8.2.291
15. Schoon I. Childhood cognitive ability and adult academic attainment: evidence from three British cohort studies. *Longit Life Course Stud.* (2010) 1:241–158. doi: 10.14301/llcs.v1i3.93

16. Machin S, Vignoles A. Educational inequality: the widening socio-economic gap. *Fisc Stud.* (2004) 25:107–28. doi: 10.1111/j.1475-5890.2004.tb00099.x
17. Palermo TM, Riley CA, Mitchell BA. Daily functioning and quality of life in children with sickle cell disease pain: relationship with family and neighborhood socioeconomic distress. *J Pain.* (2008) 9:833–40. doi: 10.1016/j.jpain.2008.04.002
18. Schwartz LA, Radcliffe J, Barakat LP. Associates of school absenteeism in adolescents with sickle cell disease. *Pediatr Blood Cancer.* (2009) 52:92–6. doi: 10.1002/pbc.21819
19. Smith KE, Patterson CA, Szabo MM, Tarazi RA, Barakat LP. Predictors of academic achievement for school-age children with sickle cell disease. *Adv Sch Ment Health Promot.* (2013) 6:5–20. doi: 10.1080/1754730X.2012.760919
20. Crosby LE, Joffe NE, Irwin MK, Strong H, Peugh J, Shook L, et al. School performance and disease interference in adolescents with sickle cell disease. *Physical Disabil: Educ Relat Serv.* (2015) 34:14. doi: 10.14434/pders.v34i1.13918
21. Bakri MH, Ismail EA, Elsedfy GO, Amr MA, Ibrahim A. Behavioral impact of sickle cell disease in young children with repeated hospitalization. *Saudi J Anaesth.* (2014) 8:504. doi: 10.4103/1658-354X.140867
22. Thompson RJ, Armstrong FD, Kronenberger WG, Scott D, McCabe MA, Smith B, et al. Family functioning, neurocognitive functioning, and behavior problems in children with sickle cell disease. *J Pediatr Psychol.* (1999) 24:491–8. doi: 10.1093/jpepsy/24.6.491
23. Schatz J. Brief report: academic attainment in children with sickle cell disease. *J Pediatr Psychol.* (2004) 29:627–33. doi: 10.1093/jpepsy/jsh065
24. Disabilities CoCw. The Pediatrician's role in development and implementation of an Individual Education Plan (IEP) and/or an Individual Family Service Plan (IFSP). *Pediatrics.* (1999) 104:124–7. doi: 10.1542/peds.104.1.124
25. Rosenfeld SJ. *Section 504 and IDEA: basic similarities and differences* (1996).
26. Prussien KV, Jordan LC, DeBaun MR, Compas BE. Cognitive function in sickle cell disease across domains, cerebral infarct status, and the lifespan: a meta-analysis. *J Pediatr Psychol.* (2019) 44:948–58. doi: 10.1093/jpepsy/jsz031
27. Prussien KV, Siciliano RE, Ciriegio AE, Anderson AS, Sathanayagam R, DeBaun MR, et al. Correlates of cognitive function in sickle cell disease: a meta-analysis. *J Pediatr Psychol.* (2020) 45:145–55. doi: 10.1093/jpepsy/jsz100
28. King AA, Strouse JJ, Rodeghier MJ, Compas BE, Casella JF, McKinstry RC, et al. Parent education and biologic factors influence on cognition in sickle cell anemia. *Am J Hematol.* (2014) 89:162–7. doi: 10.1002/ajh.23604
29. Schatz J, Finke RL, Kellett JM, Kramer JH. Cognitive functioning in children with sickle cell disease: a meta-analysis. *J Pediatr Psychol.* (2002) 27:739–48. doi: 10.1093/jpepsy/27.8.739
30. Kawadler JM, Clayden JD, Clark CA, Kirkham FJ. Intelligence quotient in paediatric sickle cell disease: a systematic review and meta-analysis. *Dev Med Child Neurol.* (2016) 58:672–9. doi: 10.1111/dmcn.13113
31. Covidence systematic review software. Melbourne, Australia: Veritas Health Innovation. Available online at: www.covidence.org
32. US Department of Commerce, Census Bureau. *Current Population Survey, October, 1994 through 2016–2017*. Available from: https://nces.ed.gov/programs/digest/d17/tables/dt17_225.90.asp?referer=raceindicators (accessed September 25, 2021).
33. U.S. Department of Education, Office of Special Education Programs. *Individuals with Disabilities Education Act (IDEA) database. National Center for Education Statistics, Common Core of Data (CCD), "State Nonfiscal Survey of Public Elementary and Secondary Education," 2000–01 through 2018–19 2020.* Available from: https://nces.ed.gov/programs/digest/d19/tables/dt19_204.40.asp (accessed September 25, 2021).
34. Viechtbauer W. Bias and efficiency of meta-analytic variance estimators in the random-effects model. *J Educ Behav Stat.* (2005) 30:261–93. doi: 10.3102/10769986030003261
35. Cochran WG. The combination of estimates from different experiments. *Biometrics.* (1954) 10:101. doi: 10.2307/3001666
36. Higgins JPT, Thompson SG. Quantifying heterogeneity in a meta-analysis. *Stat Med.* (2002) 21:1539–58. doi: 10.1002/sim.1186
37. Viechtbauer W, Cheung MWL. Outlier and influence diagnostics for meta-analysis. *Res Synth Methods.* (2010) 1:112–25. doi: 10.1002/jrsm.11
38. Sterne JAC, Egger M. Regression methods to detect publication and other bias in meta-analysis. *Publ Bias Meta-Analysis: Prevent Assess Adjustments.* (2005) 99:110. doi: 10.1002/0470870168.ch6
39. Viechtbauer W, Viechtbauer MW. Package Metafor. The Comprehensive R Archive Network Package 'Metafor'. (2017).
40. Duval S, Tweedie R. A. nonparametric "trim and fill" method of accounting for publication bias in meta-analysis. *J Am Stat Assoc.* (2000) 95:89–98. doi: 10.1080/01621459.2000.10473905
41. Duval S, Tweedie R. Trim and fill: a simple funnel-plot-based method of testing and adjusting for publication bias in meta-analysis. *Biometrics.* (2000) 56:455–63. doi: 10.1111/j.0006-341X.2000.00455.x
42. Rothstein HR, Sutton AJ, Borenstein M. Publication bias in meta-analysis: prevention, assessment and adjustments (2006). New York: John Wiley & Sons. doi: 10.1002/0470870168
43. Soares DL, Lemos GC, Primi R, Almeida LS. The relationship between intelligence and academic achievement throughout middle school: The role of students' prior academic performance. *Learn Individ Differ.* (2015) 41:73–8. doi: 10.1016/j.lindif.2015.02.005
44. Mayes SD, Calhoun SL, Bixler EO, Zimmerman DN. IQ and neuropsychological predictors of academic achievement. *Learn Individ Differ.* (2009) 19:238–41. doi: 10.1016/j.lindif.2008.09.001
45. Partanen M, Kang G, Wang WC, Krull K, King AA, Schreiber JE, et al. Association between hydroxycarbamide exposure and neurocognitive function in adolescents with sickle cell disease. *Br J Haematol.* (2020) 189:1192–203. doi: 10.1111/bjh.16519
46. Stotesbury H, Kirkham FJ, Kölbel M, Balfour P, Clayden JD, Sahota S, et al. White matter integrity and processing speed in sickle cell anemia. *Neurology.* (2018) 90:e2042–e50. doi: 10.1212/WNL.0000000000005644
47. Riehle-Colarusso T, Autry A, Razzaghi H, Boyle CA, Mahle WT, Van Naarden Braun K, et al. Congenital Heart Defects and Receipt of Special Education Services. *Pediatrics.* (2015) 136:496–504. doi: 10.1542/peds.2015-0259
48. Dupaul GJ, Chronis-Tuscano A, Danielson ML, Visser SN. Predictors of receipt of school services in a national sample of youth with ADHD. *J Atten Disord.* (2019) 23:1303–19. doi: 10.1177/1087054718816169
49. Ghafuri DL, Sanger M, Rodeghier M, DeBaun MR. Integrated psychology support and comprehensive cognitive evaluation improves access to special education services for children with sickle cell disease. *Pediatr Blood Cancer.* (2019) 66:e27755. doi: 10.1002/pbc.27755
50. Anderson GE, Whipple AD, Jimerson SR. Grade retention: achievement and mental health outcomes. *Natl Assoc School Psychol.* (2002) 1–4. Available online at: https://www.fmptic.org/download/grade_retention_achievement_and_mental_health_outcomes.pdf
51. Jimerson SR, Anderson GE, Whipple AD. Winning the battle and losing the war: Examining the relation between grade retention and dropping out of high school. *Psychol Sch.* (2002) 39:441–57. doi: 10.1002/pits.10046
52. Fine JG, Davis JM. Grade retention and enrollment in post-secondary education. *J Sch Psychol.* (2003) 41:401–11. doi: 10.1016/j.jsp.2003.07.001
53. Heitzer AM, Longoria J, Okhomina V, Wang WC, Raches D, Potter B, et al. Hydroxyurea treatment and neurocognitive functioning in sickle cell disease from school age to young adulthood. *Br J Haematol.* (2021) 195:256–66. doi: 10.1111/bjh.17687
54. Brown RT, Buchanan I, Doepke K, Eckman JR, Baldwin K, Goonan B, et al. Cognitive and academic functioning in children with sickle-cell disease. *J Clin Child Psychol.* (1993) 22:207–18. doi: 10.1207/s15374424jccp2202_7

55. Clouston SAP, Manganello JA, Richards M, A. life course approach to health literacy: the role of gender, educational attainment and lifetime cognitive capability. *Age Ageing.* (2017) 46:493–9. doi: 10.1093/ageing/afw229
56. Montez JK, Hummer RA, Hayward MD. Educational attainment and adult mortality in the United States: a systematic analysis of functional form. *Demography.* (2012) 49:315–36. doi: 10.1007/s13524-011-0082-8
57. Mathys C, Véronneau M-H, Lecocq A. Grade Retention at the transition to secondary school: using propensity score matching to identify consequences on psychosocial adjustment. *J Early Adolesc.* (2019) 39:97–133. doi: 10.1177/0272431617735651
58. Leventhal T, Graber JA, Brooks-Gunn J. Adolescent transitions to young adulthood: antecedents, correlates, and consequences of adolescent employment. *J Res Adolescence.* (2001) 11:297–323. doi: 10.1111/1532-7795.00014
59. Jimerson SR. On the failure of failure. *J Sch Psychol.* (1999) 37:243–72. doi: 10.1016/S0022-4405(99)00005-9
60. Wills KE, Nelson SC, Hennessy J, Nwaneri MO, Miskowiec J, McDonough E, et al. Transition planning for youth with sickle cell disease: embedding neuropsychological assessment into comprehensive care. *Pediatrics.* (2010) 126:S151. doi: 10.1542/peds.2010-1466J
61. Aygun B, Parker J, Freeman MB, Stephens AL, Smeltzer MP, Wu S, et al. Neurocognitive screening with the Brigance Preschool screen-II in 3-year-old children with sickle cell disease. *Pediatr Blood Cancer.* (2011) 56:620–4. doi: 10.1002/pbc.22833
62. Duncan GJ, Dowsett CJ, Claessens A, Magnuson K, Huston AC, Klebanov P, et al. School readiness and later achievement. *Dev Psychol.* (2007) 43:1428. doi: 10.1037/0012-1649.43.6.1428
63. Eisenberg N, Valiente C, Eggum ND. Self-Regulation and School Readiness. *Early Educ Dev.* (2010) 21:681–98. doi: 10.1080/10409289.2010.497451

Conflict of Interest: AK and JH receive research funding from Global Blood Therapeutics. JH receives consultancy fees from Global Blood Therapeutics, Vindico Medical Education, UpToDate and bluebird bio.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Neurology

ORIGINAL RESEARCH
published: 13 December 2021
doi: 10.3389/fneur.2021.761443

Association Between Patent Foramen Ovale and Overt Ischemic Stroke in Children With Sickle Cell Disease

Najibah A. Galadanci[1], Walter Johnson[2], April Carson[3], Gerhard Hellemann[4], Virginia Howard[5†] and Julie Kanter[1†]*

[1] Division of Hematology and Oncology, UAB School of Medicine, University of Alabama at Birmingham, Birmingham, AL, United States, [2] Department of Pediatrics, UAB School of Medicine, University of Alabama at Birmingham, Birmingham, AL, United States, [3] Jackson Heart Study, University of Mississipi Medical Center, Jackson, MS, United States, [4] Department of Biostatistics, School of Public Health, University of Alabama at Birmingham, Birmingham, AL, United States, [5] Department of Epidemiology, School of Public Health, University of Alabama at Birmingham, Birmingham, AL, United States

Ischemic stroke is one of the most devastating complications of sickle cell anemia (SCA). Previous studies have shown that intracardiac shunting including patent foramen ovale (PFO) can be a potential risk factor for stroke in children with SCA. This study investigates the association between PFO and overt ischemic stroke in the DISPLACE (Dissemination and Implementation of Stroke Prevention Looking at the Care Environment) study cohort of 5,247 children with SCA of whom 1,414 had at least one clinical non-contrast transthoracic echocardiogram. Presence of PFO was taken from the clinical report. Further, we assessed the association between PFO and other clinical and hemolytic factors in children with SCA such as history of abnormal sickle stroke screen [elevated Transcranial Doppler ultrasound (TCD) velocity] and patient's baseline hemoglobin. In 642 children for whom all data were available, the adjusted odds ratio (OR) for overt stroke was higher in those with PFO but this was not statistically significant (OR: 1.49, 95% CI: 0.20–11.03, $p = 0.6994$). With an OR of 0.85, the study suggested less PFOs in those with abnormal TCD, but this was not statistically significant (95% CI: 0.17–4.25, $p = 0.8463$). Overall, the prevalence of PFO in this large sub study of non-contrast echocardiography amongst children with SCA is much lower than previous smaller studies using bubble contrast echocardiography. Overt stroke was non-statistically more common in children with SCA and PFO, but there was no evidence that PFO was more common in those with abnormal TCD, the most important pediatric sickle stroke screen.

Keywords: sickle cell disease, children, ischemic stroke, patent foramen ovale, sickle stroke screen

OPEN ACCESS

Edited by:
Fenella Jane Kirkham, University College London, United Kingdom

Reviewed by:
Vesna Brankovic, University of Belgrade, Serbia
Juan Dario Ortigoza-Escobar, Hospital Sant Joan de Déu Barcelona, Spain

****Correspondence:***
Julie Kanter
jkanter@uabmc.edu

† These authors share senior authorship

Specialty section:
This article was submitted to Pediatric Neurology, a section of the journal Frontiers in Neurology

Received: *19 August 2021*
Accepted: *03 November 2021*
Published: *13 December 2021*

Citation:
Galadanci NA, Johnson W, Carson A, Hellemann G, Howard V and Kanter J (2021) Association Between Patent Foramen Ovale and Overt Ischemic Stroke in Children With Sickle Cell Disease. Front. Neurol. 12:761443. doi: 10.3389/fneur.2021.761443

INTRODUCTION

Sickle cell anemia (SCA) is the leading cause of overt ischemic stroke in children (1), with a stroke risk up to 10% per year without any primary stroke prevention strategy applied (2–5). Stroke is one of the most devastating complications in children with SCA leading to high morbidity and mortality (3, 4). Several risk factors have been identified for stroke in children with SCA including anemia, leukocytosis, hypertension, silent infarction, history of acute chest syndrome, and other environmental and genetic factors (4, 6).

Previous studies have shown patent foramen ovale (PFO) to be a significant risk factor for overt ischemic stroke in adults (without SCA) with a prevalence of up to 40% in young adults (7, 8). PFO leads to paradoxical embolization where emboli from the systemic venous circulation pass from the right heart directly into the left heart and eventually the brain without filtration in the lungs (8–10).

Children with SCA have been shown to be at a 4- to 100-fold increased risk for thrombosis compared with the general population (11). SCA is associated with increased activation of coagulation mechanism leading to increased circulating thrombin, platelet activation, increased activation of fibrinolysis and decreased levels of anticoagulant proteins (12–15). In SCA, the chronic anemia and pulmonary arterial hypertension may lead to increased right heart pressure thereby predisposing to right to left shunting and paradoxical embolization; this may predispose children with SCA who have a PFO to increased risk for stroke (16). Previous studies have shown that potential right-to-left shunting through the heart or the lungs, diagnosed with bubble contrast transthoracic echocardiograms, can be a risk factor for stroke in children with SCA (17, 18). Unfortunately, because of the perceived risks of bubble contrast in children with SCA without stroke, most of these studies were limited by their small sample size and the use of control groups of children without SCA (17).

The greatest advancement to date in preventing strokes in children with SCA has been using transcranial doppler ultrasound (TCD) screening (now called a sickle stroke screen) to identify children who are at risk of stroke and institute primary prevention strategy (2). The sickle stroke screen is used to detect stenosis in the distal intracranial portions of the internal carotid artery and the proximal middle cerebral artery MCA as evidence by elevated TCD velocity in the arteries (19). The STOP trial demonstrated that children with elevated TCD velocity >200 cm per second that received regular blood transfusion therapy had a 92% relative risk reduction in the rate of overt strokes when compared with standard therapy. Despite the success shown in the STOP trial, and the subsequent published guidelines, many centers continue to have poor implementation of the recommended annual TCD screening (20–24). In addition, currently it is unclear whether the etiology of strokes in children and adults occurring in the era of sickle stroke screening with TCD are related to alternative stroke mechanisms or implementation failure of the screening system. Preliminary results from the DISPLACE study suggest that the majority of ischemic strokes occurring in the current era are most often occurring in children who have not had a sickle stroke screen/TCD in the preceding 365 days (publication pending). Although causality cannot be assessed in DISPLACE, it does appear that a failure of implementation of sickle stroke screen is in part responsible for these events.

Recently, the DISPLACE (Dissemination and Implementation of Stroke Prevention Looking at the Care Environment) study was funded by National Heart Lung and Blood Institute (NHLBI) to evaluate implementation of TCD screening and assess barriers and facilitators to evidence-based stroke prevention for children with SCA across 28 sites in the USA (25). As part of DISPLACE study, data were collected on children with SCA who were aged 2–16 at the time of the study and included TCD results, MRI results, Echocardiogram information (if done for clinical assessment) as well as laboratory and demographic information. The overall goal of the current study was to investigate the association between PFO and overt ischemic stroke in the DISPLACE cohort. Further, we assessed the association between PFO and other clinical and hemolytic factors in children with SCA such as abnormal TCD, baseline hemoglobin and blood pressure.

METHODS

Study Population and Design

DISPLACE study retrospectively collected data on 5,247 children with SCA aged between 2 and 16 years from 28 centers in the US including clinical and laboratory assessments from defined study years of 2012–2016 (25). These data included baseline values of heart rate and blood pressure measurements, anthropometric measurements including height and weight and presence or absence of sickle cell disease-related therapies including chronic red cell transfusion (CRCT) and hydroxyurea (HU) therapies. Laboratory data included baseline data on complete blood count including hemoglobin and reticulocyte count.

While the clinical and anthropomorphic data focused were only collected during years 2012–2016, data from radiographic studies were uploaded from birth-present. Thus, Magnetic Resonance Imaging (MRI), TCD, and echocardiogram reports were included if available throughout the lifespan of the individuals. All of these radiographic studies including echocardiograms were ordered by the treating provider for standard of care (not for study-specific inquiry) and were not read centrally. As shown in the practice pattern questionnaire for echocardiography among participating centers in the DISPLACE study, about 50% of the centers order echocardiogram as part of routine care for children with SCD, while the remaining 50% order echo in children only secondary to other clinical circumstances including history of overt stroke, history of silent stroke, asthma, LVH on chest x-ray, murmurs that in non-typical flow murmur.

All echocardiogram studies were performed and interpreted according to the American Society of Echocardiography guidelines (26, 27). The echocardiogram reports collected ranged from 2000 to 2020. There was no central adjudication of the echocardiograms or the reports; the data were only collected as reported. The case report forms for neurologic complications and MRI findings included the options for overt ischemic stroke, silent cerebral infarct, and hemorrhagic stroke. Only the overt ischemic stroke was included in this project.

The current study was conducted as a cross-sectional analysis of children with SCA who had at least one echocardiogram and one set of laboratory and clinical assessments (during the same calendar year as the echocardiograph). Of the 5,247 children in the DISPLACE database, 3,833 were excluded who did not have an echocardiogram or accompanying lab and clinical assessment resulting in an analysis cohort of 1,414 (**Figure 1**).

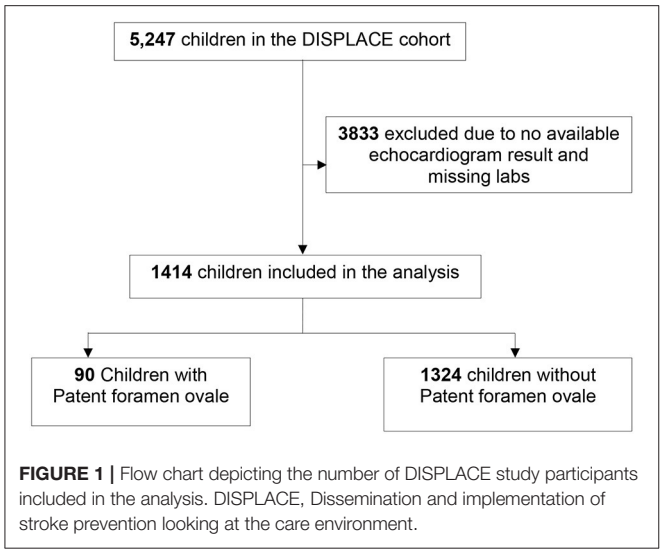


FIGURE 1 | Flow chart depicting the number of DISPLACE study participants included in the analysis. DISPLACE, Dissemination and implementation of stroke prevention looking at the care environment.

Institutional Review Board and Data Use Agreements

Institutional Review Board (IRB) approval and data use agreement for the DISPLACE study were obtained at and between each clinical institution and the sponsoring institution using a common protocol (25). All data were de-identified at time of entry and all data were retrospective; thus, consent was not required from individual patients or their families. For the current analysis, we further obtained IRB approval from the University of Alabama at Birmingham to evaluate the de-identified data.

Variable Definitions

Our primary outcome of interest was the relationship between presence of PFO and presence of overt ischemic stroke. Our secondary outcome is abnormal TCD velocity as recorded into the DISPLACE database. Our predictor variable was PFO defined as present/absent on the echocardiogram report. Our covariates included age in years, sex, systolic blood pressure (SBP), diastolic blood pressure (DBP), hemoglobin level, reticulocyte count and ever or never use of (HU) or CRCT. Selection of our covariates was based on previous knowledge on literature about their importance in the research area (28, 29). All variables were identified from the DISPLACE database.

Statistical Analysis

Descriptive analysis was carried out to examine the association between PFO and covariates. Median and interquartile range (IQR) were reported for age, hemoglobin level, reticulocyte count, SBP, and DBP, whereas frequencies and percentages were reported for sex, HU, and CRCT. In the bivariate analysis, Kruskal-Wallis test and Cochran-Mantel-Haenszel test were conducted for continuous and categorical variables respectively. To determine the association between PFO and overt ischemic stroke, a logistic regression was fit adjusting for covariates. Using the no PFO as the referent group, crude and adjusted odds ratios (ORs) with their accompanying 95% confidence intervals (CIs) were calculated for the group with PFO. Further, to determine the association between PFO and abnormal TCD velocity, we used a logistic regression and adjusted for potential covariates.

For all analysis, we used complete case analysis to handle missing data, where we include only individuals with data on all variables. Further to ensure our sample is similar in baseline characteristics with the other children in the DISPLACE cohort that were not included in our analysis, we compared the demographics and clinical characteristics of children included in our analysis to the children that were excluded due to the missing echocardiogram results.

All reported *P*-values were 2 sided. Statistical significance was defined as $p < 0.05$. All data analysis were performed using SAS 9.4 (SAS Institute, Cary, NC).

TABLE 1 | Demographics and clinical characteristics for children with sickle cell anemia comparing children with PFO to those without.

	N	PFO	NO PFO	*P*-value
Number	1,414	90	1,324	
Age (years)	1,414	4.8 ± 5.3	8.7 ± 46	<0.0001
Sex*, *n* (%)				0.2967
Male	725	41 (46.1)	684 (51.8)	
Female	685	48 (53.9)	637 (48.2)	
Hemoglobin (g/dl)†	950	8.9 (8.0, 9.9)	8.6 (7.8, 9.6)	0.3624
Reticulocyte (per 1,000)†	437	326 (137.8, 407.5)	283.0 (158.0, 395.0)	0.8371
Systolic BP (mmHg)†	642	107 (96, 117)	109 (101, 116)	0.2861
Diastolic BP (mmHg)†	642	64 (53, 68)	62 (56, 68)	0.9791
Abnormal TCD, *n* (%)	142	133 (10.5)	9 (10.0)	
History of stroke, *n* (%)	102	97 (7.3)	5 (5.6)	
Therapy group, *n* (%)				0.0960
No therapy	617	51 (58.6)	566 (46.6)	
Blood trans	190	10 (11.5)	180 (14.8)	
Hydroxyurea	494	26 (29.8)	468 (38.5)	

BP, Blood pressure (mmHg); Blood trans, Chronic red cell transfusion; PFO, Patent foramen ovale.

† Variables are reported as median (interquartile range).

**Variables are reported as the frequency and percent relative to the row attribute.*

P-values from Row mean zero scores differ using Cochran-Mantel-Haenszel test for categorical and ttest or Wilcoxon rank sum test for continuous variables.

RESULTS

Participants' Characteristics at Baseline

The baseline characteristics of the children are shown in table 1. A total of 1,414 children with SCD from DISPLACE were included in the analysis who had completed echocardiograms in the DISPLACE database. All of the children had SCA (HbSS or HbSB0 disease). All children had at least one documented echocardiogram entered in the DISPLACE database, one clinical assessment result and one complete blood count laboratory result. Of the 1,414 children, 6.3% had PFO noted by echocardiogram (**Table 1**).

The mean age of the children who had PFO was 4.8 years and this was significantly lower than those without PFO (8.7years) (*p*

TABLE 2 | Crude and adjusted odds ratios** and 95% Confidence intervals (95% CI) for the association between patent foramen ovale and ischemic stroke.

	Total number	++No PFO	PFO
Patients	1,414	1,324	90
Patients with ischemic stroke (%)	102	97 (7.3)	5 (5.6)
Crude odds ratio (95% CI)	1,414	1.0 (ref)	0.74 (0.30–1.88)
*Adjusted odds ratio (95% CI)	642	1.0 (ref)	1.49 (0.20–11.03)

CI, Confidence interval; PFO, Patent foramen ovale.
***Logistic regression was used to calculate odds ratio comparing children with PFO to those without PFO *adjusted for age, sex, systolic blood pressure, diastolic blood pressure. ++The reference group is the group with no PFO. The sample size used for the adjusted model dropped from 1,414 to 642 due to missing values in some of the covariates included in the model.*

TABLE 3 | Crude and adjusted odds ratios** and 95% Confidence intervals (95% CI) for the association between patent foramen ovale and abnormal TCD.

	Total number	++No PFO	PFO
Patients	1,414	1,324	90
Patients with abnormal TCD velocity (%)	142	133 (10.5)	9 (10.0)
Crude odds ratio (95% CI)	1,414	1.00 (ref)	0.99 (0.49–2.03)
*Adjusted odds ratio (95% CI)	642	1.00 (ref)	0.85 (0.17–4.25)

N (%), Number (percent); CI, Confidence interval; PFO, Patent foramen ovale; TCD, transcranial doppler ultrasound velocity.
***Logistic regression was used to calculate odds ratio comparing children with PFO to those without PFO *adjusted for age, sex, systolic blood pressure, diastolic blood pressure. ++The reference group is the group with no PFO. The sample size used for the adjusted model dropped from 1,414 to 642 due to missing values in some of the covariates included in the model.*

< 0.0001). There were no differences in sex, systolic or diastolic blood pressure, hemoglobin, and/or reticulocyte count between those with PFO and those without PFO (**Table 1**). A lower proportion of children with PFO had been treated on CRCT compared to children without PFO, while a higher proportion of children with PFO had been treated on HU compared to children without, but this difference did not reach statistical significance ($p = 0.0516$). About 29% of children with PFO were on HU while only about 10% of children with PFO were on CRCT.

Association Between PFO and Overt Ischemic Stroke in Children With SCA

A total of 102 (7.2%) of the 1,414 children included in the analysis had an overt ischemic stroke. Out of this 5 (5.6%) had PFO. The odds of stroke was higher in those with PFO but this was not statistically significant [OR: 1.49, 95% CI: 0.20–11.03, $p = 0.6994$; **Table 2**].

Association Between PFO and Abnormal TCD in Children With SCA

A total of 142 (10.0%) of the 1,414 included in the analysis had abnormal TCD. Out of this, only 9 (10%) had PFO. There is no evidence that the odds of having abnormal TCD is higher among those with PFO compared to those without even after adjusting for potential covariates (OR: 0.85, 95% CI: 0.17–4.25, $p = 0.8463$; **Table 3**).

DISCUSSION

In the current crossectional analysis of a large cohort of children with SCA, we found a lower than expected prevalence of PFO as reported in the routine, presumably all non-contrast, transthoracic echocardiograms. Only 6% of the children with SCA were found to have PFO on routine echocardiogram.

Our results did not support the hypothesis that PFO is associated with overt ischemic stroke in children with SCA as only ∼5% of children with PFO had overt ischemic stroke. In contrast to this study, previous studies have reported consistently higher prevalence of PFO in the general population (30) as well as in children with SCA. In a meta-analysis, Mattle et al. showed an increased risk of stroke among adults with PFO compared to those without (30). One study, in which transthoracic and/or trans esophageal echocardiography was used in more than three quarters of children with stroke, found the prevalence of PFO to be low (31). However there is evidence for a higher prevalence of right-to-left shunting, including at atrial level, in children with stroke undergoing bubble contrast, e.g., with transthoracic echocardiography or transcranial Doppler (cTCD) (32, 33); this is considered particularly important in those without vasculopathy (32). Similarly, previous studies have reported an increased risk of stroke among adults and children with SCA and PFO (16, 17, 34). In a study by Dowling et al. they found a significantly higher prevalence of right to left shunting in children with SCA compared to those without SCA. Importantly, this study included any identified Right to Left shunting as the target variable including but not limited to PFO (16). Additionally, they also found that those with any potential right to left shunting had a greater odds of being in the group of children with SCA and stroke compared to children without SCD. There were notable differences between their study population and their target variable and ours which may have contributed to the inconsistencies in the results. We included a control group of children with SCA but without stroke, while they had a control group of children without SCA or stroke.

Considering the pathophysiology of stroke in SCA, our results showed that even though PFO may be a potential risk factor for stroke in the general population, in children with SCA, other risk factors seem to play a more important role in the pathogenesis of stroke than the PFO. The presence of PFO should not be considered to be confirmatory evidence of paradoxical embolic stroke in children with SCA and overt ischemic stroke. As shown in a review of echocardiogram features and paradoxical emboli by Aggeli et al. even among the general population, the presence of PFO does not necessarily confirm the diagnosis of paradoxical embolic stroke in a patient with an overt ischemic stroke. The patient's clinical history, brain, cerebrovascular, and cardiovascular imaging should be reviewed in detail to determine whether embolic stroke is a possibility and if so, candidate sources including carotid or vertebral vasculopathy as well as right-to-left shunting (8). Further, in another review, Aggeli et al. showed that even though PFO may increase the risk of occurrence of an embolic stroke, there were not enough data to determine whether PFO closure or antiplatelet drugs prevented recurrence in these patients (35).

In the current study we found no significant association between PFO and abnormal TCD velocity. Approximately 10% of the children with PFO had abnormal TCD on their sickle stroke screen. Considering abnormal TCD velocity is the most important surrogate use to identify those at increased risk of overt ischemic stroke in children with SCA (19, 36, 37), our hypothesis was that children with SCA with abnormal TCD velocities will have higher prevalence of PFOs compared to those with normal TCD velocities. However, this was not supported by our findings.

Age was found to be significantly associated with PFO. The median age for children with PFO was significantly lower than that of children without PFO. This is expected and consistent with findings from previous studies showing that the majority of PFOs close during infancy, and for some the closure may be gradual during childhood (38). Some studies reported complete anatomic closure in up to 70–75% of adults (39).

In the present study, hemolytic factors including hemoglobin level and reticulocyte count were not significantly associated with presence of PFO. Although there is limited data on the relationship between hemolytic factors and PFO, Kucukal et al. showed that increased red cell adhesion markers correlate strongly with hemolytic markers including hemoglobin level and reticulocyte count and a history of intrapulmonary right to left shunts (40).

Limitations of the Study

As expected with a retrospective crossectional study design, our study has limitations. DISPLACE is a real-world evaluation of current practice in clinical centers in the US. All echocardiograms were performed according to standard of care for the institution. The reports collected from each center were used according to how they were reported from the center with no additional review conducted as part of this study. PFO was defined as present/absent on the echocardiogram report. Not all children had a bubble study to determine the presence of PFO. We had a low number of events compared to what was reported in previous studies. Additionally, we used only available data and therefore were not able to include echocardiograms with reasonable amount of missing data and DISPLACE participants who do not have echo results. Therefore, in the adjusted models for the logistic regression, the number of children included was only 45% of the full cohort To ensure our study population did not differ from the original DISPLACE study, we compared the baseline characteristics of the participants included in our analysis to the general DISPLACE participants and we found no significant difference in the baseline demographic and laboratory characteristics between the two populations (**Supplementary Table 1**).

The strengths of our study include the use of a large national population sample of children with SCA from 28 sites across the US, which therefore facilitates the generalizability of our findings. To our knowledge, this is one of the largest studies investigating stroke and PFO in sickle cell disease. In addition, our study used a comparison group of children with SCA without PFO and therefore making the two groups more comparable in terms of baseline characteristics.

CONCLUSION

Our study showed a much lower prevalence of PFO among children with SCA than previously published. We found no increased risk of stroke or abnormal TCD among those with PFO compared to those without PFO in children with SCA. This could be due to the fact that overall DISPLACE recorded a much lower frequency of abnormal TCD than reported in older studies (25). We believe that even though PFO may be associated with increased risk of overt ischemic stroke in the general population, in children with SCA, other known risk factors including anemia, hypertension, silent infarction and history of acute chest syndrome, likely play a more important role. Contrast echocardiography or transcranial Doppler may be considered in patients with SCA of any age who have had an overt stroke and have no evidence of vasculopathy on TCD or MRA. If treatment, e.g., closure or antiplatelet therapy, is standard of care for people with cryptogenic stroke and right-to left shunting, larger prospective studies in SCA should focus on diagnosis or exclusion of right to left shunting at cardiac or pulmonary level as a risk factor for overt and silent stroke.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because of privacy reasons. However, aggregated datasets may be shared. Requests to access the datasets should be directed to Julie Kanter, jkanter@uabmc.edu.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by University of Alabama at Birmingham Institutional Review Board. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

NG, WJ, VH, and JK designed the study. AC, GH, and VH wrote and reviewed the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This study was supported by the grant 5R01HL133896-04 to JK from NIH and Pre-doctoral grant 20PRE35210531 to NG from AHA.

ACKNOWLEDGMENTS

The authors would like to thank the National Heart Blood and Lung Institute (NHLBI) of the National Institute for Health (NIH), USA and the American Heart Association (AHA).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2021.761443/full#supplementary-material

REFERENCES

1. Baker C, Grant AM, George MG, Grosse SD, Adamkiewicz TV. Contribution of sickle cell disease to the pediatric stroke burden among hospital discharges of African-Americans-United States, 1997-2012. *Pediatr Blood Cancer.* (2015) 62:2076–81. doi: 10.1002/pbc.25655
2. Adams RJ, McKie VC, Hsu L, Files B, Vichinsky E, Pegelow C, et al. Prevention of a first stroke by transfusions in children with sickle cell anemia and abnormal results on transcranial doppler ultrasonography. *N Engl J Med.* (1998) 339:5–11. doi: 10.1056/NEJM199807023390102
3. DeBaun MR, Jordan LC, King AA, Schatz J, Vichinsky E, Fox CK, et al. American society of hematology 2020 guidelines for sickle cell disease: prevention, diagnosis, and treatment of cerebrovascular disease in children and adults. *Blood Adv.* (2020) 4:1554–8. doi: 10.1182/bloodadvances.2019001142
4. Ohene-Frempong K, Weiner SJ, Sleeper LA, Miller ST, Embury S, Moohr JW, et al. Cerebrovascular accidents in sickle cell disease: rates and risk factors. *Blood.* (1998) 91:288–94.
5. Adams RJ. Big strokes in small persons. *Arch Neurol.* (2007) 64:1567–74. doi: 10.1001/archneur.64.11.1567
6. Miller ST, Macklin EA, Pegelow CH, Kinney TR, Sleeper LA, Bello JA, et al. Silent infarction as a risk factor for overt stroke in children with sickle cell anemia: a report from the cooperative study of sickle cell disease. *J Pediatr.* (2001) 139:385–90. doi: 10.1067/mpd.2001.117580
7. Lechat P, Mas JL, Lascault G, Loron P, Theard M, Klimczac M, et al. Prevalence of patent foramen ovale in patients with stroke. *N Engl J Med.* (1988) 318:1148–52. doi: 10.1056/NEJM198805053181802
8. Aggeli C, Verveniotis A, Andrikopoulou E, Vavuranakis E, Toutouzas K, Tousoulis D. Echocardiographic features of PFOs and paradoxical embolism: a complicated puzzle. *Int J Cardiovasc Imaging.* (2018) 34:1849–61. doi: 10.1007/s10554-018-1406-1
9. Overell JR, Bone I, Lees KR. Interatrial septal abnormalities and stroke: a meta-analysis of case-control studies. *Neurology.* (2000) 55:1172–79. doi: 10.1212/WNL.55.8.1172
10. Dowling MM, Ikemba CM. Intracardiac shunting and stroke in children: a systematic review. *J Child Neurol.* (2011) 26:72–82. doi: 10.1177/0883073810383913
11. Srisuwananukorn A, Raslan R, Zhang X, Shah BN, Han J, Gowhari M, et al. Clinical, laboratory, and genetic risk factors for thrombosis in sickle cell disease. *Blood Adv.* (2020) 4:1978–86. doi: 10.1182/bloodadvances.2019001384
12. Ataga KI, Brittain JE, Desai P, May R, Jones S, Delaney J, et al. Association of coagulation activation with clinical complications in sickle cell disease. *PLoS ONE.* (2012) 7:e29786. doi: 10.1371/journal.pone.0029786
13. Colombatti R, De Bon E, Bertomoro A, Casonato A, Pontara E, Omenetto E, et al. Coagulation activation in children with sickle cell disease is associated with cerebral small vessel vasculopathy. *PLoS ONE.* (2013) 8:e78801. doi: 10.1371/journal.pone.0078801
14. Shah N, Thornburg C, Telen MJ, Ortel TL. Characterization of the hypercoagulable state in patients with sickle cell disease. *Thromb Res.* (2012) 130:e241–5. doi: 10.1016/j.thromres.2012.08.307
15. Hyacinth HI, Adams RJ, Greenberg CS, Voeks JH, Hill A, Hibbert JM, et al. Effect of chronic blood transfusion on biomarkers of coagulation activation and thrombin generation in sickle cell patients at risk for stroke. *PLoS ONE.* (2015) 10:e0134193. doi: 10.1371/journal.pone.0134193
16. Dowling MM, Quinn CT, Ramaciotti C, Kanter J, Osunkwo I, Inusa B, et al. Increased prevalence of potential right-to-left shunting in children with sickle cell anaemia and stroke. *Br J Haematol.* (2017) 176:300–8. doi: 10.1111/bjh.14391
17. Dowling MM, Lee N, Quinn CT, Rogers ZR, Boger D, Ahmad N, et al. Prevalence of intracardiac shunting in children with sickle cell disease and stroke. *J Pediatr.* (2010) 156:645–50. doi: 10.1016/j.jpeds.2009.10.012
18. Razdan S, Strouse JJ, Naik R, Lanzkron S, Urrutia V, Resar JR, et al. Patent foramen ovale in patients with sickle cell disease and stroke: case presentations and review of the literature. *Case Rep Hematol.* (2013) 2013:516705. doi: 10.1155/2013/516705
19. Adams RJ, McKie VC, Carl EM, Nichols FT, Perry R, Brock K, et al. Long-term stroke risk in children with sickle cell disease screened with transcranial doppler. *Ann Neurol.* (1997) 42:699–704. doi: 10.1002/ana.410420505
20. Kwiatkowski JL, Voeks JH, Kanter J, Fullerton HJ, Debenham E, Brown L, et al. Ischemic stroke in children and young adults with sickle cell disease in the post-STOP era. *Am J Hematol.* (2019) 94:1335–43. doi: 10.1002/ajh.25635
21. Crosby LE, Joffe NE, Davis B, Quinn CT, Shook L, Morgan D, et al. Implementation of a process for initial transcranial doppler ultrasonography in children with sickle cell anemia. *Am J Prev Med.* (2016) 51(1 Suppl. 1):S10–6. doi: 10.1016/j.amepre.2016.01.021
22. Reeves SL, Fullerton HJ, Cohn LM, Dombkowski KJ, Boulton ML, Braun TM, et al. Missed opportunities for transcranial doppler screening among children with sickle cell disease. *Clin Pediatr.* (2016) 55:1093–9. doi: 10.1177/0009922815614351
23. Weisman JK, Diamond CE, Kappa S, Nickel RS. Transcranial doppler screening adherence among children with sickle cell anemia seen in the emergency department. *J Pediatr.* (2019). 217:172–76. doi: 10.1182/blood-2018-99-115057
24. Reeves SL, Madden B, Freed GL, Dombkowski KJ. Transcranial doppler screening among children and adolescents with sickle cell anemia. *JAMA Pediatr.* (2016) 170:550–6. doi: 10.1001/jamapediatrics.2015.4859
25. Kanter J, Phillips S, Schlenz AM, Mueller M, Dooley M, Sirline L, et al. Transcranial doppler screening in a current cohort of children with sickle cell anemia: results from the DISPLACE study. *J Pediatr Hematol Oncol.* (2021) 43:e1062–8. doi: 10.1097/MPH.0000000000002103
26. Silvestry FE, Cohen MS, Armsby LB, Burkule NJ, Fleishman CE, Hijazi ZM, Interventions. Guidelines for the echocardiographic assessment of atrial septal defect and patent foramen ovale: from the American society of echocardiography and society for cardiac angiography and interventions. *J Am Soc Echocardiogr.* (2015) 28:910–58. doi: 10.1016/j.echo.2015.05.015
27. Writing Group for Echocardiography in Outpatient Pediatric C, Campbell RM, Douglas PS, Eidem BW, Lai WW, Lopez L, et al. ACC/AAP/AHA/ASE/HRS/SCAI/SCCT/SCMR/SOPE 2014 appropriate use criteria for initial transthoracic echocardiography in outpatient pediatric cardiology: a report of the American college of cardiology appropriate use criteria task force, american academy of pediatrics, American heart association, American society of echocardiography, heart rhythm society, society for cardiovascular angiography and interventions, society of cardiovascular computed tomography, society for cardiovascular magnetic resonance, and society of pediatric echocardiography. *J Am Soc Echocardiogr.* (2014) 27:1247–66. doi: 10.1016/j.echo.2014.10.002
28. Eddine AC, Alvarez O, Lipshultz SE, Kardon R, Arheart K, Swaminathan S. Ventricular structure and function in children with sickle cell disease using conventional and tissue doppler echocardiography. *Am J Cardiol.* (2012) 109:1358–64. doi: 10.1016/j.amjcard.2012.01.001
29. Faro GB, Menezes-Neto OA, Batista GS, Silva-Neto AP, Cipolotti R. Left ventricular hypertrophy in children, adolescents and young adults

with sickle cell anemia. *Rev Bras Hematol Hemoter.* (2015) 37:324–28. doi: 10.1016/j.bjhh.2015.07.001
30. Mattle HP, Meier B, Nedeltchev K. Prevention of stroke in patients with patent foramen ovale. *Int J Stroke.* (2010) 5:92–102. doi: 10.1111/j.1747-4949.2010.00413.x
31. Ganesan V, Prengler M, McShane MA, Wade AM, Kirkham FJ. Investigation of risk factors in children with arterial ischemic stroke. *Ann Neurol.* (2003) 53:167–73. doi: 10.1002/ana.10423
32. Benedik MP, Zaletel M, Meglic NP, Podnar T. A right-to-left shunt in children with arterial ischaemic stroke. *Arch Dis Child.* (2011) 96:461–7. doi: 10.1136/adc.2010.203992
33. Khan R, Chan AK, Mondal TK, Paes BA, Thrombosis and Hemostasis in Newborns G. Patent foramen ovale and stroke in childhood: a systematic review of the literature. *Eur J Paediatr Neurol.* (2016) 20:500–11. doi: 10.1016/j.ejpn.2016.04.012
34. Razdan S, Strouse JJ, Reddy A, Resar DF, Hasan RK, Resar JR, et al. Patent foramen ovale in adults with sickle cell disease and stroke. *Am J Hematol.* (2016) 91:E358–60. doi: 10.1002/ajh.24440
35. Aggeli C, Polytarchou K, Dimitroglou Y, Patsourakos D, Delicou S, Vassilopoulou S, et al. Stroke and presence of patent foramen ovale in sickle cell disease. *J Thromb Thrombolysis.* (2021) 52:889–97. doi: 10.1007/s11239-021-02398-3
36. Adams R, McKie V, Nichols F, Carl E, Zhang DL, McKie K, et al. The use of transcranial ultrasonography to predict stroke in sickle cell disease. *N Engl J Med.* (1992) 326:605–10. doi: 10.1056/NEJM199202273260905
37. Adams RJ, Nichols FT, Figueroa R, McKie V, Lott T. Transcranial doppler correlation with cerebral angiography in sickle cell disease. *Stroke.* (1992) 23:1073–7. doi: 10.1161/01.STR.23.8.1073
38. Hampton T, Alsaleem M, Murphy-Lavoie HM. Patent foramen ovale. Treasure Island, FL: StatPearls (2021).
39. Russo MG, Cirillo A, Rinelli G, Vairo U, Favilli S, Moreo A, et al. [Physiological and paraphysiological echocardiographic findings in neonatal and pediatric age]. *G Ital Cardiol.* (2019) 20:627–31. doi: 10.1714/3254.32223
40. Kucukal E, Man Y, Quinn E, Tewari N, An R, Ilich A, et al. Red blood cell adhesion to ICAM-1 is mediated by fibrinogen and is associated with right-to-left shunts in sickle cell disease. *Blood Adv.* (2020) 4:3688–98. doi: 10.1182/bloodadvances.2020001656

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers | Frontiers in Neurology

REVIEW
published: 21 June 2022
doi: 10.3389/fneur.2022.835823

Considerations for Selecting Cognitive Endpoints and Psychological Patient-Reported Outcomes for Clinical Trials in Pediatric Patients With Sickle Cell Disease

Anna M. Hood[1]*, *Lori E. Crosby*[2,3,4], *Hanne Stotesbury*[5], *Melanie Kölbel*[5] and *Fenella J. Kirkham*[5]

[1] Division of Psychology and Mental Health, Manchester Centre for Health Psychology, University of Manchester, Manchester, United Kingdom, [2] Division of Behavioral Medicine, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, United States, [3] Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, OH, United States, [4] James M. Anderson Center for Health Systems Excellence, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, United States, [5] Developmental Neurosciences Unit and Biomedical Research Centre, University College London Great Ormond Street Institute of Child Health, London, United Kingdom

OPEN ACCESS

Edited by:
Kirsten A. Donald, University of Cape Town, South Africa

Reviewed by:
Sanford Auerbach, Boston University, United States
Jeffrey Schatz, University of South Carolina, United States

***Correspondence:**
Anna M. Hood
anna.hood@manchester.ac.uk

Specialty section:
This article was submitted to Pediatric Neurology, a section of the journal Frontiers in Neurology

Received: *14 December 2021*
Accepted: *20 May 2022*
Published: *21 June 2022*

Citation:
Hood AM, Crosby LE, Stotesbury H, Kölbel M and Kirkham FJ (2022) Considerations for Selecting Cognitive Endpoints and Psychological Patient-Reported Outcomes for Clinical Trials in Pediatric Patients With Sickle Cell Disease. Front. Neurol. 13:835823. doi: 10.3389/fneur.2022.835823

Pediatric patients with sickle cell disease (SCD) experience a range of medical complications that result in significant morbidity and mortality. Recent advances in prophylactic and curative treatment approaches have highlighted the need for sensitive and clinically-meaningful trial endpoints. The detrimental effects of cognitive and psychological difficulties on social and economic mobility are well described. Although numerous reviews have assessed cognitive outcomes in other rare genetic disorders, SCD has not received the same focus. This review describes the cognitive (i.e., executive function and processing speed) and psychological domains (i.e., depression and anxiety) that are consistently associated with SCD pathology and, therefore, may be of particular interest as clinical trial endpoints. We then discuss corresponding well-validated and reliable cognitive tests and patient-reported outcomes (PROs) that may be appropriate for clinical trials given their robust psychometric properties, ease of administration, and previous use in the SCD population. Further, we provide a discussion of potential pitfalls and considerations to guide endpoint selection. In line with the move toward patient-centered medicine, we identify specific tests (e.g., NIH Toolbox Cognition Module, Wechsler Cancellation Test) and psychological PROs (e.g., PROMIS depression and anxiety scales) that are sensitive to SCD morbidity and have the potential to capture changes that are clinically meaningful in the context of patients' day to day lives. In particularly vulnerable cognitive domains, such as executive function, we highlight the advantages of composite over single-test scores within the context of trials. We also identify general (i.e., practice effects, disease heterogeneity) and SCD-specific considerations (i.e., genotype, treatment course, and disease course, including degree of neurologic, pain, and sleep morbidity) for trial measures. Executive function composites

hold particular promise as trial endpoints that are clinically meaningful, amenable to change, relatively easy to collect, and can be incorporated into the routine care of patients with SCD in various settings and countries.

Keywords: executive function, processing speed, depression, anxiety, intervention

INTRODUCTION

Sickle cell disease (SCD) is an umbrella term for a group of inherited disorders that affect the structure of hemoglobin and reduce the overall oxygen-carrying capacity of the blood (1). SCD affects ∼100,000 individuals in the United States (US) and between 50,000 and 60,000 individuals in Europe (2), who are mainly immigrants or the descendants of individuals from endemic areas such as Sub-Saharan Africa (3, 4). For many years, chronic blood transfusion and hydroxycarbamide have been the primary therapeutic tools for SCD. Chronic blood transfusion remains the gold-standard treatment for stroke prevention (5). The US and European guidelines (6, 7) also highlight that hydroxycarbamide should be available for all pediatric (>9-months of age) SCD populations, and there is abundant evidence for laboratory and clinical efficacy (8, 9). Bone marrow and stem cell transplantations have long remained the only clinically available curative treatment options, but there are significant risks, and donors must be closely matched with recipients for optimal outcomes (10, 11).

After years of stagnation, there has recently been an explosion in prophylactic and potentially curative treatment options for patients with SCD. The US Food and Drug Administration (FDA) has approved triple the number of new therapies within the past 4 years compared with the three decades prior. Among these treatments is the L-glutamine amino acid, Endari, which reduced oxidative stress and admissions for pain in a recent phase 3 trial (12). Others include Crizanlizumab, a humanized monoclonal antibody that binds to P-selectin, inhibiting adhesive interactions that may play a central role in pain episodes in SCD (13). Voxelotor (Oxbryta), a small molecule that binds to hemoglobin, inhibits hemoglobin polymerization and increases the hemoglobin's affinity for oxygen, was also recently approved (14). Although interest has also grown in curative therapies, including gene therapy (i.e., inserting genes to make healthy red blood cells) and gene editing (alteration of a selected DNA sequence in a living cell), these remain in the early stages of evaluation (15) (see **Figure 1** for an overview of treatment options). Other innovative approaches currently under investigation in clinical trials in SCD include behavioral interventions (Clinical Trial No: NCT03150433) and Montelukast (Clinical Trial No: NCT04351698) (16) for comorbid sleep-disordered breathing (16).

Given the increase in novel therapeutic and curative approaches for the treatment of SCD, identifying sensitive and clinically meaningful endpoints for clinical trials is a pressing issue. Cognitive deficits (17, 18) have been identified frequently in patients with SCD, with more profound deficits observed in those with more severe neurologic injury (i.e., infarction) (19). Additionally, patients with SCD experience disproportionately high rates of psychological difficulties (i.e., depression and anxiety) (20, 21). Although more than 100 reports have documented the effects of 82 treatments on cognitive outcomes in patients with other rare genetic disorders (22), these areas have not received the same focus in SCD. Highlighting the knowledge gap, the American Society of Hematology (ASH) and the American Food and Drug Administration (FDA) recently partnered to develop consensus recommendations for clinical trial endpoints for patients with SCD (23). The ASH report included a summary of suggested cognitive tests and psychological patient-reported outcome measures (PROs) to use as endpoints. Our goal is to build upon these broad recommendations and discuss in-depth cognitive and psychological PROs that may be appropriate for use in clinical trials whilst discussing specific factors and potential pitfalls that must be carefully considered in selecting cognitive and psychological endpoints for trials.

Systemic SCD vascular pathology may simultaneously affect multiple end-organs with direct and indirect effects on the brain (24), and cognitive outcomes represent the final common pathway (25). Therefore, cognition may be well suited to assess the functional benefit of new therapeutic approaches to vascular end-organ disease. Further, better psychological functioning is associated with improved patient-reported and functional life outcomes, including quality of life (26, 27) and scholastic and employment gains (28). Cognitive tests and psychological PROs may also have several distinct advantages as endpoints for patients with SCD, including their ability to reliably capture meaningful cognitive impairment and psychological difficulties, their rigorous validation in the population, and their sensitivity to change.

Despite the significant advances that have resulted from randomized controlled trials across all chronic illness pediatric populations, the proportion of worldwide pediatric trials remains low at 9% (29). Similarly, although children bear 25% of the global chronic disease burden (30), few medicines are approved specifically for children, with rates of off-label prescribing estimated as high as 90%. Further, one study demonstrated that 38% of pediatric studies had yet to be completed for many drugs (e.g., anti-infective) authorized for adult use up to a decade ago (31). Given the evidence that early intervention may significantly reduce the risk of acute events (32) and that over 40% of patients with SCD are children (33), pediatric clinical trials are urgently needed in this population.

With the evidence for the effects of SCD on cognition and the proliferation of disease-modifying therapies in the last few years, including cognitive and psychological endpoints in clinical trials is a vital next step that may improve the knowledge-base around clinically meaningful outcomes in pediatric patients with SCD. Although cognitive dysfunction (17, 18, 34) and psychological

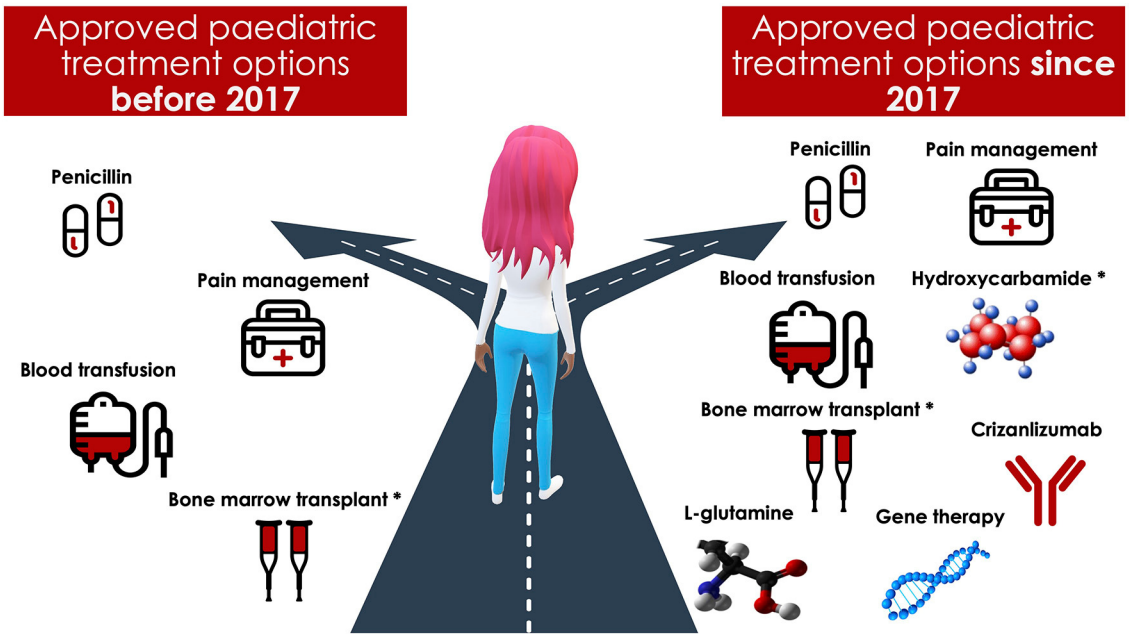


FIGURE 1 | An overview of treatment options for pediatric patients with sickle cell disease. *Hydroxycarbamide was used off label for pediatric patients with sickle cell disease before 2017. Bone marrow transplantation requires a matched donor.

difficulties (20) have been reported in pediatric patients with SCD for many years, there has been a focus on documenting *all* observed challenges. Relatively less attention has been paid to the specific domains with the most significant deficits, likely to be related to the pathophysiology and potentially preventable or even reversible, even though further focus in these areas could inform the development of targeted interventions. In view of childhood being a critical window for intervention, our paper will focus on the pediatric SCD population (0–18 years) and present a description of the cognitive and psychological domains in which patients frequently experience difficulties. Additionally, this paper will identify well-validated and reliable cognitive tests and psychological PROs that are capable of capturing changes beyond any practice effects that are clinically meaningful in the day to day lives of pediatric patients with SCD, and which may therefore hold promise as trial endpoints.

SELECTING COGNITIVE AND PSYCHOLOGICAL DOMAINS

Selecting appropriate cognitive tests and psychological PROs as endpoints in clinical trials is not trivial. Endpoints are the analyzed parameters (e.g., change from baseline to 12 weeks in standardized cognitive scores) and should be relatively easy to collect, proximal to the disease or treatment, medically significant, meaningful to patients, families and providers, and ideally be available for incorporation into routine care in a variety of settings and countries (35). Cognitive tests and psychological PROs fit all of these criteria, and patients with SCD and their advocates have called for their inclusion as critical endpoints for clinical trials assessing disease-modifying therapies (36).

Although cognitive function encompasses a variety of domains, including general intelligence, language, visual-spatial abilities, and memory, patients with SCD appear to experience particular difficulties in the domains of executive function (37–39), processing speed (40), and attention (41, 42). Psychological functioning covers an equally broad range of domains, comprising behavior, emotion, social skills, and overall mental health. In the SCD population, however, depression and anxiety symptoms appear to be the most common psychological challenges (20, 21). Below, we consider these cognitive domains and areas of psychological functioning in which difficulties have been most consistently reported in patients with SCD, and which therefore may be of particular interest as clinical trial endpoints.

Intelligence Quotient

Significantly reduced intelligence quotients (IQ) are often observed in patients with SCD, and IQ is the most frequently reported indicator of general cognitive abilities (17, 18). However, although IQ provides a single composite with robust statistical properties, we do not recommend using IQ as an endpoint in clinical trials that include pediatric patients with SCD (see **Table 1** for a detailed consideration).

Executive Function

Moving beyond IQ, domain-specific tests of cognition are likely better able to capture changes in pediatric patients with SCD within the context of a clinical trial. Executive function is the domain that has received the most attention in both the SCD and broader literature. Debates remain as to the far transfer

TABLE 1 | Intelligence quotient (IQ)—considerations for use as a cognitive endpoint in clinical trials of pediatric patients with sickle cell disease.

Description	Reasons may not be appropriate for clinical trials	Reasons may be appropriate for clinical trials
IQ is a total score derived from a set of standardized subtests (i.e., verbal and perceptual reasoning) designed to assess human intelligence. Each specific subtest (raw score) is compared to other children in the same age group (normative sample). Generally, an average IQ is 100 with a standard deviation of 15. Sixty-eight percent of population scores lie between 85 and 115.	IQ represents an aggregate or global capacity comprising a set of related but distinguishable abilities (43, 44). IQ is also not designed to measure the extent of cognitive impairment in single domains (43, 44). IQ enables a compensation model, i.e., a deficit in one subtest (e.g., Block Design) may be compensated by better performance in another, related, but distinct subtest (e.g., Vocabulary) (45). An IQ composite may obscure meaningful differences among subtest scores, conflating relative strengths and weaknesses (45). If no global IQ effect is observed investigators may accept the null hypothesis erroneously.	The Wechsler Abbreviated Scale of Intelligence Second Edition (WASI-II 2 or 4 subtest versions) takes 15–30 min to complete, respectively, can be given to pediatric patients aged as young as 6 years of age, and IQ along with verbal comprehension and perceptual reasoning indices can be obtained (47).
	Some subtests that make up the IQ are less sensitive to change during a clinical trial period, i.e., the Vocabulary and Similarities subtests rely on crystallized intelligence (accumulated knowledge based on experience) (46) that are unlikely to demonstrate an increase in IQ through a short-term intervention.	Researchers may want to screen for global intellectual disabilities obtained as a baseline outcome rather than as a primary endpoint.
	There is limited evidence that interventions to improve IQ have sustained effects after they end (48).	
	IQ also only captures a subset of cognitive abilities pertinent to everyday functioning. Scores do not comprehensively reflect abilities in areas that are particularly vulnerable in pediatric patients with sickle cell disease.	Researchers may want to match IQ across treatment and placebo arms obtained as a baseline outcome rather than as a primary endpoint.
	Full-scale IQ requires administering a minimum of 10 subtests, which can take between 1 and 2 h depending on the participant's age and the need for breaks. This assessment length may be burdensome on both the administrator and the participant.	
	Collecting and interpreting IQ data as an endpoint in a clinical trial may be logistically difficult and prohibitively expensive.	
	Pediatric patients with sickle cell disease tend to score relatively lower on subtests (i.e., Vocabulary and Similarities) included in estimates or shorter version IQ tests. These tests does not include working memory and processing speed subtests; thus, estimates of IQ may be overestimated.	

(e.g., to other skills) and length of benefits (e.g., >1 year) following interventions to improve executive function (49). The relative influence of socioeconomic status on performance is also an area of debate (50). Nevertheless, there is evidence that executive function is trainable to a certain degree and that more training leads to more significant gains (e.g., dosage effects) (51). The core executive functions comprise higher-level cognitive processes composed of three interrelated core skills: inhibitory control, working memory, and cognitive flexibility (52). Inhibitory control involves resisting the expression of an instinctive response and/or impulse to do something. Working memory involves holding information in mind while performing one or more mental operations. Cognitive flexibility is the mental ability to switch between concepts, flexibly adjust to changing demands, and look at something from a different perspective (53).

The dorsolateral prefrontal cortex is a region of the frontal lobes associated with executive function (54), and alterations in functional connectivity in this brain region have been observed in patients with SCD (55). There is evidence that deficits in switching and inhibition may be moderated by lesion type and location in patients with SCD, with one study indicating that children with frontal lesions showed the greatest impairments (56). Another study found a diminished event-related potential component difference between error and correct responses in SCD children with frontal lesions, indicating weaker response monitoring systems (57). Given that the prefrontal cortex also mediates social behavior (58), interventions that improve executive functions could, in theory, also indirectly support improved quality of life via improved social behavior, which may reciprocally enhance executive function. Support for this theory has been demonstrated through a 6-month executive function and social information intervention administered in the classroom (e.g., preschoolers without SCD) that showed improved inhibition, visual attention, and flexibility along with improved social processing skills (59).

Using executive function as a cognitive endpoint in a clinical trial in pediatric patients with SCD has several advantages. In the general population, executive function has demonstrated more predictive power than IQ, with working memory more predictive of scholastic success (60) and childhood inhibitory control revealed as more predictive of adult outcomes, including physical and mental health, criminal activity, and quality of life (61). Cognitive flexibility also predicts the ability to bounce back from and adapt to negative life events and everyday stressors (62). Specific to patients with SCD, there is considerable

empirical evidence that executive dysfunction is related to sleep (63), persistent pain (64), chronic fatigue (65), abnormal blood velocities (66), cerebral blood flow (67), and quality of life (68–70), which are all often domains that are targeted in clinical trials of patients with SCD. Additionally, non-randomized studies have demonstrated that computerized working memory training programs (38, 39) and proximity to a blood transfusion (37) improve executive function. Taken together, these studies provide evidence that executive function is amenable to change in populations with SCD and that performance in this domain may serve as a clinically meaningful endpoint in future trials.

Processing Speed

Processing speed refers to processing information that can be sensed, perceived, understood, and responded to rapidly (71) and has been identified as a core component of attention (e.g., sustained, selective, and focused). Slowed processing speed can limit cognitive function in other domains (e.g., how much information can be attended to or encoded) (72); however, although interrelated, processing speed has been shown to be separable from other cognitive processes (73). Importantly, processing speed is a sensitive and specific cognitive domain for pediatric patients with SCD (40). Several studies have found slower latency rather than poorer accuracy between patients with SCD and controls, which indicates that slowed processing speed may mediate impairments across other cognitive domains (74–77). Similar to executive function, processing speed has also been associated with SCD morbidity, including reduced arterial oxygen content and white matter integrity (40) and increased oxygen extraction fraction, a potential marker of ischemic risk (67).

A Phase 1 randomized controlled trial (RCT) has also demonstrated improved processing speed (Cancellation subtest of the WISC) in twelve patients with SCD randomized to 6 weeks of auto-adjusting positive airway pressure treatment (APAP) (78). Processing speed was also the primary endpoint for a larger, longer trial of APAP in children and adults with SCD (79), and along with executive function, is the primary endpoint of the planned trial to improve sleep-disordered breathing in young children with SCD (16). Given the current evidence that improved processing speed is related to improved functional outcomes and has demonstrated change following treatment in an RCT, it is a potentially sensitive and clinically meaningful endpoint in clinical trials for patients with SCD. Researchers could also consider including a test of processing speed as a measured outcome and then controlling for it in analyses assessing change in executive function endpoint to determine if it has explanatory power.

Attention

Attention is a complex set of processes that allow individuals to select and concentrate on relevant stimuli. There have been relatively few studies specifically on attention in pediatric patients with SCD (17). However, prevalence rates of ADHD in children with SCD appear to be higher than the general pediatric population estimate of ∼10% (80), with studies conducted in the US finding rates between 19 and 40% (81–83). In pediatric patients with SCD, a pilot RCT has also demonstrated the short-term efficacy of stimulant medication in improving attention compared with placebo (41). Given that we have less evidence about attention in pediatric patients with SCD, at this time, we suggest that it should not be considered as a trial endpoint, particularly as so many different tests and measures have been used to assess this domain in a relatively small number of studies (17). However, attention tests could be included in trials as measured outcomes to learn if deficits are as widespread and persistent as those found in the executive function and processing speed domains.

Depression and Anxiety

Depression and anxiety are diagnosable disorders that cause a persistent feeling of sadness and loss of interest, or a feeling of unease, such as worry or fear, respectively. The prevalence of depression in the pediatric SCD population is unclear, with estimates between 4 and 46% (84). These estimates are mostly much higher than the general population of Non-Hispanic Black adolescents and young adults (7–9%) (85). The prevalence of anxiety disorders is lower for children (8–17%) but may still significantly impact quality of life (86–88). Behavioral depression and anxiety-related interventions (in-person, mobile-app, pharmacological) using cognitive-behavioral therapy (CBT) (89) have been successful for patients with SCD in lowering negative thinking (90, 91) and improving coping skills (92–94). Similar to measures of attention, however, additional evidence is needed before depression and anxiety PROs (symptomology and diagnostic) should be used as endpoints in clinical trials assessing disease-modifying therapies in pediatric patients with SCD. Instead, we recommend that depression and anxiety are measured as outcomes within the context of a clinical trial to determine the relationship between cognitive domains and the therapeutic of interest.

COGNITIVE TEST AND PSYCHOLOGICAL PROs SELECTION FOR CLINICAL TRIALS

The first, though often overlooked, consideration when choosing an endpoint is assessing whether the normative data collected from the test reflects the country's broad demographic characteristics in which testing is conducted, including factors such as age, racialised identity, sex, and educational status. Choosing a test can be challenging when assessing the majority Black SCD population, particularly on the African continent, as most tests are normed in countries with majority White populations (e.g., the US and the United Kingdom) using census data to determine the number of children from racialised identities. Moreover, no test is culture-free and cognitive processes such as visual perception and spatial reasoning can develop in culturally-distinct ways (95). To overcome these challenges, clinical trials could measure the change in raw (i.e., the actual score generated on a test) rather than standardized (i.e., normative age-scaled) scores. Clinical trials in which multiple institutions across different countries collect data must also determine whether the endpoint is appropriate for

all institutions, particularly if some are high vs. low resource institutions or in countries in the Global North or South.

Many of the standardized cognitive tests and psychological PROs recommended in **Tables 1**, **2** are available in languages other than English, most often Spanish. Conducting language translations (e.g., from the original language to the target language) when the primary language of the population of focus is not available requires bicultural translators to generate culturally-responsive translations that address the discrepancies and cultural ambiguities that occur with text translations (102). Investigators should recognize that language adaptation of commercially distributed tests is not always possible as publishers may not help to facilitate this process (103) and that translating a test from one language to another does not eliminate the need to consider cultural influences.

Clinical trials of pediatric patients with SCD can follow established guidelines for other conditions in which patients experience cognitive impairment (e.g., traumatic brain injury, Neurofibromatosis Type 1). For example, FDA guidance for drug trials of patients with a traumatic brain injury requires documenting cognitive tests before trial initiation, choosing tests and PROs guided by conceptual models, and providing adequate justification of the outcome measure(s) (104). When choosing tests for pediatric patients with SCD, it is essential to consider the pathways by which novel therapies improve SCD-related pathophysiology and how these may relate to the neurophysiological processes that support brain function. For example, drugs such as Voxelotor, which reversibly binds to hemoglobin and prevents HbS polymerization by increasing the hemoglobin's affinity for oxygen, could likely improve executive function by increasing hemoglobin concentration, reducing compensatory hemodynamic stress (14). The current randomized trial of Voxelotor, HOPE Kids 2, with a primary endpoint of change in transcranial Doppler velocities in children aged 2–16 years with SCD conditional velocities, includes tests of executive function and processing speed.

Future trials must consider the extent to which any observed changes are statistically significant and the extent to which they are clinically meaningful or functionally significant. For example, a cognitive test with good reliability may show a statistically significant 1 to 2-point change. Practically, however, this might not mean that the changes translate into functionally relevant benefits (e.g., scholastic, employment) within the context of patients' daily lives. Therefore, an effect size that would demonstrate meaningful change should be determined before study initiation. Moreover, researchers can calculate the minimal clinically important difference (MCID). MCID is the smallest difference in the cognitive tests and psychological PROs used as outcomes and endpoints that patients perceive as beneficial or harmful, i.e., what is actually important to patients (105). Linking the magnitude of change to clinical trial efficacy and effectiveness reflects the intention to find a clinically important treatment effect.

MCID can be calculated through the (1) the anchor-based method, i.e., by anchoring change on the PROMIS, a numerical scale, to a categorical response (e.g., a lot better), (2) by consensus (e.g., Delphi) methods, i.e., convening an expert panel to provide independent assessments of MCID, and (3) the distribution-based method, i.e., using the distribution of the outcome or endpoint scores, particularly the variation between patients. However, this method does not center on the patient (106). For the psychological PROs, previous research in adult samples has demonstrated a 3–4 point change on Patient-Reported Outcomes Measurement Information System (PROMIS) anxiety and depression (107) or a 5-point change on the Patient Health Questionnaire (PHQ-9) (96) are considered as MCID. MCID has been utilized less for cognitive tests, but a recent study found that for older adults assessing raw scores and completion times, MCID improvement over 1-year following an exercise program was 3–5 symbols for the Digit Symbol Substitution Test (a measure of processing speed) and −11.5 to −26.0 s for the Stroop (a measure of inhibition) (97).

There are several reliable cognitive test batteries (see **Table 2**) and psychological PROs (see **Table 3**) that meet the criteria defined above and that have been used in pediatric SCD populations (17). These tests provide measures that may therefore be good candidates for endpoints in clinical trials. To aid decision making, **Tables 2**, **3** include details on the normative data used for scoring (most often based on US Census data), the cost and where to obtain tests, along with the training necessary to administer and interpret results. Researchers will also have to determine whether to obtain child-report and/or caregiver-proxy reports for psychological PROs. This decision will often be reached by considering the child's age, weighing the burden to families, the complexity of having multiple reporters, and the additional cost of gathering and analyzing data. Child and caregiver proxy reports should not be aggregated as the poor reliability (i.e., low correlations) between child and caregiver ratings represent different perspectives (98).

COGNITIVE TEST AND PSYCHOLOGICAL PROs ADMINISTRATION

Standardized test administration is critically important so that scores obtained do not over or underestimate actual ability (99). All participants need a quiet, distraction-free environment, along with a precise reading of instructions and the provision of necessary tools or stimuli. Generally, psychometrists or graduate-level students with specialized training administer and score tests according to manual instructions. Psychologists qualified to interpret scores should directly and closely supervise (100). Cognitive testing and psychological assessment should optimally occur in locations and at times that reduce the burden on participants and are consistent across trial visits. When choosing tests for clinical trials in low-resource settings, investigators should consider tests that do not require time-consuming adaptations, are inexpensive enough to be administered as a part of usual care, and do not need expensive equipment (see **Figure 2**).

Many standardized tests now have the option for administration via an iPad (see **Table 2**). Computer-aided scoring and interpretation are also available for these instruments. These adaptations (e.g., stopwatch, audio recorder) make it much easier

TABLE 2 | Neuropsychological test batteries previously used in the pediatric sickle cell population.

Battery	Cognitive domain	Tests	Test time	Ages	Languages	Norms	Cost/admin	Where to get	Training needed	Scoring
NIH toolbox cognition module (96)	*Executive*									
	Flexibility Inhibition	DCCS	4 min	3–85	English; Spanish; Cebuano	Introduced in 2012 $N = 4,859$ representative of U.S. Census population [gender, racialised identity, ethnicity (i.e., Hispanic), SES]	$500 per year (up to 10 iPads)/1 iPad per participant	Download from iTunes. Psychologist to unlock module; healthmeasures.net	Online; in-person; qualified users—psychometrist, psychology graduate students, or psychologist	On iPad—raw, age-corrected SS download to iCloud or email
	Working memory	Flanker	3 min	3–85						
		List sorting	7 min	7–85						
	Processing Speed	Pattern comparison	3 min	7–85						
NIH examiner (97)	*Executive*									
	Flexibility	Set shifting	5 min	3–90	English; Spanish	Normed 2006–2010 $N = 1,113$ from 9 sites in the U.S. (range of sex, racialised identity, ethnicity). Range of disorders including 117 patients with SCD	Free/record forms; Computer with PsychoPy Version; has alternate forms	Available to qualified users upon email request http://memory.ucsf.edu/resources/examiner.	Training videos; qualified users—psychometrist, psychology graduate students, or psychologist	R (Statistical Software) included on the distribution CD
	Inhibition	Flanker	5 min							
		CPT	14 min							
		Anti-saccades	5 min							
		Errors	7 min							
	Working memory	Dot counting	5 min							
		N back	2 min							
	Fluency	Phonemic	2 min							
		Category	2 min							
	Planning	Unstructured	6 min							
D-KEFS (98)	*Executive*									
	Flexibility	Trail making	10 min	8–89	English; Dutch; Danish; Norwegian; Swedish	Normed in 2000 $N = 1,750$ representative of U.S. Census population [gender, racialised identity, ethnicity (i.e., Hispanic), SES]	$1,000 kit and scoring, additional cost record forms; Annual license fee plus $1.25 per subtest/uses Q interactive on 2 iPads	Pearson clinical is available to qualified users, e.g., clinical psychologists	Training supervised by licensed/registered clinical psychologists	By hand; computerized scoring kit; q-interactive (reports can be generated online)
	Inhibition	Color interference	12 min							
	Initiation	Design fluency	10 min							
	Fluency	Verbal fluency	7 min							
	Planning	Tower	15 min							
NEPSY-II (99)	*Executive*									

(Continued)

TABLE 2 | Continued

Battery	Cognitive domain	Tests	Test time	Ages	Languages	Norms	Cost/admin	Where to get	Training needed	Scoring
	Flexibility	Animal sorting	10 min	7–16	English	Normed in 2006 $N = 1,200$ representative of U.S. Census population [gender, racialised identity, ethnicity (i.e., Hispanic), SES]	$1,000 kit and scoring, additional cost record forms; Annual license fee plus $1.25 per subtest/uses Q interactive on 2 iPads	Pearson Clinical is available to qualified users, e.g., clinical psychologists	Training supervised by licensed/registered clinical psychologists	By hand; computerized scoring kit; q-interactive (reports can be generated online)
	Inhibition	Inhibition	11 min	5–16						
	Fluency	Design fluency	4 min	5–12						
	Attention	Auditory attention and response set	11 min	5–16						
Wechsler tests (WPPSI, WISC, WASI) (100)	*Processing Speed*	Coding	5 min	6–90	21 languages including English; Spanish; French; German Arabic; Chinese	Ns = 2,000–2,500 Representative of U.S. and U.K. based on census data (gender, racialised identity, ethnicity, SES). Separate norms available for other translations	$1,400 kit and scoring, $60 per year for scored reports; Annual license fee plus $1.25 per subtest using Q interactive on 2 iPads	Pearson Clinical is available to qualified users, e.g., clinical psychologists	Training supervised by licensed/ registered clinical psychologists	By hand; computerized scoring Q global; q- interactive (reports can be generated online)
		Animal Coding	5 min	4–7						
		Symbol Search	5 min	6–90						
		Bug Search	5 min	4–7						
		Cancellation	5 min	4–90						
Continuous performance test (101)	*Attention*	Kiddie CPT CPT	8 min 14 min	4–7 8–90	English	$N = 1,400$ representative of U.S. Census population [gender, racialised identity, ethnicity (i.e., Hispanic), SES]	$2,900 for USB with unlimited use of both tests	Pearson Clinics al available to qualified users, e.g., clinical psychologists	Training supervised by licensed/registered clinical psychologists	Computerized scoring on USB (reports are generated)

SCD, sickle cell disease; Admin, Administration; DCCS, Dimensional Change Card Sort; Flanker, Flanker Inhibitory Control and Attention; SES, socioeconomic status; SS, standard scores; CPT, Continuous Performance Test; D-KEFS, Delis-Kaplan Executive Function System™; WPPSI, Wechsler Preschool & Primary Scale of Intelligence; WISC, Wechsler Intelligence Scale for Children; WAIS, Wechsler Adult Intelligence Scale.

TABLE 3 | Psychological patient-reported outcomes frequently used in the pediatric sickle cell population.

Measures	Psychological domain	Test time	Ages	Languages	Norms	Cost/admin	Where to get	Training needed	Scoring
PROMIS (self-report and caregiver-proxy report) (108)	Depression Anxiety	5 min 5 min	5–90 5–90	English; Spanish; Other language translations on request	Ongoing validation; a range of disorders including patients with SCD	Free; on paper; computer; with an app $750 fee for other language translations (can request a waiver)	NIH Toolbox app; PROMIS app; REDCap; EPIC; OBERD; healthmeasures.net	Can train research staff, students quickly. Interpretation of scores from a psychologist	By hand; on iPad, download and score using statistical software
Beck depression inventory-II (BDI – II) (109)	Depression	5 min	13–80	English; Spanish;	*N* = 500 adult psychiatric outpatients in the US and a student sample of 120 college students in Canada as the control group	$40 per year for a single scoring subscription; $100 for starter kit; $60 for 25 paper record forms	Pearson clinical-available to qualified users, e.g., clinical psychologists	Can train research staff, students quickly. Interpretation of scores from a psychologist	By hand; on iPad, download and score using statistical software
Patient Health Questionnaire-−9 (PHQ-9) Adolescent version available (110)	Depression	2–5 mins	11–17 18 and over	40 languages including English; Spanish; Arabic; Chinese	*N* = 6,000 patients (3,000 from general internal medicine and family practice clinics *N* = 3,000 from obstetrics-gynecology clinics in the US	Freely available	Download online	Can train research staff, students quickly. Interpretation of scores from a psychologist	By hand; on iPad, download and score using statistical software
State-trait anxiety inventory (111)	Anxiety	10 min	15 and over	40 languages including English; Spanish; Arabic; Chinese	Large US sample of college and high school students Small groups of psychiatric, general and medical patients, and young prisoners	$100 Adult complete kit—manual with scoring key and 25 record forms	Mind Garden-available to qualified users, e.g., clinical psychologists	Can train research staff, students quickly. Interpretation of scores from a psychologist	By hand; on iPad, download and score using statistical software
Generalized Anxiety Disorder – 7 (GAD – 7) (112)	Anxiety	2–5 min	13 and over	40 languages including English; Spanish; Arabic; Chinese	*N* = 2,740 primary care patients *N*= 5,030 general population in Germany reported sex and other demographic characteristics, but not racialised identity or ethnicity	Freely available	Download online	Can train research staff, students quickly. Interpretation of scores from a psychologist	By hand; on iPad, download and score using statistical software
Child behavior checklist (CBCL) (113)	Emotional and behavioral problems	10 min	6–18	English; French; Translation into 60 languages	*N* = 2,300 children assessed at 42 mental health agencies in the US (this is used in the scoring software). The Multicultural Family Assessment Module on the Progress and Outcomes App provides norms from 50 other countries	$500 for the computerized starter kit	ASEBA—available to qualified users, e.g., clinical psychologists	Can train research staff, students quickly. Interpretation of scores from a psychologist	By hand; on iPad, download and score using statistical software

SCD, sickle cell disease; Admin, Administration; PROMIS, Patient-Reported Outcomes Measurement Information System; EPIC, Electronic Portfolio of International Credentials; ASEBA, The Achenbach System of Empirically Based Assessment.

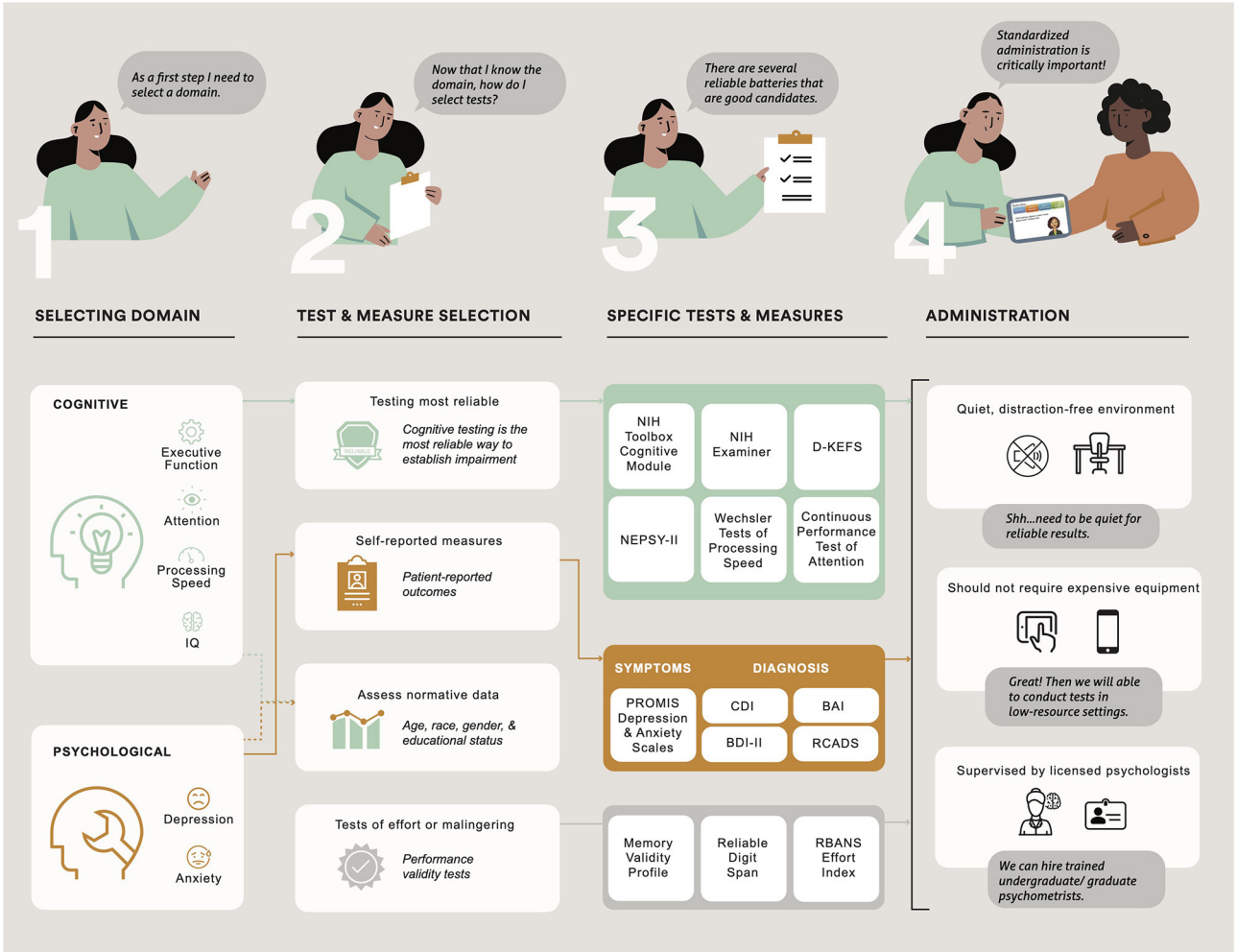


FIGURE 2 | Description of the process in choosing cognitive domains and psychological function and administering cognitive tests and psychological patient-reported outcomes in clinical trials of pediatric patients with sickle cell disease. NIH, National Institutes of Health; D-KEFS, Delis Kaplan Executive Function System; NEPSY-II, A Developmental NEuroPSYchological Assessment; PROMIS, Patient Reported Outcome Measurement Information System; CDI, Children's Depression Inventory; BAI, Beck Anxiety Inventory, BDI, Beck Depression Inventory-II; RCADS, Revised Children's Anxiety and Depression Scale.

to test at multiple locations (e.g., SCD clinic), easing the burden on patients and their families who likely have many routine medical appointments to attend. Additionally, computer-based batteries appear to reduce administration errors and increase efficiency in testing (101). Disadvantages associated with iPad administration of some tests include the need to configure Wi-Fi to the test battery and the need for a blue-tooth connection for between-device communication during administration. There are several options for completing psychological PROs, including paper and pencil, phone, or online (e.g., REDCap or Qualtrics surveys).

The use of digital and remote assessment of psychological PROs in clinical trials has accelerated since the start of the COVID-19 pandemic (108). Although remote assessment may improve reliability and reduce variability if continuous or multiple data points are gathered, may be cost-effective, and appear more inclusive as they reduce transportation burden, there are disadvantages that researchers in clinical trials of pediatric patients with SCD need to scrutinize before making their decision. Remote assessment of psychological PROs may reduce control, require participants to have Wi-Fi connections, and personal computers, tablets or mobile phones, which may not be available to all families. Providing in-person (conducted during usual care appointments) and online options to complete psychological PROs is one way to lessen digital exclusion for patients and families (109).

Practice Effects

Practice effects refer to improvements in performance due to increased familiarity with and exposure to test materials, differing test-taking strategies, and less anxiety in the test-taking environment. If not considered, such effects may be difficult to disentangle from effects related to the intervention of interest. Neuropsychologists generally schedule cognitive testing

1–2 years apart in clinical practice. Serial cognitive testing and psychological assessment occur more frequently in clinical trials, making practice effects a genuine concern. If not correctly integrated into the interpretation of results, practice effects can easily lead to false conclusions. Despite this, these effects are often underappreciated (100). Timed tests, psychomotor processing tests, and novel tests are more susceptible to practice effects (110). Unfortunately, these are the tests that patients with SCD generally find the most challenging (37). Additionally, practice effects tend to occur more often in younger vs. older participants (111). It is also possible that practice effects may differ between those with SCD and controls with disease severity (e.g., SCD genotype, pain episodes, sleep difficulties, brain abnormalities) in the pediatric SCD population serving to modify results. However, this possibility has not yet been empirically tested. When comprising their samples, researchers should weigh the potential for practice effects (e.g., large age range, choice of tests) when choosing appropriate endpoints for the clinical trial.

There are approaches to reduce practice effects in clinical trials (112). First, massed practice in a pre-baseline period (e.g., showing all participants the test materials, providing instructions, completing test practice items) may reduce the effects of novelty and any anxiety related to the test-taking environment. The precise number of pre-baseline assessments necessary to achieve habituation (i.e., for cognitive performance to stabilize) has not been established, but previous research in non-SCD populations has indicated that 1 to 2 practice trials before baseline can result in habituation (113). Using massed practice would have to be considered carefully, however, given the additional cost and time burden. Second, if it is possible to counterbalance the administration of tests (e.g., systematic variation of the order of conditions), it can help to reduce practice effects as well as the influence of other nuisance variables. Third, computerized adaptive testing (e.g., tests that adapt to the participant's ability) can reduce practice effects (114). Fourth, using tests with multiple similar items or alternate forms of tests at baseline and post-intervention can minimize item exposure. Specific to patients with SCD, researchers could consider using the Wechsler Cancellation subtest when assessing processing speed as previous research demonstrates that this test appears more resistant to practice effects in this population (78). Each approach has strengths so that the final decision will depend on economic and pragmatic considerations (112).

OTHER CONSIDERATIONS

Age

A clinical trial of pediatric patients with SCD will need to consider the influence of normal growth and development on cognitive processes. For instance, children between the ages of 3–5 years have more difficulty with tests of executive function, and different executive functions mature at different rates (53). Attentional control emerges first in infancy and then develops quickly in early childhood. Cognitive flexibility and information processing take longer to develop and have a critical period between 7 and 9 years. They are relatively mature by 12 years of age. After a transitional period that begins in adolescence, "executive control" is thought to emerge (115). These developmental changes make assessing executive function in a pediatric population more challenging. For example, if a child is 7 years old at baseline and 9 years old at the end of a clinical trial, any improvements in executive function may be due to expected developmental growth rather than the disease-modifying therapy or intervention under consideration. As treatment effects are typically averaged across a sample, further challenges may arise if the sample includes children at very different developmental stages. In the example above, scores in those over the age of 12 may be expected to remain relatively unchanged. A specific examination of scores and/or a well-matched control sample (ethnicity, age, socioeconomic status) can help to determine if the change is related to age or the intervention.

Researchers may choose to analyze both raw and standardized scores. Raw scores might best fit the data (116) but are also susceptible to regression to the mean (the tendency for a person's score to move toward the population mean test score with retesting) and practice effects (117). Although changes in age-corrected standard scores are likely more understandable to the audience, their interpretation can be misleading. For example, a negative change in standard scores can either represent an actual decline in raw scores or a failure for raw scores to improve at the expected rate for age, with no change in raw scores or performance. In the context of a clinical trial, researchers may need to consider standardizing post-treatment scores using baseline age or assessing patients across a narrower age range (e.g., 12–16 rather than 6–16 years), examining developmental trajectories for the particular test.

Genotype and Treatment Course

Several SCD-specific factors need to be considered when using cognitive tests as endpoints in clinical trials. Foremost, although most clinical trials target pediatric patients with the HbSS genotype, they also include other genotypes (e.g., HbSC, HbSβ0 thalassemia, HbSβ+ thalassemia). The patients with the HbSS genotype often experience the most clinical severity and consequently have more cognitive challenges than other patients with SCD (17). It is unclear if these patients also have more psychological challenges. Moreover, if patients continue to receive treatments (e.g., chronic blood transfusion, HU) as part of standard care during the clinical trial, cognitive endpoints may differ related to treatment type (118) or when the patient received treatment in relation to cognitive testing (37). As such, researchers may need to account for genotype and treatment course and decide whether to minimize for these factors to ensure balance across trial arms, divide samples into subgroups, or control for these factors in their analyses.

Neurologic Complications

Previous research has shown that compared to controls, children with SCD with and without overt neurologic complications have a higher burden of white matter hyperintensities (119), reduced cortical and subcortical gray matter volume (120), and widespread reductions in the microstructural integrity of white matter (40, 121). Past work also demonstrates that

pediatric patients with SCD who have experienced overt stroke consistently have poorer cognitive outcomes than other patients with SCD and non-SCD controls (19). The literature is more mixed concerning cognitive differences between patients with SCD and silent cerebral infarction (SCI) and those with "normal-appearing MRI." Although older studies have tended to find a detriment in cognitive performance in patients with SCI (17, 122), more recent studies have found few, if any, differences (40, 123–125). Discrepancies in the literature may relate not only to differences in the precise definition of SCI, sample characteristics, and cognitive domains studied but also to advances in MRI technology, with higher resolution scans identifying a greater number of patients (and controls) with SCI (126). With higher resolution techniques, other characteristics such as lesion volume may provide a more sensitive metric.

Given these challenges, if mixed neurologic groups are included in a clinical trial, data from patients with extensive structural tissue injury could be analyzed separately to determine if they differ significantly from the larger sample. Alternatively, investigators may control or minimize for neurologic status in analyses. Deciding whether to analyse data from patients with and without SCI will require deeper investigation of findings and, ideally, reporting why data have been grouped (e.g., scores were very similar) or not grouped (e.g., clinically meaningful differences). However, researchers conducting analyses in subgroups of pediatric patients with SCD (e.g., stroke vs. SCI vs. no infarct on MRI) will have to balance the additional knowledge gained with reduced statistical power, as sample sizes will be made smaller by subgrouping. Another possible route includes using block randomization so that there is more than one patient group with equal numbers of participants with overt stroke and SCI (presence/volume) assigned to each group.

Pain

Although pain causes significant morbidity for those living with SCD, our understanding of the impact on cognition remains limited. Nevertheless, the few studies conducted show that persistent pain and executive dysfunction are significantly related (64). Coordinating cognitive testing only when patients are not experiencing pain can be a goal. However, this may generally not be achievable given that many patients with SCD experience persistent pain (127, 128). One potential option is to use pain-related PROs to determine potential pain interference and intensity and their relation to cognitive endpoints. Pain experience/frequency may then be included as a control variable in analyses (129). Minimization for baseline pain is another alternative.

Sleep

Previous research indicates that pediatric patients with SCD experience sleep-disordered breathing (130), including obstructive sleep apnoea (131), along with a high prevalence of sleep-onset insomnia (132). Other sleep disturbances (133), including nocturnal enuresis (134) and leg movement (135), occur in about one-third of patients. There is evidence that sleep may impact cognitive performance (136). In clinical trials of patients with SCD, including a measure of sleepiness as an outcome would therefore be useful (137). Researchers should also consider whether they will use a predefined "sleepiness" cut-off score to determine if cognitive testing can be conducted. Researchers might consider choosing the most suitable time of day for each patient to be tested (138). Utilizing these measures might increase the time burden and require additional coordination with families, as the timing of testing would need to be coordinated with greater precision and ought ideally to be matched at both baseline and study exit. Similar to our suggestions related to pain, accounting for sleepiness in statistical analyses is another way to control for this factor.

COGNITIVE COMPOSITE ENDPOINTS

Combining test scores or item responses from relevant subdomains into a composite score creates a single endpoint with several advantages for clinical trials in pediatric patients with SCD. Multiple endpoints may appear to improve the explanatory power and provide additional specificity; however, they also require assumptions about the magnitude of the treatment effect across outcome domains and increase the possibility of *post-hoc* "cherry-picking" of significant results (104). Composite endpoints may simplify decision making around selecting a specific primary outcome from multiple plausible tests or measures (139). The FDA requires a single outcome, defined *a priori,* to license new drugs (140). In a rare disorder like SCD, the additional statistical power afforded by a single endpoint cannot be discounted. The easiest and most widely used method to create a composite is to place the scores on a common metric (e.g., z, T, scaled score, or other standard scores) and then average them (141). An executive composite created using this method differentiated between children with SCD receiving chronic transfusions, HU, and demographically-matched controls (37). As this method does not assess inter-correlations between tests or the factor structure, it can provide a summary score of overall cognitive performance in a particular domain (141).

An alternative approach is to use confirmatory factor analysis to model latent ability based on a set of scores. The latent ability model captures variance across tests or measures and accounts for covariance attributable to method effects or theoretical similarities. For example, if two tests both measure cognition with precision, but one test measures higher-order functioning and the other lower-order functioning, the latent ability model can measure the underlying trait across the full range of ability. An additional advantage is that this methodology can control for and quantify differences in performance based on demographics (e.g., age, sex at birth). Once computed, a latent ability composite score (mean = 0, standard deviation = 1) is available for each participant in the study (104). Latent ability composite scores have been created for Alzheimer's disease studies (142) but not yet for adult or pediatric SCD studies.

We advocate for a single cognitive endpoint in clinical trials of patients with SCD, like an executive function composite. Notably, a domain-specific composite is not the same as an IQ, which is an aggregate or global index that reflects performance

across a wide variety of cognitive domains. There are inherent challenges in developing composites as tests in the same domain are not identical and may capture distinct abilities. Batteries of executive function tasks balance both unity and diversity, but the three target executive functions (e.g., cognitive flexibility, inhibition, working memory) do tap into an underlying common ability (52, 143), and there is evidence of dysfunction in all three areas of executive function in SCD (17). The NIH EXAMINER already provides an executive composite score that has been shown to differentiate between groups of children with SCD with and without silent infarction and stroke (144). However, most neuropsychological batteries with demonstrated sensitivity to executive dysfunction in SCD do not provide a specific executive function composite, so either the averaging or latent approach will need to be considered.

CONCLUSION

This paper identifies specific tests (e.g., NIH Toolbox Cognition Module, Wechsler Cancellation Test) and psychological PROs (e.g., PROMIS depression and anxiety scales) that can potentially capture clinically changes meaningful in the context of patients' day to day lives. For cognition, executive function and processing speed are the domains in which pediatric patients with SCD have the most difficulty. There is preliminary evidence that executive function composite scores are amenable to disease-modifying therapies (37–39); therefore, they hold particular promise as endpoints for future clinical trials, with batteries such as the NIH Toolbox and the NIH Examiner providing valid and reliable measures with demonstrated sensitivity to change. A significant proportion of pediatric patients with SCD have widespread brain abnormalities, including in the prefrontal cortex of the brain (145), executive function encompasses those cognitive processes that underlie goal-directed behavior mediated by activity within the prefrontal cortex, and executive function has been demonstrated to be related to cerebral hemodynamic parameters (67). Therefore, it is unsurprising that executive function is a plausible candidate endpoint for a clinical trial.

Cognitive tests and psychological PROs do not come without limitations and specific considerations for the SCD population, but thoughtful adaptations to study design and statistical analyses may help address potential challenges. Although not intended as an exhaustive list, this review provides an overview of recommended tests and PROs that are relatively easy to collect, associated with SCD morbidity, meaningful to patients and families, and can be incorporated into routine care in various settings and countries.

AUTHOR CONTRIBUTIONS

AH conceptualized the paper, completed the review, and drafted the manuscript. HS and MK contributed to the review and provided feedback on the manuscript. LC and FK conceptualized the paper, contributed to the review, and provided feedback on the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

AH was supported in part by a grant from the National Heart, Lung, and Blood Institute (1F32HL143915). LC was funded through a Patient-Centered Outcomes Research Institute (PCORI) Award (CDR-1609-36055).

ACKNOWLEDGMENTS

The authors would like to thank Neha Mann for her assistance with visualizing **Figure 2**.

REFERENCES

1. Rees DC, Williams TN, Gladwin MT. Sickle-cell disease. *Lancet.* (2010) 376:2018–31. doi: 10.1016/S0140-6736(10)61029-X
2. Angastiniotis M, Cannon L, Antoniou E, Brunetta AL, Constantinou G, Knoll EM, et al. Hemoglobin disorders in Europe: a systematic effort of identifying and addressing unmet needs and challenges by the Thalassemia International Federation. *Thalass Rep.* (2021) 11:13–21. doi: 10.4081/thal.2021.9803
3. Modell B, Darlison M. Global epidemiology of haemoglobin disorders and derived service indicators. *Bull World Health Organ.* (2008) 86:480–7. doi: 10.2471/BLT.06.036673
4. Hassell KL. Population estimates of sickle cell disease in the U.S. *Am J Prev Med.* (2010) 38:S512–21. doi: 10.1016/j.amepre.2009.12.022
5. Brawley OW, Cornelius LJ, Edwards LR, Gamble VN, Green BL, Inturrisi C, et al. National Institutes of Health Consensus Development Conference statement: hydroxyurea treatment for sickle cell disease. *Ann Intern Med.* (2008) 148:932–8. doi: 10.7326/0003-4819-148-12-200806170-00220
6. Yawn BP, Buchanan GR, Afenyi-Annan AN, Ballas SK, Hassell KL, James AH, et al. Management of sickle cell disease: summary of the 2014 evidence-based report by expert panel members. *JAMA.* (2014) 312:1033–48. doi: 10.1001/jama.2014.10517
7. Qureshi A, Kaya B, Pancham S, Keenan R, Anderson J, Akanni M, et al. Guidelines for the use of hydroxycarbamide in children and adults with sickle cell disease: a British Society for Haematology Guideline. *Br J Haematol.* (2018) 181:460–75. doi: 10.1111/bjh.15235
8. Ware RE, Helms RW. Stroke with transfusions changing to hydroxyurea (SWiTCH). *Blood.* (2012) 119:3925–32. doi: 10.1182/blood-2011-11-392340
9. Wang WC, Ware RE, Miller ST, Iyer R V, Casella JF, Minniti CP, et al. A multicenter randomised controlled trial of hydroxyurea (hydroxycarbamide) in very young children with sickle cell anaemia. *Lancet.* (2011) 377:1663. doi: 10.1016/S0140-6736(11)60355-3
10. Bernaudin F, Socie G, Kuentz M, Chevret S, Duval M, Bertrand Y, et al. Long-term results of related myeloablative stem-cell transplantation to cure sickle cell disease. *Blood.* (2007) 110:2749–56. doi: 10.1182/blood-2007-03-079665
11. Shenoy S, Angelucci E, Arnold SD, Baker KS, Bhatia M, Bresters D, et al. Current results and future research priorities in late effects after hematopoietic stem cell transplantation (HCT) for children with sickle cell disease and thalassemia: a consensus statement from the second pediatric blood and marrow transplant consortium. *Biol Blood Marrow Transplant.* (2017) 23:552–61. doi: 10.1016/j.bbmt.2017.01.009
12. Niihara Y, Miller ST, Kanter J, Lanzkron S, Smith WR, Hsu LL, et al. A phase 3 trial of l-glutamine in sickle cell disease. *N Engl J Med.* (2018) 379:226–35. doi: 10.1056/NEJMoa1715971
13. Han J, Saraf SL, Gordeuk VR. Systematic review of crizanlizumab: a new parenteral option to reduce vaso-occlusive pain crises in patients with sickle cell disease. *Pharmacother J Hum Pharmacol Drug Ther.* (2020) 40:535–43. doi: 10.1002/phar.2409
14. Vichinsky E, Hoppe CC, Ataga KI, Ware RE, Nduba V, El-Beshlawy A, et al. A phase 3 randomized trial of voxelotor in sickle cell disease. *N Engl J Med.* (2019) 381:509–19. doi: 10.1056/NEJMoa1903212

15. Orkin SH, Bauer DE. Emerging genetic therapy for sickle cell disease. *Annu Rev Med.* (2019) 70:257–71. doi: 10.1146/annurev-med-041817-125507
16. Hood AM, Stotesbury H, Kölbel M, DeHaan M, Downes M, Kawadler JM, et al. Study of montelukast in children with sickle cell disease (SMILES): a study protocol for a randomised controlled trial. *Trials.* (2021) 22:1–18. doi: 10.1186/s13063-021-05626-6
17. Prussien K V, Jordan LC, DeBaun MR, Compas BE. Cognitive function in sickle cell disease across domains, cerebral infarct status, and the lifespan: a meta-analysis. *J Pediatr Psychol.* (2019) 44:948–58. doi: 10.1093/jpepsy/jsz031
18. Berkelhammer LD, Williamson AL, Sanford SD, Dirksen CL, Sharp WG, Margulies AS, et al. Neurocognitive sequelae of pediatric sickle cell disease: a review of the literature. *Child Neuropsychol.* (2007) 13:120–31. doi: 10.1080/09297040600800956
19. Kawadler JM, Clayden JD, Clark CA, Kirkham FJ. Intelligence quotient in paediatric sickle cell disease: a systematic review and meta-analysis. *Dev Med Child Neurol.* (2016) 58:672–9. doi: 10.1111/dmcn.13113
20. Benton TD, Ifeagwu JA, Smith-Whitley K. Anxiety and depression in children and adolescents with sickle cell disease. *Curr Psychiatry Rep.* (2007) 9:114–21. doi: 10.1007/s11920-007-0080-0
21. Jerrell JM, Tripathi A, McIntyre RS. Prevalence and treatment of depression in children and adolescents with sickle cell disease: a retrospective cohort study. *Prim Care Companion CNS Disord.* (2011) 13:27132. doi: 10.4088/PCC.10m01063
22. van der Vaart T, Overwater IE, Oostenbrink R, Moll HA, Elgersma Y. Treatment of cognitive deficits in genetic disorders: a systematic review of clinical trials of diet and drug treatments. *JAMA Neurol.* (2015) 72:1052–60. doi: 10.1001/jamaneurol.2015.0443
23. Farrell AT, Panepinto J, Carroll CP, Darbari DS, Desai AA, King AA, et al. End points for sickle cell disease clinical trials: patient-reported outcomes, pain, and the brain. *Blood Adv.* (2019) 3:3982–4001. doi: 10.1182/bloodadvances.2019000882
24. Stotesbury H, Kawadler JM, Saunders DE, Hales PW, Clark CA, Kirkham FJ. Vascular instability and neurological morbidity in sickle cell disease; an integrative framework. *Front Neurol.* (2019) 10:871. doi: 10.3389/fneur.2019.00871
25. Hulbert ML, Ford AL. Understanding sickle cell brain drain. *Blood, J Am Soc Hematol.* (2014) 124:830–1. doi: 10.1182/blood-2014-06-582403
26. Graves JK, Hodge C, Jacob E. Depression, anxiety, and quality of life in children and adolescents with sickle cell disease. *Pediatr Nurs.* (2016) 42:113.
27. Sil S, Cohen LL, Dampier C. Psychosocial and functional outcomes in youth with chronic sickle cell pain. *Clin J Pain.* (2016) 32:527–33. doi: 10.1097/AJP.0000000000000289
28. Williams H, Silva S, Cline D, Freiermuth C, Tanabe P. Social and behavioral factors in sickle cell disease: employment predicts decreased health care utilization. *J Health Care Poor Underserved.* (2018) 29:814. doi: 10.1353/hpu.2018.0060
29. Pasquali SK, Lam WK, Chiswell K, Kemper AR, Li JS. Status of the pediatric clinical trials enterprise: an analysis of the US ClinicalTrials. gov registry. *Pediatrics.* (2012) 130:e1269–77. doi: 10.1542/peds.2011-3565
30. Ritchie H, Roser M. *Causes of Death. Our World Data* (2018). Available online at: https://ourworldindata.org/causes-of-death (accessed December 2, 2021).
31. Hwang TJ, Tomasi PA, Bourgeois FT. Delays in completion and results reporting of clinical trials under the Paediatric Regulation in the European Union: a cohort study. *PLoS Med.* (2018) 15:e1002520. doi: 10.1371/journal.pmed.1002520
32. Rankine-Mullings AE, Logan TM, King L-G, Cunningham-Myrie CA, Scott CR, Knight-Madden JM. The risk of acute events among patients with sickle cell disease in relation to early or late initiation of care at a specialist center: evidence from a retrospective cohort study. *BMC Pediatr.* (2020) 20:1–8. doi: 10.1186/s12887-020-02270-y
33. Brousseau DC, Panepinto J, Nimmer M, Hoffmann RG. The number of people with sickle-cell disease in the United States: national and state estimates. *Am J Hematol.* (2010) 85:77–8. doi: 10.1002/ajh.21570
34. Schatz J, Finke RL, Kellett JM, Kramer JH. Cognitive functioning in children with sickle cell disease: a meta-analysis. *J Pediatr Psychol.* (2002) 27:739–48. doi: 10.1093/jpepsy/27.8.739
35. Check D. *Choosing and Specifying Endpoints and Outcomes.* NIH Collaboratory Living Textbook of Clinical Trials (2019). Available online at: https://rethinkingclinicaltrials.org/chapters/design/choosing-specifying-end-points-outcomes/choosing-and-specifying-endpoints-and-outcomes-introduction/ (accessed November 20, 2021).
36. Tambor E, Robinson M, Hsu L, Chang H-Y, Al Naber J. coreSCD: multi-stakeholder consensus on core outcomes for sickle cell disease clinical trials. *BMC Med Res Methodol.* (2021) 21:1–9. doi: 10.1186/s12874-021-01413-8
37. Hood AM, King AA, Fields ME, Ford AL, Guilliams KP, Hulbert ML, et al. Higher executive abilities following a blood transfusion in children and young adults with sickle cell disease. *Pediatr Blood Cancer.* (2019) 66:e27899. doi: 10.1002/pbc.27899
38. Hardy SJ, Hardy KK, Schatz JC, Thompson AL, Meier ER. Feasibility of home-based computerized working memory training with children and adolescents with sickle cell disease. *Pediatr Blood Cancer.* (2016) 63:1578–85. doi: 10.1002/pbc.26019
39. Hardy SJ, Bills SE, Meier ER, Schatz JC, Keridan KJ, Wise S, et al. A randomized controlled trial of working memory training in pediatric sickle cell disease. *J Pediatr Psychol.* (2021) 46:1001–14. doi: 10.1093/jpepsy/jsab030
40. Stotesbury H, Kirkham FJ, Kölbel M, Balfour P, Clayden JD, Sahota S, et al. White matter integrity and processing speed in sickle cell anemia. *Neurology.* (2018) 90:e2042–50. doi: 10.1212/WNL.0000000000005644
41. Daly B, Kral MC, Brown RT, Elkin D, Madan-Swain A, Mitchell M, et al. Ameliorating attention problems in children with sickle cell disease: a pilot study of methylphenidate. *J Dev Behav Pediatr.* (2012) 33:244. doi: 10.1097/DBP.0b013e31824ba1b5
42. Hood AM, Reife I, King AA, White DA. Brief screening measures identify risk for psychological difficulties among children with sickle cell disease. *J Clin Psychol Med Settings.* (2020) 27:651–61. doi: 10.1007/s10880-019-09654-y
43. Carroll JB. Psychometrics, intelligence, and public perception. *Intelligence.* (1997) 24:25–52. doi: 10.1016/S0160-2896(97)90012-X
44. Wechsler D. *The Measurement of Adult Intelligence*. 3rd ed. Baltimore, MD: Williams & Wilkins Co. (1944).
45. Wegenschimmel B, Leiss U, Veigl M, Rosenmayr V, Formann A, Slavc I, et al. Do we still need IQ-scores? Misleading interpretations of neurocognitive outcome in pediatric patients with medulloblastoma: a retrospective study. *J Neurooncol.* (2017) 135:361–9. doi: 10.1007/s11060-017-2582-x
46. Morrison AB, Chein JM. Does working memory training work? The promise and challenges of enhancing cognition by training working memory. *Psychon Bull Rev.* (2011) 18:46–60. doi: 10.3758/s13423-010-0034-0
47. Wechsler D. *WASI-II: Wechsler Abbreviated Scale of Intelligence*. San Antonio, TX: PsychCorp (2011).
48. Protzko J. The environment in raising early intelligence: a meta-analysis of the fadeout effect. *Intelligence.* (2015) 53:202–10. doi: 10.1016/j.intell.2015.10.006
49. Diamond A, Ling DS. Conclusions about interventions, programs, and approaches for improving executive functions that appear justified and those that, despite much hype, do not. *Dev Cogn Neurosci.* (2016) 18:34–48. doi: 10.1016/j.dcn.2015.11.005
50. Hackman DA, Gallop R, Evans GW, Farah MJ. Socioeconomic status and executive function: developmental trajectories and mediation. *Dev Sci.* (2015) 18:686–702. doi: 10.1111/desc.12246
51. Jaeggi SM, Buschkuehl M, Jonides J, Perrig WJ. Improving fluid intelligence with training on working memory. *Proc Natl Acad Sci.* (2008) 105:6829–33. doi: 10.1073/pnas.0801268105
52. Miyake A, Friedman NP, Emerson MJ, Witzki AH, Howerter A. The unity and diversity of executive functions and their contributions to complex "frontal lobe" tasks: a latent variable analysis. *Cogn Psychol.* (2000) 41:49–100. doi: 10.1006/cogp.1999.0734
53. Diamond A. Executive functions. *Annu Rev Psychol.* (2013) 64:135–68. doi: 10.1146/annurev-psych-113011-143750
54. Curtis CE, D'Esposito M. Persistent activity in the prefrontal cortex during working memory. *Trends Cogn Sci.* (2003) 7:415–23. doi: 10.1016/S1364-6613(03)00197-9
55. Bhatt RR, Zeltzer LK, Cologner J, Wood JC, Coates TD, Labus JS. Patients with sickle-cell disease exhibit greater functional connectivity and centrality

in the locus coeruleus compared to anemic controls. *NeuroImage Clin.* (2019) 21:101686. doi: 10.1016/j.nicl.2019.101686
56. Craft S, Schatz J, Glauser TA, Lee B, DeBaun MR. Neuropsychologic effects of stroke in children with sickle cell anemia. *J Pediatr.* (1993) 123:712–7. doi: 10.1016/S0022-3476(05)80844-3
57. Hogan AM, Vargha-Khadem F, Saunders DE, Kirkham FJ, Baldeweg T. Impact of frontal white matter lesions on performance monitoring: ERP evidence for cortical disconnection. *Brain.* (2006) 129:2177–88. doi: 10.1093/brain/awl160
58. Kolb B, Mychasiuk R, Muhammad A, Li Y, Frost DO, Gibb R. Experience and the developing prefrontal cortex. *Proc Natl Acad Sci.* (2012) 109:17186–93. doi: 10.1073/pnas.1121251109
59. Honoré N, Houssa M, Volckaert A, Noël M-P, Nader-Grosbois N. Training inhibition and social cognition in the classrooms. *Front Psychol.* (2020) 11:1974. doi: 10.3389/fpsyg.2020.01974
60. Alloway TP, Alloway RG. Investigating the predictive roles of working memory and IQ in academic attainment. *J Exp Child Psychol.* (2010) 106:20–9. doi: 10.1016/j.jecp.2009.11.003
61. Moffitt TE, Arseneault L, Belsky D, Dickson N, Hancox RJ, Harrington H, et al. A gradient of childhood self-control predicts health, wealth, and public safety. *Proc Natl Acad Sci.* (2011) 108:2693–8. doi: 10.1073/pnas.1010076108
62. Genet JJ, Siemer M. Flexible control in processing affective and non-affective material predicts individual differences in trait resilience. *Cogn Emot.* (2011) 25:380–8. doi: 10.1080/02699931.2010.491647
63. Hollocks MJ, Kok TB, Kirkham FJ, Gavlak J, Inusa BP, DeBaun MR, et al. Nocturnal oxygen desaturation and disordered sleep as a potential factor in executive dysfunction in sickle cell anemia. *J Int Neuropsychol Soc.* (2012) 18:168–73. doi: 10.1017/S1355617711001469
64. Connolly ME, Bills SE, Hardy SJ. Neurocognitive and psychological effects of persistent pain in pediatric sickle cell disease. *Pediatr Blood Cancer.* (2019) 66:e27823. doi: 10.1002/pbc.27823
65. Anderson LM, Allen TM, Thornburg CD, Bonner MJ. Fatigue in children with sickle cell disease: association with neurocognitive and social-emotional functioning and quality of life. *J Pediatr Hematol Oncol.* (2015) 37:584–9. doi: 10.1097/MPH.0000000000000431
66. Kral MC, Brown RT. Transcranial Doppler ultrasonography and executive dysfunction in children with sickle cell disease. *J Pediatr Psychol.* (2004) 29:185–95. doi: 10.1093/jpepsy/jsh020
67. Prussien K V, Compas BE, Siciliano RE, Ciriegio AE, Lee CA, Kassim AA, et al. Cerebral hemodynamics and executive function in sickle cell anemia. *Stroke.* (2021) 52:1830–4. doi: 10.1161/STROKEAHA.120.032741
68. Ludwig NN, Sil S, Khowaja MK, Cohen LL, Dampier C. Executive functioning mediates the relationship between pain coping and quality of life in youth with sickle cell disease. *J Pediatr Psychol.* (2018) 43:1160–9. doi: 10.1093/jpepsy/jsy057
69. Allen TM, Anderson LM, Rothman JA, Bonner MJ. Executive functioning and health-related quality of life in pediatric sickle cell disease. *Child Neuropsychol.* (2017) 23:889–906. doi: 10.1080/09297049.2016.1205011
70. Hood AM, Kölbel M, Stotesbury H, Kawadler J, Slee A, Inusa B, et al. Biopsychosocial Predictors of Quality of Life in Paediatric Patients With Sickle Cell Disease. *Front Psychol.* (2021) 12:681137. doi: 10.3389/fpsyg.2021.681137
71. Salthouse TA. Aging and measures of processing speed. *Biol Psychol.* (2000) 54:35–54. doi: 10.1016/S0301-0511(00)00052-1
72. Silva MA, Lee JM. *Neurocognitive Testing.* Elsevier (2021). doi: 10.1016/B978-0-12-822963-7.00047-5
73. Agelink van Rentergem JA, de Vent NR, Schmand BA, Murre JMJ, Staaks JPC, Huizenga HM. The factor structure of cognitive functioning in cognitively healthy participants: a meta-analysis and meta-analysis of individual participant data. *Neuropsychol Rev.* (2020) 30:51–96. doi: 10.1007/s11065-019-09423-6
74. Montanaro M, Colombatti R, Pugliese M, Migliozzi C, Zani F, Guerzoni ME, et al. Intellectual function evaluation of first generation immigrant children with sickle cell disease: the role of language and sociodemographic factors. *Ital J Pediatr.* (2013) 39:36. doi: 10.1186/1824-7288-39-36
75. Berg C, Edwards DF, King A. Executive function performance on the children's kitchen task assessment with children with sickle cell disease and matched controls. *Child Neuropsychol.* (2012) 18:432–48. doi: 10.1080/09297049.2011.613813
76. Puffer ES, Schatz JC, Roberts CW. Relationships between somatic growth and cognitive functioning in young children with sickle cell disease. *J Pediatr Psychol.* (2010) 35:892–904. doi: 10.1093/jpepsy/jsp124
77. Kral MC, Brown RT, Curé JK, Besenski N, Jackson SM, Abboud MR. Radiographic predictors of neurocognitive functioning in pediatric sickle cell disease. *J Child Neurol.* (2006) 21:37–44. doi: 10.1177/08830738060210010701
78. Marshall MJ, Bucks RS, Hogan AM, Hambleton IR, Height SE, Dick MC, et al. Auto-adjusting positive airway pressure in children with sickle cell anemia: results of a phase I randomized controlled trial. *Haematologica.* (2009) 94:1006–10. doi: 10.3324/haematol.2008.005215
79. Howard J, Lee SA, Inusa B, Cheng MYE, Bavenjit C, Reading IC, et al. Prevention of morbidity in sickle cell disease (POMS2a)—overnight auto-adjusting continuous positive airway pressure compared with nocturnal oxygen therapy: a randomised crossover pilot study examining patient preference and safety in adults and children. *Trials.* (2019) 20:1–15. doi: 10.1186/s13063-019-3461-x
80. Xu G, Strathearn L, Liu B, Yang B, Bao W. Twenty-year trends in diagnosed attention-deficit/hyperactivity disorder among US children and adolescents, 1997-2016. *JAMA Netw Open.* (2018) 1:e181471. doi: 10.1001/jamanetworkopen.2018.1471
81. Lance EI, Comi AM, Johnston M V, Casella JF, Shapiro BK. Risk factors for attention and behavioral issues in pediatric sickle cell disease. *Clin Pediatr.* (2015) 54:1087–93. doi: 10.1177/0009922815594356
82. Acquazzino MA, Miller M, Myrvik M, Newby R, Scott JP. Attention deficit hyperactivity disorder in children with sickle cell disease referred for an evaluation. *J Pediatr Hematol Oncol.* (2017) 39:350–4. doi: 10.1097/MPH.0000000000000847
83. Benton TD, Boyd R, Ifeagwu J, Feldtmose E, Smith-Whitley K. Psychiatric diagnosis in adolescents with sickle cell disease: a preliminary report. *Curr Psychiatry Rep.* (2011) 13:111–5. doi: 10.1007/s11920-011-0177-3
84. Moody KL, Mercer K, Glass M. An integrative review of the prevalence of depression among pediatric patients with sickle cell disease. *Soc Work Public Health.* (2019) 34:343–52. doi: 10.1080/19371918.2019.1606754
85. Mojtabai R, Olfson M, Han B. National trends in the prevalence and treatment of depression in adolescents and young adults. *Pediatrics.* (2016) 138:e20161878. doi: 10.1542/peds.2016-1878
86. Cobham VE, Hickling A, Kimball H, Thomas HJ, Scott JG, Middeldorp CM. Systematic review: anxiety in children and adolescents with chronic medical conditions. *J Am Acad Child Adolesc Psychiatry.* (2019) 59:595–618. doi: 10.1016/j.jaac.2019.10.010
87. Anim MT, Osafo J, Yirdong F. Prevalence of psychological symptoms among adults with sickle cell disease in Korle-Bu Teaching Hospital, Ghana. *BMC Psychol.* (2016) 4:53. doi: 10.1186/s40359-016-0162-z
88. Levenson JL, McClish DK, Dahman BA, Bovbjerg VE, Citero V de A, Penberthy LT, et al. Depression and anxiety in adults with sickle cell disease: the PiSCES project. *Psychosom Med.* (2008) 70:192–6. doi: 10.1097/PSY.0b013e31815ff5c5
89. Anie KA. Psychological complications in sickle cell disease. *Br J Haematol.* (2005) 129:723–9. doi: 10.1111/j.1365-2141.2005.05500.x
90. Gil KM, Wilson JJ, Edens JL, Workman E, Ready J, Sedway J, et al. Cognitive coping skills training in children with sickle cell disease pain. *Int J Behav Med.* (1997) 4:364–77. doi: 10.1207/s15327558ijbm0404_7
91. Powers SW, Mitchell MJ, Graumlich SE, Byars KC, Kalinyak KA. Longitudinal assessment of pain, coping, and daily functioning in children with sickle cell disease receiving pain management skills training. *J Clin Psychol Med Settings.* (2002) 9:109–19. doi: 10.1023/A:1014940009788
92. Thomas VJ, Dixon AL, Milligan P, Thomas N. Cognitive-behaviour therapy for the management of sickle cell disease pain: an evaluation of a community-based intervention. *Br J Health Psychol.* (1999) 4:209–29. doi: 10.1348/135910799168588
93. Broome ME, Maikler V, Kelber S, Bailey P, Lea G. An intervention to increase coping and reduce health care utilization for school-age children and adolescents with sickle cell disease. *J Natl Black Nurses Assoc JNBNA.* (2001) 12:6–14. doi: 10.1111/j.1744-6155.2001.tb00240.x

94. Schatz J, Schlenz A, McClellan CB, Puffer ES, Hardy S, Pfeiffer M, et al. Changes in coping, pain and activity following cognitive-behavioral training: a randomized clinical trial for pediatric sickle cell disease using smartphones. *Clin J Pain.* (2015) 31:536. doi: 10.1097/AJP.0000000000000183
95. Fernández AL, Abe J. Bias in cross-cultural neuropsychological testing: problems and possible solutions. *Cult Brain.* (2018) 6:1–35. doi: 10.1007/s40167-017-0050-2
96. Löwe B, Unützer J, Callahan CM, Perkins AJ, Kroenke K. Monitoring depression treatment outcomes with the patient health questionnaire-9. *Med Care.* (2004) 42:1194–201. doi: 10.1097/00005650-200412000-00006
97. Jehu DA, Davis JC, Madden K, Parmar N, Liu-Ambrose T. Minimal clinically important difference of executive function performance in older adults who fall: a secondary analysis of a randomized controlled trial. *Gerontology.* (2021) 67:1–9. doi: 10.1159/000518939
98. Morris C, Gibbons E, Fitzpatrick R. *Child and Parent Reported Outcome Measures: A Scoping Report Focusing on Feasibility for Routine Use in the NHS*. Oxford: Department of Public Health, University of Oxford (2009).
99. Lezak M, Howieson D, Loring D. *Neuropsychological Assessment*. 5th edn. Oxford, New York: Oxford University Press (2012).
100. Committee on Psychological Testing IV. *Psychological Testing in the Service of Disability Determination* (Washington, DC) (2015).
101. Clark SW, Gulin SL, Heller MB, Vrana SR. Graduate training implications of the Q-interactive platform for administering Wechsler intelligence tests. *Train Educ Prof Psychol.* (2017) 11:148. doi: 10.1037/tep0000155
102. Ford CL, Takahashi LM, Chandanabhumma PP, Ruiz ME, Cunningham WE. Anti-racism methods for big data research: lessons learned from the HIV testing, linkage, & retention in care (HIV TLR) study. *Ethn Dis.* (2018) 28:261. doi: 10.18865/ed.28.S1.261
103. Fernandez AL. Modern neuropsychological tests for a diversity of cultural contexts. *Clin Neuropsychol.* (2019) 33:438–45. doi: 10.1080/13854046.2018.1560501
104. Silverberg ND, Crane PK, Dams-O'Connor K, Holdnack J, Ivins BJ, Lange RT, et al. Developing a cognition endpoint for traumatic brain injury clinical trials. *J Neurotrauma.* (2017) 34:363–71. doi: 10.1089/neu.2016.4443
105. Jaeschke R, Singer J, Guyatt GH. Measurement of health status: ascertaining the minimal clinically important difference. *Control Clin Trials.* (1989) 10:407–15. doi: 10.1016/0197-2456(89)90005-6
106. McGlothlin AE, Lewis RJ. Minimal clinically important difference: defining what really matters to patients. *Jama.* (2014) 312:1342–3. doi: 10.1001/jama.2014.13128
107. Lee AC, Driban JB, Price LL, Harvey WF, Rodday AM, Wang C. Responsiveness and minimally important differences for 4 patient-reported outcomes measurement information system short forms: physical function, pain interference, depression, and anxiety in knee osteoarthritis. *J Pain.* (2017) 18:1096–110. doi: 10.1016/j.jpain.2017.05.001
108. Waterhouse DM, Harvey RD, Hurley P, Levit LA, Kim ES, Klepin HD, et al. Early impact of COVID-19 on the conduct of oncology clinical trials and long-term opportunities for transformation: findings from an American Society of Clinical Oncology Survey. *JCO Oncol Pract.* (2020) 16:417–21. doi: 10.1200/OP.20.00275
109. Hood AM, Strong H, Nwankwo C, Johnson Y, Peugh J, Mara CA, et al. Engaging caregivers and providers of children with sickle cell anemia in shared decision making for hydroxyurea: protocol for a multicenter randomized controlled trial. *JMIR Res Protoc.* (2021) 10:e27650. doi: 10.2196/27650
110. McCabe D, Langer KG, Borod JC, Bender HA. Practice effects. In: Kreutzer JS, DeLuca J, Caplan B, editors. *Encyclopedia of Clinical Neuropsychology*. New York, NY: Springer New York (2011). p. 1988–9.
111. Lo AHY, Humphreys M, Byrne GJ, Pachana NA. Test–retest reliability and practice effects of the Wechsler Memory Scale-III. *J Neuropsychol.* (2012) 6:212–31. doi: 10.1111/j.1748-6653.2011.02023.x
112. Goldberg TE, Harvey PD, Wesnes KA, Snyder PJ, Schneider LS. Practice effects due to serial cognitive assessment: implications for preclinical Alzheimer's disease randomized controlled trials. *Alzheimers Dement.* (2015) 1:103–11. doi: 10.1016/j.dadm.2014.11.003
113. Collie A, Maruff P, Darby DG, McSTEPHEN M. The effects of practice on the cognitive test performance of neurologically normal individuals assessed at brief test-retest intervals. *J Int Neuropsychol Soc.* (2003) 9:419. doi: 10.1017/S1355617703930074
114. Sommer M, Arendasy ME, Schützhofer B, Knessel G. Comparing the effectiveness of different methods to reduce the effect size of the practice effect in traffic psychological assessment. *Transp Res Part F Traffic Psychol Behav.* (2018) 58:955–68. doi: 10.1016/j.trf.2018.06.042
115. Anderson P. Assessment and development of executive function (EF) during childhood. *Child Neuropsychol.* (2002) 8:71–82. doi: 10.1076/chin.8.2.71.8724
116. Duff K. Evidence-based indicators of neuropsychological change in the individual patient: relevant concepts and methods. *Arch Clin Neuropsychol.* (2012) 27:248–61. doi: 10.1093/arclin/acr120
117. Martin R, Sawrie S, Gilliam F, Mackey M, Faught E, Knowlton R, et al. Determining reliable cognitive change after epilepsy surgery: development of reliable change indices and standardized regression-based change norms for the WMS-III and WAIS-III. *Epilepsia.* (2002) 43:1551–8. doi: 10.1046/j.1528-1157.2002.23602.x
118. Puffer ES, Schatz J, Roberts CW. The association of oral hydroxyurea therapy with improved cognitive functioning in sickle cell disease. *Child Neuropsychol.* (2007) 13:142–54. doi: 10.1080/09297040600584626
119. Land V, Mutsaerts HJMM, Engelen M, Heijboer H, Roest M, Hollestelle MJ, et al. Risk factor analysis of cerebral white matter hyperintensities in children with sickle cell disease. *Br J Haematol.* (2016) 172:274–84. doi: 10.1111/bjh.13819
120. Kawadler JM, Clayden JD, Kirkham FJ, Cox TC, Saunders DE, Clark CA. Subcortical and cerebellar volumetric deficits in paediatric sickle cell anaemia. *Br J Haematol.* (2013) 163:373–6. doi: 10.1111/bjh.12496
121. Kawadler JM, Kirkham FJ, Clayden JD, Hollocks MJ, Seymour EL, Edey R, et al. White matter damage relates to oxygen saturation in children with sickle cell anemia without silent cerebral infarcts. *Stroke.* (2015) 46:1793–9. doi: 10.1161/STROKEAHA.115.008721
122. Armstrong FD, Thompson RJ, Wang W, Zimmerman R, Pegelow CH, Miller S, et al. Cognitive functioning and brain magnetic resonance imaging in children with sickle cell disease. *Pediatrics.* (1996) 97:864–70. doi: 10.1542/peds.97.6.864
123. Choi S, O'Neil SH, Joshi AA, Li J, Bush AM, Coates TD, et al. Anemia predicts lower white matter volume and cognitive performance in sickle and non-sickle cell anemia syndrome. *Am J Hematol.* (2019) 94:1055–65. doi: 10.1002/ajh.25570
124. Downes M, Keenan L, Duane Y, Duffy K, Fortune G, Geoghegan R, et al. Executive function in children with sickle cell anemia on transfusion: NIH toolbox utility in the clinical context. *Clin Neuropsychol.* (2020) 62:1–16. doi: 10.1080/13854046.2020.1847325
125. Chen R, Krejza J, Arkuszewski M, Zimmerman RA, Herskovits EH, Melhem ER. Brain morphometric analysis predicts decline of intelligence quotient in children with sickle cell disease: a preliminary study. *Adv Med Sci.* (2017) 62:151–7. doi: 10.1016/j.advms.2016.09.002
126. Stotesbury H, Kawadler JM, Clayden JD, Saunders DE, Hood AM, Kolbel M, et al. Quantification of silent cerebral infarction on high-resolution FLAIR and cognition in sickle cell anemia. *Front Neurol.* (In Press). doi: 10.3389/fneur.2022.867329
127. Dampier C, Palermo TM, Darbari DS, Hassell K, Smith W, Zempsky W. AAPT diagnostic criteria for chronic sickle cell disease pain. *J Pain.* (2017) 18:490–8. doi: 10.1016/j.jpain.2016.12.016
128. Kawadler JM, Slee A, Stotesbury H, Koelbel M, Balfour P, Saunders DE, et al. Index of pain experience in sickle cell anaemia (IPESCA): development from daily pain diaries and initial findings from use with children and adults with sickle cell anaemia. *Br J Haematol.* (2019) 186:360–3. doi: 10.1111/bjh.15841
129. Cella D, Riley W, Stone A, Rothrock N, Reeve B, Yount S, et al. The Patient-Reported Outcomes Measurement Information System (PROMIS) developed and tested its first wave of adult self-reported health outcome item banks: 2005–2008. *J Clin Epidemiol.* (2010) 63:1179–94. doi: 10.1016/j.jclinepi.2010.04.011
130. Rogers VE, Lewin DS, Winnie GB, Geiger-Brown J. Polysomnographic characteristics of a referred sample of children with sickle cell disease. *J Clin sleep Med JCSM.* (2010) 6:374. doi: 10.5664/jcsm.27880

131. Rosen CL, Debaun MR, Strunk RC, Redline S, Seicean S, Craven DI, et al. Obstructive sleep apnea and sickle cell anemia. *Pediatrics.* (2014) 134:273–81. doi: 10.1542/peds.2013-4223
132. Hankins JS, Verevkina NI, Smeltzer MP, Wu S, Aygun B, Clarke DF. Assessment of sleep-related disorders in children with sickle cell disease. *Hemoglobin.* (2014) 38:244–51. doi: 10.3109/03630269.2014.919941
133. Wallen GR, Minniti CP, Krumlauf M, Eckes E, Allen D, Oguhebe A, et al. Sleep disturbance, depression and pain in adults with sickle cell disease. *BMC Psychiatry.* (2014) 14:207. doi: 10.1186/1471-244X-14-207
134. Lehmann GC, Bell TR, Kirkham FJ, Gavlak JC, Ferguson TF, Strunk RC, et al. Enuresis associated with sleep disordered breathing in children with sickle cell anemia. *J Urol.* (2012) 188:1572–7. doi: 10.1016/j.juro.2012.02.021
135. Cabañas-Pedro AC, Roizenblatt S, de Souza AAL, Tufik S, Figueiredo MS. Periodic limb movement in sleep and sickle cell disease: a neglected association? *Br J Haematol.* (2017) 179:154–7. doi: 10.1111/bjh.14811
136. Liguoro I, Arigliani M, Tan H, Gupta A. The burden of sleep disordered breathing in children with sickle cell disease. *Pediatr Pulmonol.* (2021) 56:3607–33. doi: 10.1002/ppul.25632
137. Kölbel M, Kirkham FJ, Dimitriou D. Developmental profile of sleep and its potential impact on daytime functioning from childhood to adulthood in sickle cell anaemia. *Brain Sci.* (2020) 10:981. doi: 10.3390/brainsci10120981
138. Schmidt C, Collette F, Cajochen C, Peigneux P. A time to think: circadian rhythms in human cognition. *Cogn Neuropsychol.* (2007) 24:755–89. doi: 10.1080/02643290701754158
139. Unnikrishnan AG, Bhattacharyya A, Baruah MP, Sinha B, Dharmalingam M, Rao P V. Importance of achieving the composite endpoints in diabetes. *Indian J Endocrinol Metab.* (2013) 17:835. doi: 10.4103/2230-8210.117225
140. Brody T. *Clinical Trials: Study Design, Endpoints and Biomarkers, Drug Safety, and FDA and ICH Guidelines*. Minneapolis, MN: Academic Press (2016).
141. Iverson GL, Karr JE, Terry DP, Garcia-Barrera MA, Holdnack JA, Ivins BJ, et al. Developing an executive functioning composite score for research and clinical trials. *Arch Clin Neuropsychol.* (2020) 35:312–25. doi: 10.1093/arclin/acz070
142. Gibbons LE, Carle AC, Mackin RS, Harvey D, Mukherjee S, Insel P, et al. A composite score for executive functioning, validated in Alzheimer's Disease Neuroimaging Initiative (ADNI) participants with baseline mild cognitive impairment. *Brain Imaging Behav.* (2012) 6:517–27. doi: 10.1007/s11682-012-9176-1
143. Lehto JE, Juujärvi P, Kooistra L, Pulkkinen L. Dimensions of executive functioning: evidence from children. *Br J Dev Psychol.* (2003) 21:59–80. doi: 10.1348/026151003321164627
144. Schatz J, Stancil M, Katz T, Sanchez CE. EXAMINER executive function battery and neurologic morbidity in pediatric sickle cell disease. *J Int Neuropsychol Soc.* (2014) 20:29–40. doi: 10.1017/S1355617713001239
145. Stotesbury H, Kawadler JM, Saunders DE, Kirkham FJ. MRI detection of brain abnormality in sickle cell disease. *Expert Rev Hematol.* (2021) 14:473–91. doi: 10.1080/17474086.2021.1893687

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers | Frontiers in Neurology

ORIGINAL RESEARCH
published: 29 June 2022
doi: 10.3389/fneur.2022.867329

Quantification of Silent Cerebral Infarction on High-Resolution FLAIR and Cognition in Sickle Cell Anemia

Hanne Stotesbury[1], Jamie M. Kawadler[1], Jonathan D. Clayden[1], Dawn E. Saunders[1], Anna M. Hood[1,2], Melanie Koelbel[1], Sati Sahota[1], David C. Rees[3], Olu Wilkey[4], Mark Layton[5], Maria Pelidis[6], Baba P. D. Inusa[6], Jo Howard[6], Subarna Chakravorty[3], Chris A. Clark[1] and Fenella J. Kirkham[1,7]*

[1] Developmental Neurosciences, UCL Great Ormond Street Institute of Child Health, London, United Kingdom, [2] Division of Psychology and Mental Health, Manchester Centre for Health Psychology, University of Manchester, Manchester, United Kingdom, [3] King's College London, London, United Kingdom, [4] North Middlesex University Hospital NHS Foundation Trust, London, United Kingdom, [5] Haematology, Imperial College Healthcare NHS Foundation Trust, London, United Kingdom, [6] Department of Haematology and Evelina Children's Hospital, Guy's and St Thomas' NHS Foundation Trust, London, United Kingdom, [7] Clinical and Experimental Sciences, University of Southampton, Southampton, United Kingdom

OPEN ACCESS

Edited by:
Guillaume Sébire, McGill University, Canada

Reviewed by:
Monica Hulbert, Washington University in St. Louis, United States
Antoine Garnier-Crussard, Hospices Civils de Lyon, France

***Correspondence:**
Hanne Stotesbury
hanne.stotesbury.15@ucl.ac.uk

Specialty section:
This article was submitted to Stroke, a section of the journal Frontiers in Neurology

Received: *01 February 2022*
Accepted: *27 April 2022*
Published: *29 June 2022*

Citation:
Stotesbury H, Kawadler JM, Clayden JD, Saunders DE, Hood AM, Koelbel M, Sahota S, Rees DC, Wilkey O, Layton M, Pelidis M, Inusa BPD, Howard J, Chakravorty S, Clark CA and Kirkham FJ (2022) Quantification of Silent Cerebral Infarction on High-Resolution FLAIR and Cognition in Sickle Cell Anemia. Front. Neurol. 13:867329. doi: 10.3389/fneur.2022.867329

Research in sickle cell anemia (SCA) has used, with limited race-matched control data, binary categorization of patients according to the presence or absence of silent cerebral infarction (SCI). SCI have primarily been identified using low-resolution MRI, with radiological definitions varying in lesion length and the requirement for abnormality on both fluid attenuated inversion recovery (FLAIR) and T1-weighted images. We aimed to assess the effect of published SCI definitions on global, regional, and lobar lesion metrics and their value in predicting cognition. One hundred and six patients with SCA and 48 controls aged 8–30 years underwent 3T MRI with a high-resolution FLAIR sequence and Wechsler cognitive assessment. Prevalence, number, and volume of lesions were calculated using a semi-automated pipeline for SCI defined as: (1) Liberal: any length (L-SCI); (2) Traditional: >3 mm in greatest dimension (T-SCI); (3) Restrictive; >3 mm in greatest dimension with a corresponding T1-weighted hypo-intensity (R-SCI). Globally, as hypothesized, there were large effects of SCI definition on lesion metrics in patients and controls, with prevalence varying from 24–42% in patients, and 4–23% in controls. However, contrary to hypotheses, there was no effect of any global metric on cognition. Regionally, there was a consistent distribution of SCI in frontal and parietal deep and juxta-cortical regions across definitions and metrics in patients, but no consistent distribution in controls. Effects of regional SCI metrics on cognitive performance were of small magnitude; some were paradoxical. These findings expose the challenges associated with the widespread use of SCI presence as a biomarker of white-matter injury and cognitive dysfunction in cross-sectional high-resolution MRI studies in patients with SCA. The findings indicate that with high-resolution MRI: (1) radiological definitions have a large effect on resulting lesion groups, numbers, and volumes; (2) there is a non-negligible prevalence of lesions in young healthy controls; and (3) at the group-level, there is no cross-sectional association between global lesion metrics and general cognitive impairment irrespective of lesion definition and metric. With high-resolution multi-modal MRI, the dichotomy

of presence or absence of SCI does not appear to be a sensitive biomarker for the detection of functionally significant pathology; the search for appropriate endpoints for clinical treatment trials should continue.

Keywords: anemia, sickle cell, silent cerebral infarction, ischemia, white matter hyperintensities, magnetic resonance imaging, cognition, intelligence quotient

INTRODUCTION

People with sickle cell anemia (SCA) are at risk of silent cerebral infarction (SCI) (1). Traditionally, SCI in children with SCA has been defined using the Silent Infarct Transfusion trial criteria: an area of abnormally high signal intensity on T2-weighted (T2-w) and/or fluid-attenuated-inversion-recovery (FLAIR) MRI (1.5T) measuring at least 3 mm in greatest dimension, visible on two planes, and with no corresponding focal deficit (i.e., traditional SCI definition, T-SCI; **Figure 1**) (2, 3). The majority of SCI are "deep" white matter hyperintensities (WMH), but "juxtacortical" and "periventricular" SCI have also been described (4, 5).

SCI occur in patients with SCA as young as 7 months (6), and are associated with future neurological complications, including new and enlarging SCI as well as ischemic stroke (7). Prevalence appears to increase throughout life, with SCI detected in 25% of patients by 6 years (5), 39% by 18 years (8), and 53% by 30 years (9). Using low-resolution sequences, presence of SCI has also frequently been associated with cognitive impairment across several domains (10), which may manifest as poor academic attainment (11). The American Society for Hematology (ASH) guidelines have therefore proposed that patients with SCA or HbSβ_0 thalassemia should undergo MRI to detect SCI (12), while the use of presence of SCI as a biomarker for risk of cognitive difficulties was recently discussed at the Food and Drug Administration (FDA/ASH) meeting on endpoints for clinical trials in SCA (13). Findings have, however, been mixed, with several more recent studies failing to detect differences between patients with and without SCI in various cognitive domains, including general intelligence (IQ), processing speed and working memory (14–16).

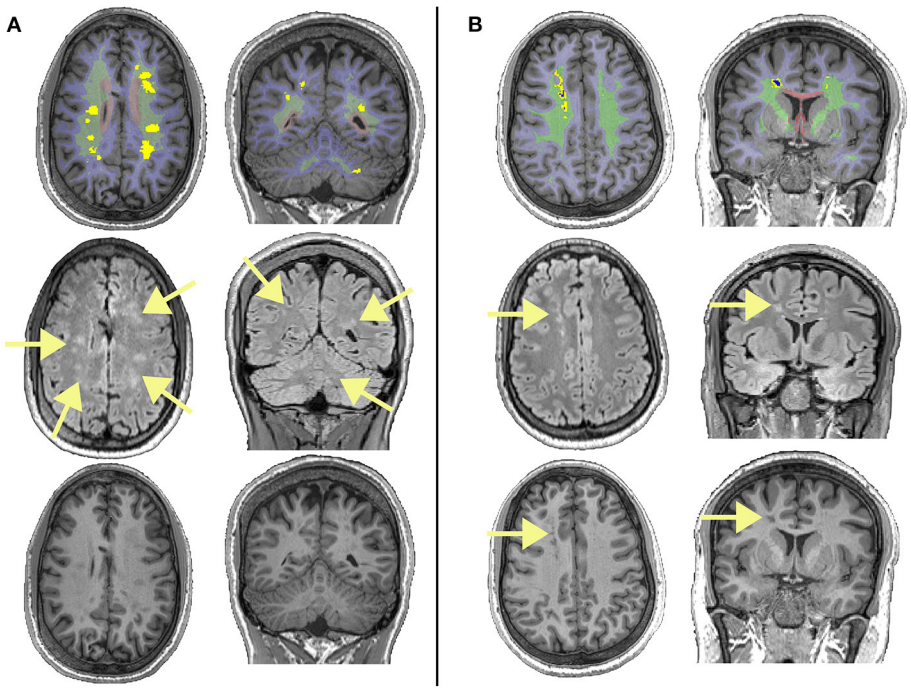


FIGURE 1 | Examples of different lesions in two patients with sickle cell anemia. The top row shows regions of interest overlaid on T1-weighted images, with the juxta-cortical region shown in light blue, the deep region shown in light green, the periventricular region shown in light red, lesions that survived the FLAIR threshold (equation 1) shown in bright yellow, and lesions that survived the T1 threshold (equation 2) shown in dark blue. The middle row shows corresponding FLAIR images, with arrows showing the locations of lesions. The bottom row shows corresponding T1w images, with arrows showing the locations of lesions that survived the T1 threshold and restrictive definition. **(A)** Showing deep lesions meeting the liberal and traditional definitions in a male patient with sickle cell anemia aged 19. Despite having high FLAIR lesion burden for both the liberal and traditional definitions, this patient had relatively low lesion burden on T1, with none of the lesions shown meeting the restrictive definition. **(B)** Showing deep lesions meeting the liberal, traditional, and restrictive definitions in a male patient with sickle cell anemia aged 20. This patient had a high FLAIR lesion burden, with some voxels within lesion masks meeting the restrictive definition.

Beyond the pediatric T-SCI definition, a more restrictive SCI definition (R-SCI) (**Figure 1**), based on studies in adults without SCA, was proposed in the first large-scale study of cognition in adults with SCA, requiring a hyperintensity of more than 5 mm in greatest dimension on T2w-MRI along with a corresponding hypo-intensity on T1-weighted (T1w) MRI (17). However, presence of R-SCI does not appear to predict general cognitive performance (i.e., IQ) in adults (17) or children (18) with SCA independently. Several studies employing a more liberal definition of SCI (e.g., hyperintensity of any length, L-SCI) have also failed to find associations with cognition (14, 19, 20).

Discrepancies in prevalence and association with cognition may relate to differences in the precise definition of SCI, clinical care, sample characteristics including age and cognitive range, and advances in technology (21). Studies using lower field strength magnets and lower-resolution sequences (e.g., 3–5 mm slice thicknesses with a slice gap) will by definition miss some lesions meeting liberal and traditional criteria, and there is evidence that lesion detectability increases with increasing magnet strength; in a study of adults with SCA, the prevalence of L-SCI increased from 60% at 3T (1 mm resolution) to 90% at 7T (0.8 mm resolution) (22).

There are relatively few data on the prevalence of SCI or any association with cognition in age- and race- matched controls and it is unclear whether the combination of increased lesion detectability and more liberal criteria improves or disrupts the ability to predict cognitive outcomes based on SCI presence alone in SCA. Greater detection of SCI in patients may include those with associated impairment, but smaller WMHs, also reported in children without SCA (23), and associated with healthy aging (24), may be less functionally significant.

In other populations where WMHs are common, including healthy older adults and those with dementia, there is typically no minimum lesion length requirement, and neurobehavioral correlates vary with lesion burden, regional classification, and lobar distribution (24, 25). Several recent studies employing higher resolution sequences in SCA populations have therefore considered one T-SCI per decade of life to be "normal," and thus grouped participants according to whether they have an abnormal burden of T-SCI for age (15, 26). However, if the overall burden is important, L-SCI volume may plausibly serve as a more sensitive combination of lesion definition and metric. Whilst a few studies have considered relationships between global SCI volume and cognitive outcome in SCA (27–29), they all are based on relatively low resolution MRI sequences with thick slices (3–5 mm). Only one considered the potential impact of lesion distribution, albeit by grouping patients according to whether they had frontal ($n = 7$) or more widely distributed ($n = 18$) lesions (27).

Although the majority of clinical studies are now performed at 3T, and the global and regional burden of SCI can be quantified using semi-automated methods, no high-resolution imaging studies have investigated the effect of published SCI definitions, quantification metrics, and regional classification criteria on prediction of cognitive outcome in SCA. Determining the most sensitive combination of definition and metric using modern scanners is important, particularly as clinical trials have included or proposed including the appearance of new SCI as endpoints, partly on the basis that they are traditionally associated with cognitive impairment in SCA (13, 30, 31). In addition, an SCI endpoint may appear less subjective than a patient-reported outcome, and may also be more cost effective than full cognitive assessment requiring considerable time and effort for performance and interpretation. This was echoed in the aforementioned FDA meeting on endpoints, but the need for further validation using higher-resolution 3D sequences was also emphasized (13).

Therefore, the purpose of this study was to investigate, using previous radiological definitions, high-resolution FLAIR and semi-automated methods, the potential clinical utility of SCI as a biomarker of general cognition. Specifically, we hypothesized that there would be significant differences in global lesion metrics depending on the definition employed, with the most liberal definition (L-SCI) identifying significantly greater lesion burden. We also hypothesized that global lesion metrics based on the L-SCI definition would best predict general cognitive performance. In addition, we explored regional and lobar lesion metrics based on different SCI definitions and their value in predicting general cognition.

MATERIALS AND METHODS

Sample

People with homozygous SCA (HbSS) or HbSβ_0-thalassemia were recruited to three on-going and concurrent studies at UCL Great Ormond Street Institute of Child Health between 2015 and 2019: the Sleep and Asthma Cohort follow-up (SAC) (32), the Prevention of Morbidity in Sickle Cell Anaemia baseline investigation (POMS) (31) and a study of sleep in SCA (33). Controls were siblings and race-matched peers (i.e., Black British) of patients recruited to SAC. Patients were ineligible for SAC and POMS study participation if they were receiving nocturnal respiratory support at the time of enrollment, participating in a clinical trial evaluating blood transfusion or oxygen therapy, or had chronic lung disease (other than asthma) or existing respiratory failure. Additional exclusion criteria for the POMS study were hospital admissions for acute sickle complications within 1 month of enrollment, more than 6 hospital admissions for acute sickle complications within 12 months of enrollment, overnight oximetry showing mean overnight saturation of <90% for more than 30% of total sleep time, severe sleep apnea defined by 4% oxygen desaturation index >15/h, and chronic blood transfusion or transfusion within 3 months of enrollment. For the SAC and sleep studies patients were enrolled without regard to past sickle- or sleep-related morbidity or transfusion status. West London NHS (SAC; 05/Q0408/42, 11/EM/0084, 15/LO/0347), Yorkshire NHS, (POMS; 15/YH/0213), and University College London (community recruitment 14475/001) research ethics committees provided ethical approval, and participants/parents provided written informed consent.

Cognitive Measures

IQ was estimated using the two-subtest Wechsler Abbreviated Scale of Intelligence (WASI; POMS participants) (34), the

Wechsler Intelligence Scale for Children (WISC-IV; SAC participants <16 years) (35), or the Wechsler Adult Intelligence Scale (WAIS-IV; SAC participants >16 years) (36). Subtests from the WISC-IV (POMS and SAC participants <16 years old) or WAIS-IV (POMS and SAC participants >16 years old) measuring working memory (Digit Span, Arithmetic) and processing speed (Coding, Symbol Search) were used to calculate composite indices (working memory index, WMI and processing speed index, PSI, respectively). Participants were classified as cognitively impaired if either their WMI, PSI, or IQ score fell below 70, corresponding to the 2nd percentile (i.e., 2 standard deviations below the normative mean). For 1 patient and 1 control with invalid performance on the coding subtest, and 1 control with invalid performance on the Symbol Search subtest, Cancellation subtest scores were used to calculate PSI. Participants were assessed as close to the date of MRI as possible, with 76% undergoing both on the same day, and all undergoing both within 4.5 months.

Hematological Measures

Peripheral oxygen saturation (SpO_2) was recorded at rest using a single fingertip pulse oximeter (Masimo Pronto) reading for at least 3 min on the day of MRI (SAC) or the closest trial visit (POMS). For patients with SCA, use of disease-modifying treatments (i.e., chronic blood transfusion, hydroxyurea) and blood counts were collected from medical records using the closest scheduled routine clinic visit to date of MRI.

Socioeconomic Measures

Educational attainment, estimated from UK postcode using the English Indices of Deprivation (37) provided an index of socio-economic status. This measure has demonstrated sensitivity in SCA patients (16), and reflects educational attainment in local areas based on several indicators: (1) average scores for pupils in state-funded schools at ages 7–11 and 14–16 years, (2) absence from state-funded secondary schools, (3) the proportion of people staying on in education/training post 16 years, entry to higher education, and (4) proportion of working adults with no/low qualifications and language proficiency. Total scores are ranked from 1 to 10, with 1 representing the most deprived.

Magnetic Resonance Imaging

MRI was performed on a 3T Siemens Prisma (Erlangen, Germany) with 80 mT/m gradients and a 64-channel receive head coil. The protocol included coronal high-resolution 3D FLAIR (TR = 5,000 ms, TE = 395 ms, voxel size = 0.65 × 0.65 × 1.0 mm, scan time = 6 min 22 s), axial 2D T2-w turbo spin echo (TR = 8 420 ms, TE = 68 ms, voxel size = 0.50 × 0.50 × 4.0 mm, scan time = 2 min, 50 s), T1-w magnetization-prepared rapid acquisition gradient echo (MPRAGE; TR = 2,300 ms, TE = 2.74 ms, TI = 909 ms, flip angle = 8°, voxel size = 1 × 1 × 1 mm, scan time = 5 min, 21 s) and 3D time-of-flight MRA sequences (TR = 21.0 ms, TE = 3.4 ms, scan time= 5 min, 33 s).

SCI Definitions

The full pipeline for definition, quantification, and classification of SCI in participants without focal neurological symptoms is shown in **Figure 2**. First, an experienced pediatric neuroradiologist, blind to disease status, identified regions of abnormally high signal intensity indicating an ischemic lesion and recorded the lobe in which lesions were located. Lesions were identified on the coronal FLAIR and confirmed on the axial T2-weighted image, i.e., visible in two planes as lesions in accordance with the silent infarct transfusion (SIT) trial protocol (2). Clearly distinguishable SCI "mimics" were excluded (e.g., linear perivascular spaces running in the direction of small veins). Using Horos (https://horosproject.org), lesions were then manually measured on the coronal FLAIR, counted, and considered against three definitions;

(1) L-SCI: any length (19, 29).
(2) T-SCI: at least 3 mm in greatest dimension (i.e., SIT-trial definition) (2).
(3) R-SCI: at least 3 mm in greatest dimension with a corresponding hypo-intensity on T1w MRI (17).

Quantification of Global SCI

To extract participant-specific estimates of total intracranial volume, as well as masks of the cortex, white matter, and ventricles (lateral and fourth), cortical reconstruction and volumetric segmentation were performed on T1-w images using Freesurfer (Center for Biomedical Imaging, Massachusetts, USA; http://surfer.nmr.mgh.harvard.edu/). Regions of interest (ROIs) were then manually drawn around the identified lesions on native FLAIR images, before FLAIR and T1w images were bias-corrected using SPM (Wellcome Trust Centre for Neuroimaging, London, UK; http://www.fil.ion.ucl.ac.uk/spm), and linearly affine-aligned using FSL (FMRIB, Oxford, UK; https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSL). Based on a previously published SCA-specific pipeline (29), for each participant the mean FLAIR intensity across cortex was calculated, and the following lower threshold was used to determine which voxels within ROIs should be included in a total FLAIR lesion burden mask (**Figure 2**);

$$\mathrm{LowerThr}_{flair} = 1.02 \times \mathrm{mean}_{\mathrm{FLAIR}cortex} \quad (1)$$

Next, the FSL cluster tool separated the total FLAIR lesion mask into individual FLAIR lesion masks that corresponded with each lesion initially identified by the neuroradiologist. For consistency with the FLAIR hyperintensity definition, and to operationalize and automate the visual definition of a corresponding T1 hypo-intensity provided by Choudhury et al. (18) (i.e., that lesions appear either iso- or hypo- intense to surrounding gray matter), the mean cortical T1 intensity for each participant was calculated. The following upper threshold was then used to determine which, if any, voxels within individual FLAIR lesion masks should be included in a T1-thresholded FLAIR lesion cluster mask (**Figure 2**):

$$\mathrm{UpperThr}_{t1} = 1.02 \times \mathrm{mean}_{\mathrm{T1}cortex} \quad (2)$$

The total number and volume of lesions surviving the semi-automated pipeline were then calculated for lesions that met the L-SCI, T-SCI, and R-SCI definitions.

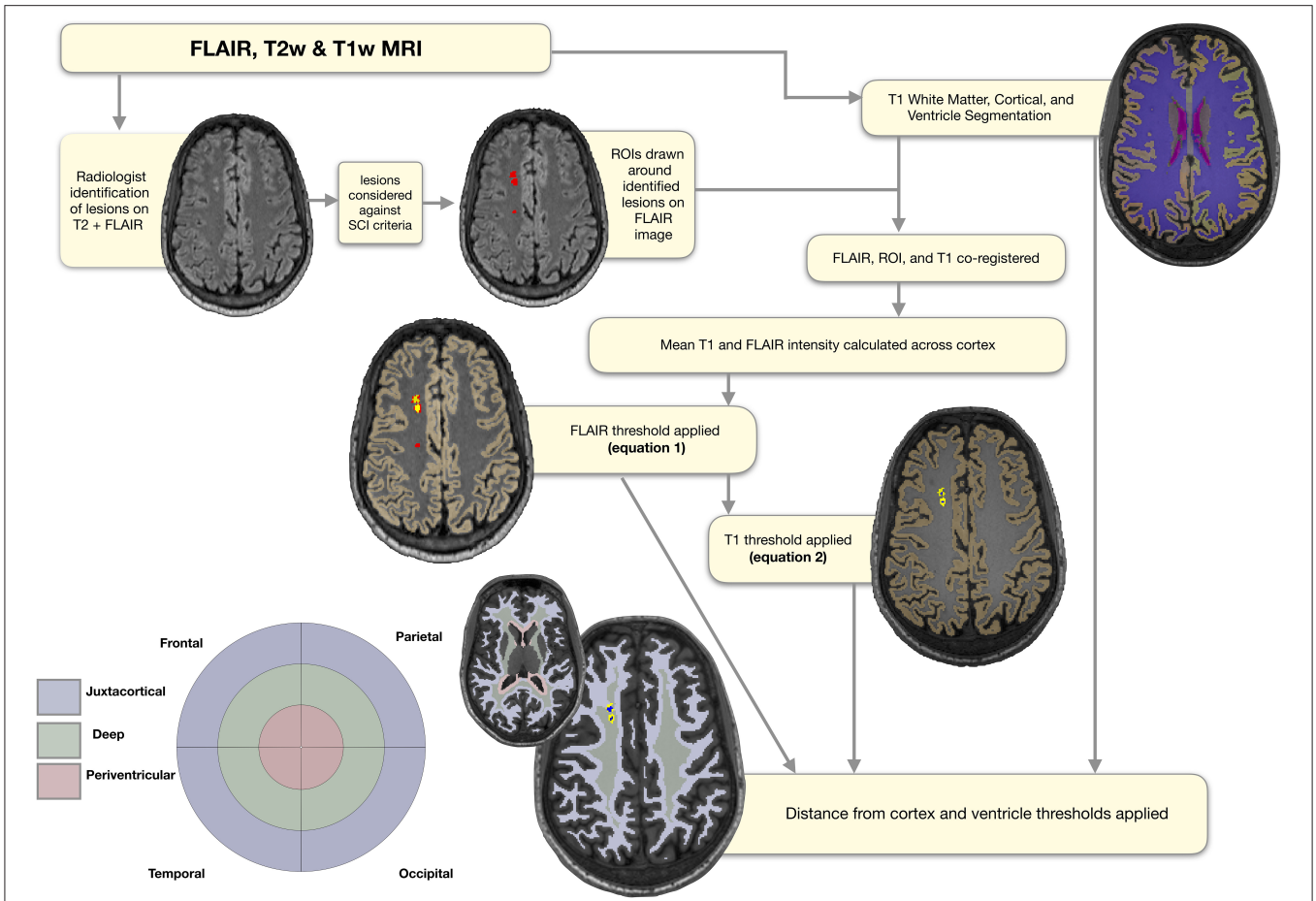


FIGURE 2 | Semi-automated pipeline. Showing regions of interest (ROIs) at each stage of the semi-automated pipeline overlaid on a FLAIR image from a representative participant with sickle cell anemia (male, 25 years old). The initial ROIs drawn around the lesions identified by the neuroradiologist are shown in red. The ROIs following application of the FLAIR threshold are shown in yellow, and the ROIs following application of the T1 threshold in blue. The masks used for regional classification, along with the corresponding simplified bullseye plot, are shown in light blue for the juxta-cortical region, light green for the deep region, and light pink for the periventricular region.

Classification and Quantification of Regional SCI

To classify lesions as periventricular, deep, or juxtacortical, FSL was used to create participant-specific maps quantifying distances from the ventricle and cortex masks from Freesurfer. Although established absolute distance rules for classification of lesions exist from work with adult non-SCA samples, we elected not to apply these given the younger age range of our sample. Instead, for each participant's "distance from ventricle" map, an upper threshold corresponding to the 5th percentile of voxels in white matter was used to create a periventricular region mask (**Figure 2**). Voxels within the periventricular region mask were then excluded from the participant-specific cortex distance map to avoid overlap. Next, an upper threshold corresponding to the 75th percentile of voxels in white matter was applied to the cortex distance map to create a juxtacortical region mask. Remaining white matter voxels were then used to generate a deep white matter region mask. These distance thresholds were chosen as they gave the most accurate classification compared with neuroradiologist classification for a random subsample of patients ($N = 10$, pipeline accurately classified lesions in all cases). We had also experimented with the 10th percentile of white matter for the distance from ventricles mask, and the 85th percentile of voxels for the distance from cortex mask. For the 10th percentile periventricular threshold, 1 lesion that was classed as periventricular by the neuroradiologist fell in the deep regional mask. Similarly, for the 85th percentile juxta-cortical threshold, 1 lesion that was classed as deep by the neuroradiologist fell in the juxta-cortical mask.

The number and volume of lesions of each class were then quantified. For the number of lesions, in the event of overlap between FLAIR lesion masks and periventricular, juxtacortical, and/or deep region masks, lesions were classified according to whichever regional mask contained the greatest number of FLAIR lesion voxels (**Figure 1**). If regions contained an equal number of lesion voxels, the neuroradiologist was consulted

TABLE 1 | Representation of lesion metrics included in regression models.

Model	Lesion metrics included as predictors
1.	$SCI_{y/n}$
2.	$SCI_{y/n} + SCI_{y/n} * SCA_{y/n}$
3.	$SCI_{y/n} + SCI_{number}$
4.	$SCI_{y/n} + SCI_{y/n} * SCA_{y/n} + SCI_{number} + SCI_{number} * SCA_{y/n}$
5.	$SCI_{y/n} + SCI_{volume}$
6.	$SCI_{y/n} + SCI_{y/n} * SCA_{y/n} + SCI_{volume} + SCI_{volume} * SCA_{y/n}$

*+ = addition; * = multiplication.*
$SCI_{y/n}$, binary variable representing presence or absence of silent cerebral infarction; SCI_{number}, continuous variable representing the number of lesions; SCI_{volume}, continuous variable representing the volume of lesions; $SCA_{y/n}$, binary variable representing presence or absence of sickle cell anemia.

for classification ($N = 3$ cases). For the volume of lesions, the number of voxels within each regional mask was used, allowing for within-lesion overlap between regions. A simplified "bulls-eye" representation of lesion burden (38) in 12 regional-lobar zones was created (**Figure 2**), with the lobe as designated by the neuroradiologist.

Statistical Analysis

Analyses were performed in RStudio Desktop 1.0.153 using packages including "companion to applied regression" (39) and "global validation of linear models" (40). Prior to statistical analyses, variables were assessed for normality and equality of variance. Non-normal lesion metrics (i.e., number and volume) were log-transformed. Estimates of global lesion metrics (i.e., total prevalence, number, and volume) were compared between L-SCI, T-SCI, and R-SCI definitions using Cochran's-Q and Friedman tests, and between patients and controls using Chi-Squared, Fishers Exact ($N < 10$), and Mann-Whitney U tests. Effect sizes were estimated using maximum-corrected measure of effect size (η^2_Q), Kendall's W(w), Cramér's-phi (ϕ), Cohens d (d), and correlation coefficients (r), and interpreted using Cohen's conventions (small d = 0.2, η^2_Q/w/ϕ/r = 0.1, medium d = 0.5, $\eta^2_Q/w/\phi/r/ = 0.3$, large d = 0.8, η^2_Q $w/\phi/r = 0.5$) (41).

Global Models

For each SCI definition, regression models estimating IQ, WMI, PSI, and cognitive impairment from an increasingly complex combination of global lesion metrics were computed (**Table 1**). Models including binary indicators denoting the presence of SCI or the presence of at least 1 SCI that met the relevant definition per decade of life (**Table 1**; Model 1) were compared to models where binary indicators were included alongside continuous quantifiers, representing either the global number (**Table 1**; Model 3) or volume (**Table 1**; Model 5) of SCI according to the relevant definition.

To explore potential effects of SCA status on the effect of SCI metric, where distributions allowed, models were also computed with interaction terms composed of [mean-centered SCA status] X [lesion metric] (**Table 1**; Models 2, 4, and 6). Models were also re-computed in a subset including SCA patients only.

Regional Models

As for the global lesion models, exploratory regional models including binary indicators denoting regional (i.e., deep, juxta-cortical and periventricular) or lobar (i.e., frontal, parietal, temporal, and occipital) lesion presence, were compared to corresponding models including indicator variables alongside continuous quantifiers denoting SCI number or volume. Models were also computed with a predictor based on the total number of regional-lobar zones with lesion voxels. Previous reports indicate a significantly unequal regional and lobar distribution of lesions in patients with SCA (4). Therefore, we expected a bimodal distribution of relatively commonly affected regions/lobes vs. relatively rarely affected regions/lobes. To avoid large between-region/lobe differences in statistical power and statistical inferences based on very small numbers of participants, only the most commonly affected regions and lobes were included in regional models.

Model Statistics

Across all models, a probability threshold of $p < 0.05$ was considered statistically significant, and model assumptions and variance inflation factors (VIF) were assessed. If there was significant multi-collinearity (i.e., VIF > 5) between binary indicator or continuous quantifier variables, binary indicator variables were dropped from models. The predictive value of definitions was assessed by comparing beta-values and semi-partial correlation coefficients for any significant lesion predictors between the models. Based on prior literature (16, 18, 42), pre-selected covariates comprising age, sex, total intracranial volume, SpO_2, and education deciles, were included in all models. In models re-computed in the SCA patient subset, hemoglobin was additionally included as a covariate. Prior to their inclusion in models, exploratory correlation analyses were performed between variables. Correlations for comparison of the number and volume of T-SCI, L-SCI, and R-SCI with FSIQ, WMI and PSI were additionally computed separately for children ≤15.99 years and adults >16 years. The relevant group means were imputed for four patients with missing hemoglobin and seven controls with missing SpO_2. To match prior research (18), and maximize our ability to detect effects of SCI should they exist, we did not correct for multiple comparisons.

RESULTS

Participants

Of 172 recruited participants, 106 patients with SCA (53 male, age range 8–29 years, 105 HbSS, 1 HbSß$_0$-thalassemia) and 48 race- and age- matched controls (20 male, age range 8–30 years; 27 HbAA, 19 HbAS, 2 HbAC; 31 siblings of patients) were included in the final sample (**Figure 3**).

Two patients had large vessel vasculopathy, six were receiving chronic transfusion, 16 had been transfused within 3 months, and 42 were prescribed hydroxyurea. SpO_2 was significantly lower in patients than controls, and hemoglobin was lower than normative values, but there were no significant differences in age, sex, or education deciles (**Table 2**).

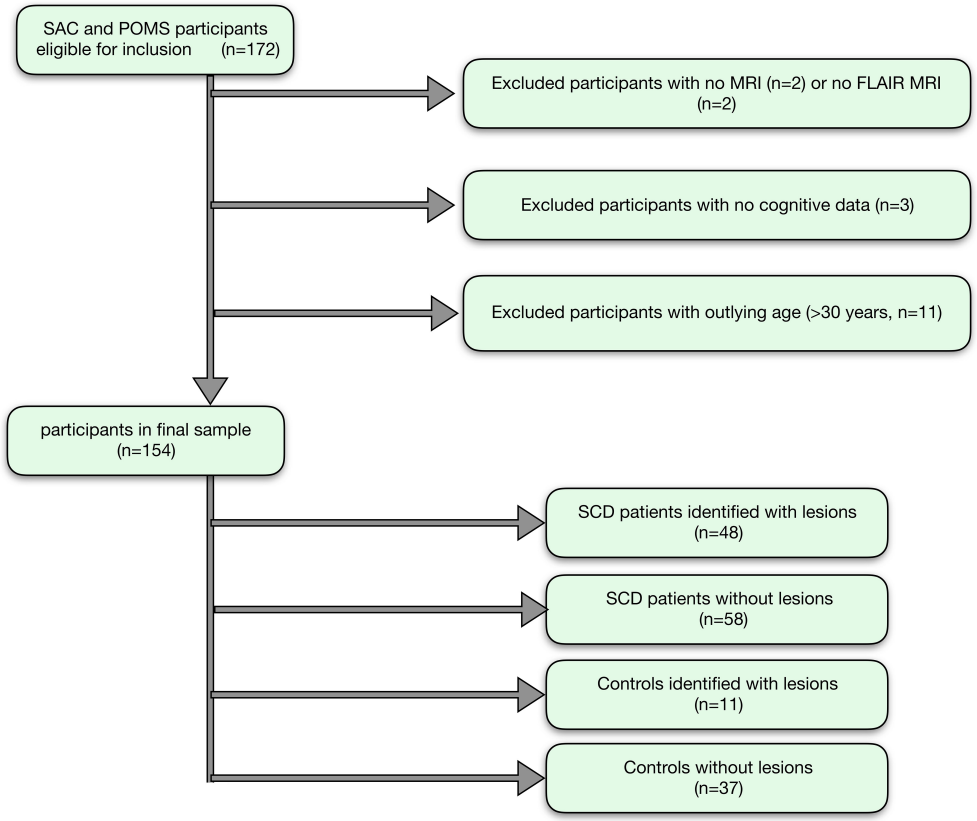


FIGURE 3 | Participant flow-chart. Showing the flow of participants, with reasons for exclusion, and the final groups for analysis.

Cognitive Performance

Analyses that assessed differences in cognitive performance between patients with SCA and controls found that patients had lower scores of 7 WMI points, 8 PSI points (*ps* < 0.005), and 4 IQ points ($p = 0.06$, **Table 2**; **Figure 4**) than controls. Effect sizes for these cognitive differences were moderate to large. For controls, there were no significant differences in cognition between those with and without sickle cell trait (all *ps* > 0.5). 11.3% of people with SCA (12/106) were identified as having cognitive impairment (i.e., WMI, PSI, or IQ score < 70) compared with 4.2% (2/48) of controls (see **Table 2**). As shown in **Figure 4**, the raw data demonstrated greater variation in cognitive performance within than between lesion groups, with no clear dose-dependent effect of the total lesion number or volume, in patients or controls.

Global SCI Metrics

Forty-five patients (42%, 23 male, median age = 16.9, range = 8–27 years) and 11 controls (23%, 2 male, median age = 14.8, range = 9–21 years, HbAS = 7) had lesions that met the L-SCI definition and survived the semi-automated pipeline. Cochran's Q tests revealed significant effects of SCI definition on lesion prevalence in both patients and controls; the L-SCI definition identified three more patients (7%) and six more controls (120%) with lesions compared to the T-SCI definition, and 20 more patients (80%) and nine more controls (450%) compared to the R-SCI definition (*ps* < 0.05; **Table 3**). A similar pattern was observed for the additional requirement that at least 1 lesion be allowed per decade of life and the median number and volume of lesions. Significant effects of SCA status on global lesion metrics were also observed, with a greater global prevalence, number, and volume of lesions observed in patients, irrespective of the SCI definition employed (*ps* < 0.005; **Table 3**).

T-SCI were detected in controls with sickle cell trait as young as 9 years of age, and in those with HbAA/AC as young as 12 years of age. A non-significantly greater proportion of controls with HbAS were identified as having SCI (L-SCI HbAA/AC: 4/29 [14%, 0 AC] vs. HbAS: 7/19 [37%]), irrespective of the definition (*ps* > 0.05).

Global SCI Metrics and Cognition

Exploratory Spearman's rank correlations revealed several significant relationships (**Figure 5**, **Supplementary Figure 1**). In addition to expected associations between closely related variables, decreases in SpO2 were modestly associated with increases in L-SCI and T-SCI lesion number and volume (rs = −0.23 to −0.28, ps < 0.05; **Figure 5**). When these

TABLE 2 | Sample demographics and cognitive performance.

	SCA ($n = 106$)	Control ($n = 48$)	*Between-group differences*
Demographic variables	**Count (percentage)**		
Sex	53 Male (50.00%)	20 Male (41.67%)	*Chi-Squared test* $X_1^2 = 0.62$, $p = 0.43$, $\phi = 0.06$
		Median (range)	*Mann-Whitney U test*
Age (yr)	16.56 (8–29)	16.55 (8–30)	U = 2,594.5, $p = 0.85$, 95% CI = −1.64–1.98, $r = 0.02$
Education Decile	5.00 (1–10)	5.00 (2–10)	U = 2,353.5, $p = 0.45$, 95% CI = −0.99–0.00, $r = 0.06$
Clinical variables	**Count (percentage)**		***Mann-Whitney U test***
Chronic Transfusion	6 (5.66%)	–	–
Acute Transfusion <3 months	16 (15.10%)	–	
Hydroxyurea	42 (39.62%)	–	
Hemoglobin (g/dl)	87.70 (60–134)	–	
SpO_2 (%)	97.00 (89–100)	99.00 (93–100)	U = 3,742.5, $p < 0.005$*, 95% CI = 1.00–2.00, r = 0.38
Cognitive variables		**Mean (SD)**	***Students t-test***
Intelligence quotient (IQ)	93.15 (13.28)	*All* 97.29 (11.78) *HbAA/HbAC 98.31 (10.52)* *HbAS 95.74 (13.64)*	$t_{(101.6)} = 1.94$, $p = 0.06$∧, 95% CI = −0.09–8.37, d = 0.33 *$t_{(31.7)} = 0.70$, $p = 0.49$, 95% CI = −4.94–10.10, $d = 0.21$*
Working memory index (WMI)	92.04 (14.18)	*All* 98.90 (13.34) *HbAA/HbAC 99.03 (13.54)* *HbAS 98.68 (13.40)*	$t_{(96.1)} = 2.90$, $p < 0.005$*, 95% CI = 2.16–11.56, d = 0.50 *$t_{(38.9)} = 0.09$, $p = 0.93$, 95% CI = −7.68–8.38, $d = 0.03$*
Processing speed index (PSI)	89.56 (12.99)	*All* 97.10 (13.03) *HbAA/HbAC 97.62 (13.24)* *HbAS 96.32 (13.01)*	$t_{(90.6)} = 3.33$, $p < 0.005$*, 95% CI = 3.05–12.05, d = 0.58 *$t_{(39.1)} = 0.33$, $p = 0.74$, 95% CI = −6.52–9.13, $d = 0.74$*
		Count (percentage)	***Fishers Exact test***
Cognitive impairment (CI)	12 (11.32%)	*HbAA* 1 (2.08%) *HbAC 0 (0.00%)* *HbAS 1 (5.26%)*	OR = 5.95, $p = 0.06$^, 95% CI = 0.83–261.56 –

Values are summary and test statistics. For between group-differences in cognitive variables, the top row compares patients and controls, while the second row compares controls with and without sickle cell trait.

*^$p < 0.1$; *$p < 0.05$.*

SCA, sickle cell anemia; HbAA/HbAC, control with hemoglobin A; HbAs, control with hemoglobin S, sickle cell trait; SpO_2, peripheral oxygen saturation; SD, standard deviation; 95% CI, 95% confidence interval.

analyses were conducted in only the SCA subsample, these associations remained significant, and other modest associations between decreases in hemoglobin and decreases in IQ ($r = 0.29$, $p < 0.05$) and PSI ($r = 0.20$, $p < 0.05$) were observed (**Supplementary Figure 1**). No univariate relationships were observed between lesion variables and any measure of cognition, and there were no relationships with total intracranial volume, age, or education deciles. When the SCA subsample was further stratified by age, there were no correlations for the L-SCI or T-SCI definitions, but volume and number of R-SCI were paradoxically positively correlated with IQ in children with SCA aged <16 years (**Table 4**). There were no correlations between volume or number of T-SCI, L-SCI or R-SCI and IQ in adults, nor any correlations with WMI or PSI in either age group (**Table 4**).

The results from the univariate correlation analyses were echoed in regression models estimating IQ, WMI, PSI, and cognitive impairment from global lesion metrics, where no significant effects emerged (**Table A1**), irrespective of the definition employed, the inclusion of interaction terms for patient status, the model complexity, or whether analyses were run across the whole sample or in people with SCA only.

Regional SCI Metrics

In patients, across SCI definitions and lesion metrics (**Figures 6**, **7**, **Supplementary Figures 2**, **3**), the distribution of lesions was relatively consistent. Following the L-SCI definition (**Figures 6**, **7**), the deep frontal and parietal regional-lobar zones were the most commonly affected (36.8 and 19.8%, respectively), and accounted for the greatest proportion of the total number (69%) and volume (72%) of lesions in people with SCA. The juxta-cortical frontal and parietal zones were also among the most commonly affected in people with SCA (29.2 and 17.9%, respectively), but accounted for a relatively smaller proportion of the total number (24%) and volume (19%) of lesions, with the remaining regional-lobar zones rarely affected (all < 10%). In controls, there was far less consistency between lesion metrics based on the L-SCI definition. Whilst the juxta-cortical frontal zone accounted for the greatest proportion of the total lesion number (31%; **Figure 7**), the deep occipital zone accounted for the greatest proportion of lesion volume (51%; **Figure 7**). SCI were not particularly common in either zone, with the juxta-cortical frontal zone affected in six controls (12.5%), and the deep occipital zone in two (4.2%; **Figure 7**).

Lesion distributions in both patients and controls were similar between the L-SCI (**Figure 7**) and T-SCA

TABLE 3 | Global lesion characteristics.

	L-SCI		T-SCI		R-SCI		
	SCA	**HC**	**SCA**	**HC**	**SCA**	**HC**	***Within-group differences***
Lesion prevalence							
Total number of participants with lesions (% of total group)	45 (42.45%)	11 (22.92%)	42 (39.62%)	5 (10.42%)	25 (23.58%)	2 (4.17%)	*Cochrans Q test* SCA: $Q_2 = 34.90$, $p < 0.005$*, $\eta^2_Q = 0.11$ HC: $Q_2 = 14.00$, $p < 0.005$*, $\eta^2_Q = 0.15$
Between-group differences	*Chi-squared test* $X^2_1 = 4.64$, $p = 0.031$*, $\phi = 0.17$		*Chi-squared test* $X^2_1 = 11.90$, $p < 0.005$*, $\phi = 0.28$		*Fishers Exact test* OR = 7.03, $p < 0.005$*		
Total number of participants with >1 lesion per decade of life (% of total group)	37 (34.91%)	9 (18.75%)	32 (30.19%)	5 (10.42%)	12 (11.32%)	0 (0.00%)	SCA: $Q_2 = 42.00$, $p < 0.005$*, $\eta^2_Q = 0.14$ HC: $Q_2 = 13.56$, $p < 0.005$*, $\eta^2_Q = 0.14$
Between-group differences	*Chi-squared test* $X^2_1 = 3.38$, $p = 0.07$^, $\phi = 0.15$		*Chi-squared test* $X^2_1 = 6.03$, $p = 0.01$*, $\phi = 0.20$		*Fishers Exact test* OR = NA, $p = 0.02$*		
Lesion number							*Friedman test*
Median total number of lesions in participants with lesion meeting definition (range)	4 (1–29)	3 (1–12)	3 (1–26)	2 (1–8)	2 (1–10)	1 (1–1)	SCA: $Q_2 = 76.80$, $p < 0.005$*, W = 0.36 HC: $Q_2 = 19.50$, $p < 0.005$*, W = 0.20
Between-group differences	*Mann-Whitney U test* U = 2,000.00, $p = 0.01$*, r = −0.18		*Mann-Whitney U test* U = 1,781.00, $p < 0.005$*, r = −0.28		*Mann-Whitney U test* U = 2,036.00, $p < 0.005$*, r = −0.22		
Lesion volume							
Median total lesion burden (volume, mm^3) in participants with any lesion burden (range)	75 (1–5177)	22 (2–1282)	63.5 (1–5177)	19 (3–1075)	4 (1–268)	2 (2–2)	*Friedman test* SCA: $Q_2 = 81.10$, $p < 0.005$*, W = 0.38 HC: $Q_2 = 19.50$, $p < 0.005$*, W = 0.20
Between-group differences	*Mann-Whitney U test* U = 1,938.00, $p < 0.005$*, r = −0.20		*Mann-Whitney U test* U = 1,774.00, $p < 0.005$*, r = −0.28		*Mann-Whitney U test* U = 2,036.00, $p < 0.005$*, r = −0.22		

*Values are summary and test statistics comparing lesion metrics between patients and controls groups (columns; Chi-Squared and Mann-whitney U tests) and within groups as a function of SCI definition (rows; Cochran's-Q and Friedman tests) ^$p < 0.1$; *$p < 0.05$. L-/T-/R-SCI, Liberal/Traditional/Restrictive silent cerebral infarction definitions; HC, healthy control; SCA, sickle cell anemia.*

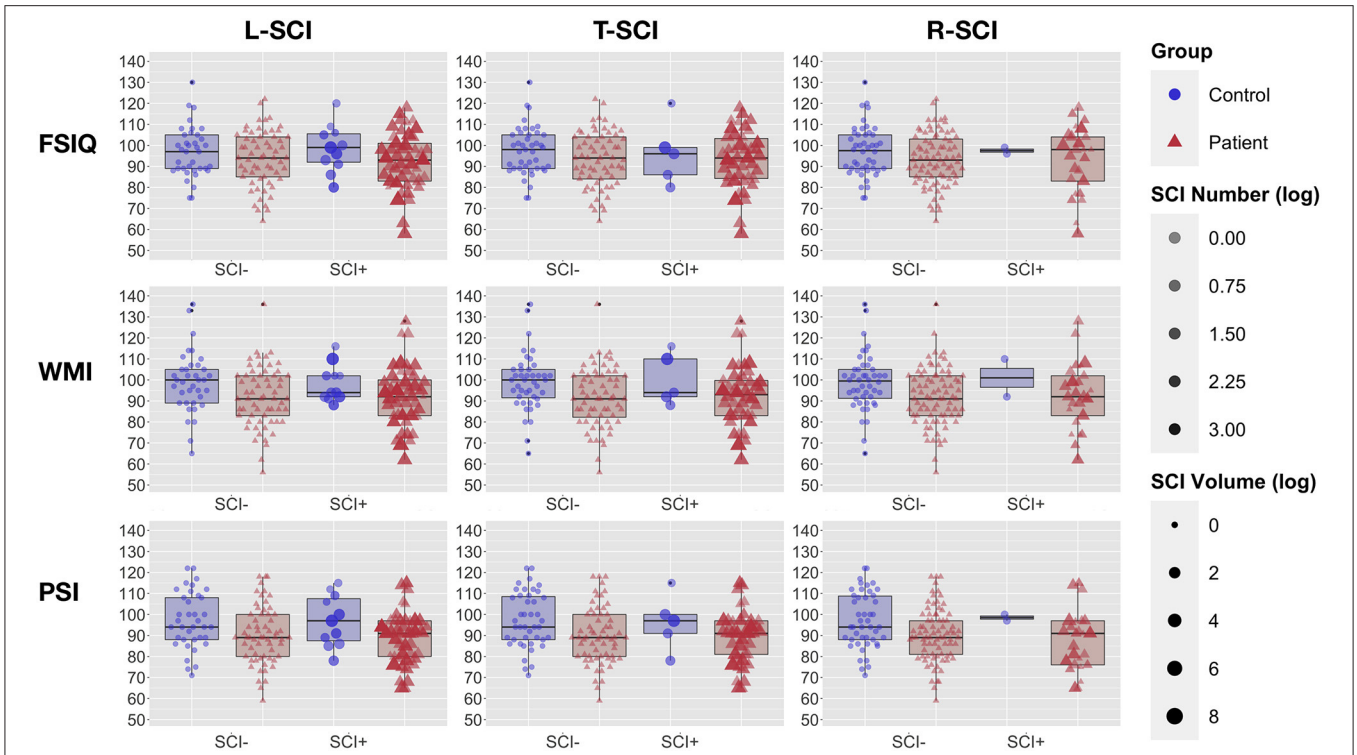


FIGURE 4 | Cognitive Performance and Global Lesion Presence. Showing full-scale IQ (FSIQ: top row), working memory index (WMI: middle row), and processing speed index (PSI: bottom row) in patients with sickle cell anemia (red triangles) and controls (blue circles). Participants are grouped by silent cerebral infarct status (SCI+ = with silent cerebral infarction, SCI– without silent cerebral infarction) for the liberal definition (left column), traditional definition (middle column), and restrictive definition (right column). For each definition, point intensity is weighted by the log-transformed number of lesions, and point size by the log transformed volume of lesion.

definitions (**Supplementary Figure 2**) but varied with the R-SCI definition (**Supplementary Figure 3**). Although the overall pattern was similar in patients, frontal lesions were more likely to survive the R-SCI criteria. There was no obvious pattern as to the location of the two surviving lesions in controls.

Regional SCI Metrics and Cognition

Exploratory regression models estimating cognitive performance from regional and lobar lesion metrics yielded results similar to those using global lesion metrics (**Supplementary Table 1**), although a couple of significant effects emerged in models predicting PSI. First, in models using regional lesion metrics to predict PSI, there was a significant negative effect of the presence of juxta-cortical lesions for the R-SCI definition. The effect was significant across the whole sample, and in the patient subsample, but was modest in size ($r = 0.16–0.19$), and disappeared in the more complex models (i.e., models 2 and 3; **Table 1**), where a significant positive effect of restrictively-defined deep lesion volume emerged in the SCA subsample (**Supplementary Table 1**). Second, in complex models using lobar lesion metrics to predict PSI, there were significant negative effects of parietal lesion volume for both the L-SCI and T-SCI definitions in the SCA subsample, but not for the R-SCI definition, or for any definition across the whole sample. For the R-SCI definition, a positive effect of frontal lesion volume was, however, observed in the SCA subsample.

Across all models, coefficients for the lesion volume variables that reached significance were very small in magnitude, corresponding to a 0.17–0.19 decrease in PSI points for every 10% increase in L-SCI or T-SCI defined parietal lesion volume, and a 0.43–0.44 increase in PSI for every 10% increase in restrictively defined frontal or deep lesion volume (**Supplementary Table 1**). Effect sizes were also modest ($r = 0.20–0.22$).

DISCUSSION

Using high-resolution FLAIR, we assessed the effect of three published SCI definitions on global and regional lesion metrics and explored their value in predicting general cognitive performance. As hypothesized, we observed large effects of SCI definition on estimates of lesion metrics in both patients and controls. However, contrary to hypotheses, neither the presence, nor the total number, nor the total volume of lesions based on any SCI definition predicted general cognitive performance or impairment. These findings underscore the numerous challenges surrounding the widespread use of binary SCI status as a biomarker of cognition in cross-sectional high-resolution MRI studies in the SCA field, including challenges related to the

definition, quantification, distribution, specificity, and timing. Taken together, our findings suggest that with high-resolution MRI: (1) radiological definitions have a large effect on resulting lesion groups, numbers, and volumes; (2) there is a non-negligible prevalence of lesions also in healthy controls; and (3) at the group-level, there is no association between global lesion metrics and general cognitive outcome irrespective of lesion definition and metric.

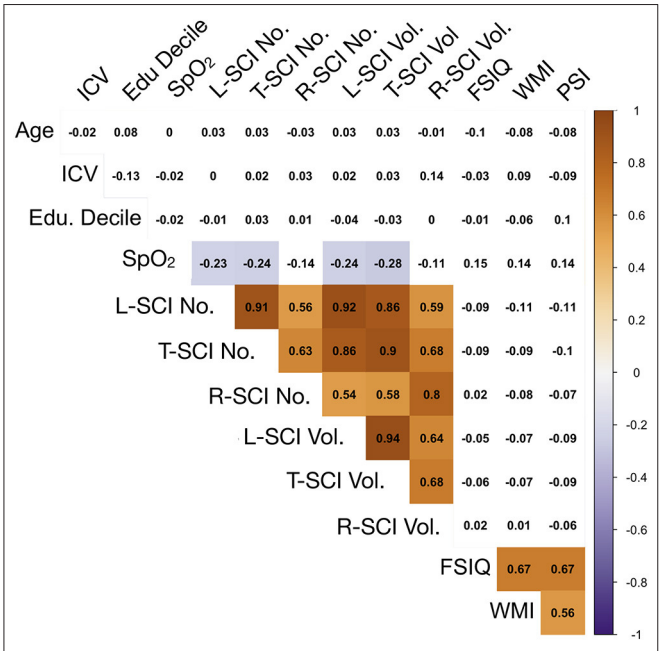


FIGURE 5 | Correlations between model predictors. Showing relationships between continuous variables included in the global regression models. Values are zero-order Spearman's rank correlation coefficients. Shaded areas represent significant relationships (i.e., $p < 0.05$), with blue used to represent negative relationships, and orange used to represent positive relationships, and color intensity used to represent the strength of the relationships. ICV, intra-cranial volume; Edu. Decile, education decile; SpO_2, peripheral oxygen saturation; SCI Lib, SCI liberal definition; SCI Trad., SCI traditional definition; SCI Rest, SCI Restrictive definition; No., Number; Vol, Volume; FSIQ, full-scale IQ; WMI, working memory index; PSI, processing speed index.

SCI Definitions and Global Lesion Metrics

We observed large effects of SCI definition on global lesion metrics, with the resulting lesion subgroups varying widely, particularly in controls. Estimates of SCI prevalence based on the L-SCI and T-SCI definitions in patients were in broad agreement (43% and 40%, respectively), and were broadly comparable with prior SCA studies employing higher field-strength MRI (43). Prevalence in patients based on the R-SCI definition was significantly lower (24%) but in line with a prior lower-resolution pediatric study (18). The total number and volume of lesions were less consistent between definitions, with 69 patient lesions (24%) and 26 control lesions (62%) not meeting the T-SCI definition, and a further 158 patient lesions (55%) and 14 control lesions (33%) not meeting the R-SCI definition. Of note, excluded lesions were often from individuals who had other lesions meeting the T1w hypointensity and/or minimum length criteria.

While radiological SCI definitions that include minimum length criteria may be justifiable in studies employing lower-resolution sequences where reliability may be a concern, there is less justification in higher resolution studies unless increased sensitivity to a specific pathology and/or outcome can be demonstrated. For prediction of cognitive performance, the present study indicated little utility of the length requirement of the T-SCI definition, or the additional T1 hypo-intensity requirement of the R-SCI definition, though both definitions had significant effects on resulting lesion groups, numbers, and volumes. These findings demonstrate the need for a standardized definition and quantification approach that is reliable and replicable for further exploration in high-resolution MRI studies. Ideally, future studies should also take into account the definitions used in studies of cerebral small vessel disease (CSVD) in adults (44–46).

For comparability with studies in CSVD, and to avoid excluding smaller lesions before attempting to explore and

TABLE 4 | Spearman correlations for comparison of cognitive outcomes and silent cerebral infarct volume and number using the Liberal (L-SCI), Traditional (T-SCI) and Restrictive (R-SCI) definitions.

	Children (<16 y) with sickle cell anemia *N* = 48						Adults (≥16 y) with sickle cell anemia *N* = 57					
	IQ		WMI		PSI		IQ		WMI		PSI	
Silent cerebral infarct definition	**r**	***p***	**r**	***p***	**r**	***p***	**r**	***p***	**r**	***p***	**r**	***p***
L-SCI total volume	0.172	0.2	0.006	0.9	−0.064	0.7	−0.191	0.2	−0.007	0.9	0.015	0.9
T-SCI total volume	0.182	0.2	−0.022	0.9	−0.040	0.8	−0.156	0.2	0.029	0.8	0.027	0.8
R-SCI total volume	0.308	0.03*	0.090	0.5	−0.093	0.5	−0.115	0.4	0.017	0.9	−0.024	0.9
L-SCI number	0.162	0.3	−0.007	0.9	−0.064	0.2	−0.204	0.1	0.012	0.9	0.019	0.9
T-SCI number	0.177	0.2	−0.029	0.8	−0.025	0.9	−0.159	0.2	0.061	0.7	0.038	0.8
R-SCI number	0.309	0.03*	0.088	0.6	−0.099	0.5	−0.152	0.3	−0.038	0.8	−0.048	0.7

*$p < 0.05$.

y, years; IQ, intelligence quotient; WMI, working memory index; PSI, processing speed index; L-/T-/R-SCI, Liberal/Traditional/Restrictive silent cerebral infarction definitions.

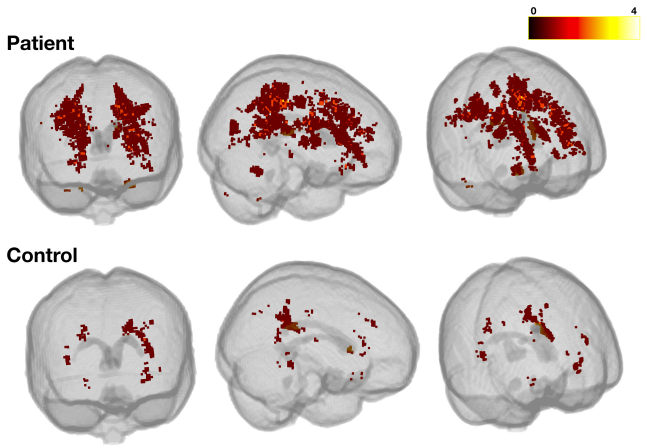


FIGURE 6 | Heatmap for liberally-defined SCI. Showing the distribution and frequency of lesions for the liberal definition in patients (top row) and controls (bottom row). All lesions were registered to a standard space template (MNI 152) using non-linear registrations from the *FSL_anat* pipeline (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/fsl_anat). The color bar shows the number of participants with lesions in a particular voxel.

understand the features of such lesions, it may be useful to consider the greatest dimension and T1-intensity not as criteria for inclusion, but as features worthy of further exploration alongside other imaging features. Future studies should therefore consider employing L-SCI definitions and semi-automated segmentation techniques. Although substantial progress has been made in the development of automated segmentation techniques in other populations (47), the sensitivity of these techniques to the often smaller and less well-defined lesions observed in younger populations with and without SCA has yet to be investigated.

Global Lesion Metrics and Cognitive Performance

Globally, our results indicate greater variation in cognition within than between lesion groups, with no observable dose-dependent effect of the total lesion burden or the lesion burden for age, in patients or controls. These findings align with those of several recent high-resolution MRI studies that have failed to detect an effect of the presence of T-SCI on cognition in patients with SCA (15, 16, 48) (**Table 5**). However, they contradict lower-resolution pediatric MRI studies where small to moderate effects have been widely reported (**Supplementary Table 2**) (10, 11, 28, 55, 56). These lower resolution studies include children enrolled in the Co-operative Study of Sickle Cell Disease (CSSCA) from 1989 to 1999 (56), and a subset of those enrolled in the SIT trial from 2005 to 2013 (42). Given their relatively large samples, prior meta-analyses have been heavily weighted by their inclusion [e.g., (10)].

Of 11 studies conducted at 3T examining effects of SCI on cognitive outcome, the only study to detect an effect used a sequence with a 5 mm slice thickness (**Table 5**), 17 out of 27 studies conducted at 1.5T found a negative effect of SCI on cognition (**Supplementary Table 2**). Taken together, these findings are consistent with the notion that the use of higher magnetic field strengths (i.e., 3T vs 1.5T) and higher resolution FLAIR sequences (i.e., 0.65 × 0.65 × 1 mm vs. 1.8 × 1.8 × 5 mm) increases lesion detectability, and that this in turn may skew or nullify prediction of cognitive impairment based on a binary categorization according to presence or absence of SCI, irrespective of the stringency of the definition employed. Increased lesion detectability is consistent with the relatively large proportion of controls we identified with SCI meeting L-SCI and T-SCI criteria (23 and 10%, respectively), the majority of whom were children. Other recent high-resolution MRI studies have similarly reported a non-negligible prevalence of T-SCI in healthy young adults (**Table 6**).

This interpretation raises the possibility that there may be a tipping point beyond which lesions become large and prominent and begin to have an effect. It is also possible that the inclusion of young adults in our sample reduced our sensitivity to potential age-dependent effects of SCI across specific domains. Although we excluded participants >30 years, used cognitive scores that were scaled for age, included age as a covariate in our regression models, and observed no relationships between age and global lesion metrics, we cannot exclude the possibility of age-dependent effects of the global presence and burden of lesions on individual cognitive trajectories in specific domains.

Discrepancies in the literature may also be accounted for by differences in sample characteristics between prior and more recent studies. The lesions observed at 1.5T MRI in the majority of prior studies are likely to have met the traditional or restrictive criteria (**Supplementary Table 2**). These studies included untreated patients with clinically significant cerebral vasculopathy, which may have contributed to both their SCIs and their cognitive deficits. Therapies such as hydroxyurea and transfusion were rarely used during the CSSCA but were prescribed in 40 and 21%, respectively of our cohort with SCA. In a recent study, executive function improved soon-after compared with long-after a blood transfusion in 27 people with SCA, >80% of whom had infarction on MRI (52). However, although the incidence of recurrent infarcts, including overt strokes and T-SCIs, was reduced, blood transfusion for 3 years did not improve IQ in SIT (3). One of the exclusion criteria was treatment with hydroxyurea within 3 months of SIT enrollment but there is now evidence for a protective effect on IQ (61, 62). There is therefore some evidence that the discrepancy between our data and previous studies could be related to therapy. It is also possible that the strategic position of SCI in relation to particularly eloquent tracts plays a role in functional outcomes.

Regional Lesion Metrics and Cognitive Performance

Our regional analyses yielded several interesting findings. We detected a consistent regional distribution of lesions across metrics and definitions in patients, but not controls. Whilst lesions in patients were similarly prevalent in deep and juxta-cortical frontal and parietal zones, lesions in deep frontal and parietal zones were greater in number and size, echoing prior

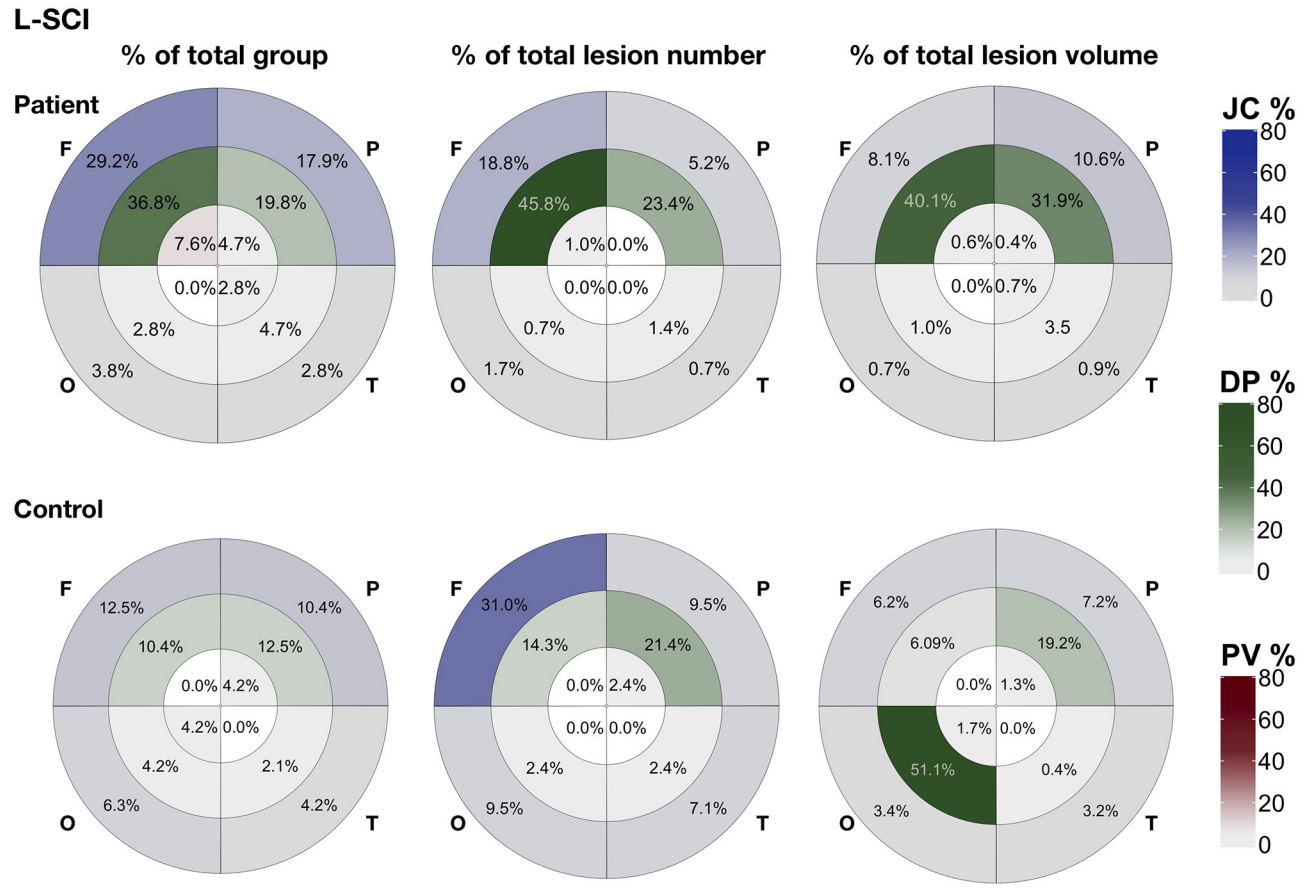


FIGURE 7 | Lesion distribution for liberally-defined SCI. Showing the distribution of lesions using bullseye plots, where sectors represent the four lobes (F, frontal; P, parietal; O, occipital; T, temporal) and rings represent the three regions; periventricular (PV; interior ring; red), deep (DP; middle ring; green), and juxta-cortical (JC; exterior ring; blue), yielding 12 regional lobar zones. Distribution is represented using three different metrics for patients (top row) and controls (bottom row): (1) the proportion of the total group with lesion voxels in particular zone (% of total group), (2) the proportion of the total group lesion number classed as belonging to regional-lobar zone (middle - % of total lesion number), and (3) the proportion of the total group lesion voxels present in a particular regional-lobar zone (right - % of total lesion volume).

SCA studies (4, 63, 64). In exploratory models, we detected some significant effects of regional and lobar lesion metrics on PSI. The most consistent finding was a moderate negative effect on PSI of parietal lesion volume for both the L-SCI and T-SCI definitions in the SCA subsample. However, confidence intervals were wide, and coefficients very small for these effects, highlighting the need to interpret them with caution. Findings for the restrictive definition were less consistent. There was a moderate negative effect of the presence of juxta-cortical R-SCI in simple models, but the effect disappeared in the more complex models, where moderate positive effects of deep and frontal R-SCI volume emerged, again with wide confidence intervals and small coefficients.

It is possible that the paradoxical effects we observed using the R-SCI definition were related to our operationalization of the visual definition of a T1-hypointensity (18), which was novel and may have been too stringent. Given that the R-SCI definition requires a corresponding FLAIR hyperintensity, and we applied the T1 threshold after the FLAIR threshold, it is also possible that some T1 hypo-intensities were missed and/or excluded (**Figure 1**). Alternatively, T1 hypo-intensities may represent older, more established lesions, with effects diminishing over time.

These possibilities could also account for the paradoxical univariate association observed between higher volume and larger number of R-SCI and higher, rather than lower, IQ in children with SCA <16 years. Another possibility is that in the child's brain, adaptive physiology which may enable brain growth and development, e.g., higher cerebral blood flow (CBF), might eventually favor the development of SCI in the borderzone regions (64, 65) related to vascular instability secondary to exhaustion of cerebral reserve mechanisms, shunting or steal. Such effects may plausibly improve or worsen over time depending on age, along with the severity of the disease course. It might be possible to follow the trajectory in patients longitudinally from a young age by comparing the prevalence, number and distribution of T-SCI, L-SCI, and R-SCI. This would enable exploration of trajectories and could help establish

TABLE 5 | Overview of high-resolution MRI studies examining impact of SCI on cognitive outcomes in patients with SCA.

Study details			SCA (N)	SCA (age)	SCI definition	MRI	Sequence Res.	Lesion Metric	% SCA w/ SCI	Cognitive Tests	Neg. effect of SCI—FSIQ, WMI, or PSI	Neg. effect of SCI—any domain	Results: Assoc w/cognition or other neuroimaging outcome
Hijmans et al. (14)	Netherlands	2010	21	6–18 y	*No explicit definition*	1.5T, 3T	*Not reported*	Prevalence (i.e., binary y/n)	57%	WISC-III (composite scores), Stop-task, Tower of London, N-back task, Beery VMI	N	N	No significant differences found between SCI+ and SCI– groups
Krejza et al. (49)	US	2012	46	3–13 y	*No explicit definition*	3T	*Not reported*	Prevalence (i.e., binary y/n)	34%	K-BIT (Kaufman Brief Intelligence Test)	–	N	No significant differences in any K-bit score between SCI+ and SCI- groups, lesion volume included in models as covariate; significance and effect sizes not reported
van der Land et al. (29)	Netherlands	2015	38	8–17.1 y	L-SCI: area of abnormally increased signal on T2 and FLAIR	3T	T2 = 0.58 × 0.72 mm, 29 slices with 5 mm thickness; FLAIR = 1.03 × 1.68 mm, 29 slices with 5 mm thickness.	Prevalence (i.e., binary y/n) and volume (rank score)	50%	WISC-III (composite scores), Trail-making test, Beery VMI	Y	Y	SCI+ group significantly reduced FSIQ (81 vs. 89), VIQ (84 vs. 93), and PSI (83 vs. 97). Effects of SCI volume rank also observed in regression models for these scores.
Chen et al. (50)	US	2017	25	Decline M = 9.67, SD = 1.41. No Decline M = 9.38, SD = 2.94	T-SCI: area of abnormally increased signal on FLAIR—min 3 mm greatest diameter		FLAIR = 1 × 1 × 1 mm	Prevalence (i.e., binary y/n)	56%	K-BIT	–	N	SCI presence did not improve model fits for IQ decline over time
Downes et al. (51)	Ireland	2020	28	8–18 y	*No explicit definition*	*Not reported*	*Not reported*	Prevalence (i.e., binary y/n)	36%	NIH toolbox tests of executive function, language, and memory	N	N	No significant differences between SCI+ and SCI- groups in EF or memory tasks, significantly better performance in the picture vocabulary task
Stotesbury et al. (16)	UK	2018	83	8–37 y	T-SCI: area of abnormally increased signal T2 and FLAIR—min 3 mm greatest diameter	3T	FLAIR = 0.65 × 1 × 0.65 mm, T2 = 0.51 × 0.51 × 5.6 mm	Prevalence (i.e., binary y/n)	45%	WASI, WAIS/WISC-IV (composite scores)	N	N	No significant differences found between SCI+ and SCI- groups in FSIQ or PSI
Hood et al. (52)^	US	2019	61	3–22 y	T-SCI	3T	*Not reported*	Prevalence (i.e., binary y/n)	41%	NIH toolbox tests of executive function, language, and memory	N	N	No significant differences found between SCI+ and SCI- groups for overall cognition (83.12 vs. 83.92), executive function (90.81 vs. 87.90) or non-executive function (89.51 vs. 91.90) composites
Choi et al. (15)	US	2019	52	M = 21.4, SD = 7.7	T-SCI w/age: increased signal FLAIR—min 3 mm—>1 per decade of age	3T	FLAIR = 1.3 × 1.0 × 1.0 mm	Prevalence (i.e., binary y/n)	48%	WASI, WISC-IV	N	N	No significant differences between those with a normal and abnormal burden of SCI for age observed for FSIQ or matrix reasoning (others not reported)
Farris et al. (53)	US	2015	15	18–55 y	L-SCI: area of abnormally increased signal on FLAIR	3T	*Not reported,*	Number	60%	Cogstate battery: GMLT	–	N	No association between SCI number and performance on GMLT
Farris et al. (48)	US	2016	15	18–55 y	L-SCI: area of abnormally increased signal on FLAIR	3T	*Not reported, but slice thickness mentioned as included in volume estimate*	Volume	–	Cogstate battery: GMLT	–	N	No association between SCI volume and performance on GMLT
Sanger et al. (54)	US	2016	48	19–59 y	*No explicit definition*	–	*Not reported*	Prevalence (i.e., binary y/n)	37.5%	–	–	–	No differences in SCI prevalence as a function of employment status

Studies identifies from Pubmed searches in July 2021 using the terms "sickle" paired with "cognitive," or "IQ" or "executive function" or "MRI"—supplemented with literature known to the co-authors. Note: there may be between-study overlap in participants. ^personal communication with author. SCA, Sickle Cell Anemia; C, Control; Res, Resolution; N, no; Y, yes; WISC/WAIS, Wechsler intelligence scale for children/ adults; WASI, Wechsler abbreviated scale of intelligence; K-BIT, Kaufman Brief Intelligence Test; GMLT, Groton Maze Learning Test of executive function; VMI, visual motor integration; L-/T-/R-SCI, Liberal/Traditional/Restrictive silent cerebral infarction definitions.

TABLE 6 | Overview of SCI prevalence in prior high-resolution imaging studies in patients with SCA and controls.

Study details			SCA (N)	SCA (age)	C (N)	C (age)	SCI definition	MRI	Sequence Res.	% SCA w/SCI	%C w/SCI
Farris et al. (53)	US	2015	15	18–55 y	7	18–55 y	L-SCI: area of abnormally increased signal on FLAIR	3T	*Not reported,*	60%	57%
van der Land et al. (22)	Netherlands	2015	10	18–25 y	10	19–25 y	L-SCI: area of abnormally increased signal on FLAIR and T2	7T	FLAIR = 0.8 mm isotropic T2 = 0.7 mm isotropic	90%	70%
Choi et al. (26)	US	2017	33	11–41 y	32	12–41 y	T-SCI w/age: area of abnormally increased signal FLAIR - min 3mm - >1 per decade of age	3T	FLAIR = 1.3 × 1.0 × 1.0 mm	39%	13%
Coloigner et al. (57)	US	2017	20	12–34 y	19	17–41 y	T-SCI w/age: area of abnormally increased signal FLAIR - min 3mm greatest diameter - more than 1 per decade of age	3T	FLAIR = 1.3 × 1.0 × 1.0 mm	20%	0%
Choi et al. (15)	US	2019	52	M = 21.4, SD = 7.7	40	M = 27.7, SD = 11.3	T-SCI w/age: increased signal FLAIR—min 3 mm – > 1 per decade of age	3T	FLAIR = 1.3 × 1.0 × 1.0 mm	48%	26%
Václavu et al. (58)	Netherlands + US	2020	36	M = 37.4, SD = 15.43	9	M = 32.08, SD = 11.14	T-SCI: area of abnormally increased signal on FLAIR—min 2 mm greatest diameter	3T	FLAIR = 0.98 × 0.98 × 1.12 mm	82%	45%
Chai et al. (59)	US	2021	26	M = 24.2, SD = 9.7	21	M = 22.6, SD = 8.9	T-SCI: area of abnormally increased signal on FLAIR—min 3 mm greatest diameter	3T	FLAIR = 1.3 × 1 × 1 mm	54%	33%
Wang et al. (60)	US	2021	34	19–28 y	49	28–37 y	*No explicit definition*	3T	FLAIR = 1.0 × 0.9 × 3.0 mm	76%	39%

Studies identifies from Pubmed searches in July 2021 using the terms "sickle" paired with "cognitive," or "IQ" or "executive function" or "MRI"—supplemented with literature known to the co-authors. There may be between-study overlap in participants. SCA, Sickle Cell Anemia; C, Control; Res, Resolution; L-/T-/R-SCI, Liberal/Traditional/Restrictive silent cerebral infarction definitions.

whether R-SCI, defined by T1 hypointensities, represent older, more established lesions.

Limitations

It is worth re-iterating that for the few effects of SCI that we did detect, there was considerable variability with associated small coefficients and modest *p*-values. Given the high number of models computed, these effects would not have survived correction for multiple comparisons, irrespective of method. In line with prior studies [e.g., (18)], and as we wanted to maximize our ability to detect effects of SCI should they be present, we elected not to apply correction for multiple comparisons in this study. For this reason, it is particularly noteworthy that despite not correcting for multiple comparisons, we observed no effects of SCI metrics on cognitive difficulties in our global models. One drawback to this approach is that the few effects that we did detect in univariate correlations and regional models may have been spurious.

Beyond our decision to not correct for multiple comparisons, limitations of our study include our lack of direct measures of socio-economic status and of hemoglobin in controls. Unless direct benefit can be demonstrated, invasive measurements are often considered inappropriate by UK ethical committees. Our patient sample was also heterogeneous, with patients on a mix of disease-modifying treatment types (i.e., hydroxyurea and chronic transfusion regimens) for unknown and likely variable durations. These limitations may have decreased the sensitivity of our regression models, but given the raw data, are unlikely to have had a major impact on the results. Unlike prior studies (2), we also did not have the resources for central adjudication of SCIs across a panel of neuroradiologists. This limitation was partially mitigated by our neuroradiologist's expertise, along with our use of a semi-automated pipeline, which ensured that previously-established (29) intensity thresholds were consistently applied.

A further limitation is that we did not include further multi-modal analyses on the etiology or consequences of SCI in this manuscript. It is however worth noting that although the name "SCI" implies infarction, white matter hyperintensities on FLAIR are non-specific, and other underlying pathologies are possible, both in patients and controls. Although the pathophysiology is unknown, the distinction between periventricular, deep, and juxta-cortical lesions is thought to be etiologically meaningful (66). The anatomical distinction is however based on relatively arbitrary distance thresholds, and there is scope for further optimization. Exploring regional and lobar effects is challenging in SCA because most patients have multiple lesions, often affecting multiple regional-lobar zones, and there is substantial variability in terms of lesion number, volume, and spatial distribution. Given that our findings suggest a peak voxel lesion density of 9% ($n = 4$; **Figure 5**), the statistical power for voxel-wise approaches is likely to be limited.

Future Directions

Using quantitative multi-modal methods to explore the haemodynamic, susceptibility, and microstructural characteristics of lesions, and of normal-appearing white-matter in anatomical regions "at-risk" of haemodynamic compromise (63, 65, 67–69), may enable better lesion classification, and may also shed further light on the underlying pathology. Understanding relationships between the microstructural tissue characteristics of lesions and normal-appearing white matter is particularly relevant in SCA, where studies have shown widespread and functionally significant reductions in the integrity of normal-appearing white matter (16, 70–72), also in patients without lesions. Another advantage of quantitative multi-modal approaches is that they provide continuous measures that are more normally distributed, and more powerful to work with statistically.

In parallel with quantification of SCI, documenting the effect of reduced arterial oxygen content secondary to anemia and hypoxia on cerebral blood flow (65) and oxygen extraction fraction (73), may shed light on the etiology of cognitive difficulties in SCA, alongside connectivity studies (74). In addition, regional brain volumes may be reduced in SCA (75, 76); we did not find an effect of total intracranial volume in our regressions but further regional analyses are planned.

Future quantitative multi-modal approaches may also help overcome another major challenge, which is that lesions are not detected at the time they occur. As a result, there is no way to determine the presence or absence of transient focal neurological symptoms at the time of insult. Elegant work has demonstrated a high prevalence of lesions on diffusion-weighted imaging in acutely anemic, as well as in steady-state (77) patients with SCA, with only some of these acute lesions later transitioning into SCI observable on FLAIR. Longitudinal quantitative multi-modal MRI studies may help identify what determines this tipping point. Such studies may also shed light on whether acute lesions are reversible or produce permanent damage below the resolution of qualitative MRI.

CONCLUSION

In conclusion, our results highlight the challenges surrounding SCI definition and quantification and suggest limited utility of SCI metrics as biomarkers of cognitive dysfunction in cross-sectional high-resolution MRI studies in patients with SCA. We have demonstrated that with high-resolution MRI: (1) radiological definitions have a large effect on resulting lesion groups, numbers, and volumes; (2) there is a non-negligible prevalence of lesions in young healthy race-matched controls; and (3) at the group-level, there is no cross-sectional association between global lesion metrics and general cognitive outcome irrespective of lesion definition and metric. As high-resolution multi-modal MRI is more widely available, the dichotomy of presence or absence of T-SCI does not appear to be a sensitive biomarker for detection of functionally significant pathology. There is therefore a need to move beyond the focus on the SCI dichotomy, with further standardization regarding definitions and methods, toward a better understanding of the broader underlying pathology. As such, the search should continue for appropriate endpoints for clinical treatment trials, including brief cognitive assessments in domains important for school and work performance, such as processing speed and executive function (52) as well as patient-reported outcomes (e.g., fatigue, pain).

DATA AVAILABILITY STATEMENT

The data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by West London NHS (SAC; 05/Q0408/42, 11/EM/0084, 15/LO/0347), Yorkshire NHS, (POMS; 15/YH/0213), and University College London (community recruitment 14475/001) research Ethics Committees. Written informed consent to participate in this study was provided by participants or their legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

HS: conceptualization, data curation, investigation, methodology, formal analysis, visualization, and writing—original draft. JK: conceptualization, investigation, methodology, and writing—review and editing. JC: resources, methodology, formal analysis, validation, and writing—review and editing. DS: investigation, and writing—review and editing. AH: resources, methodology, and writing—review and editing. MK: data curation, investigation, and writing—review and editing. SS: data curation, investigation, and project administration. DR, OW, ML, MP, BI, JH, and SC: investigation and writing—review and editing. CC: supervision and writing—review and editing. FK: conceptualization, investigation, supervision, funding acquisition, methodology, and writing—review and editing. All authors contributed to the article and approved the submitted version.

FUNDING

HS and MK were funded by Action Medical Research (GN2509) and JK by Great Ormond Street Children's Charity (V4615). AH was supported by an NIH grant (1F32HL143915). The National Institute for Health Research (PB-PG-1112-29099) and NIH (R01HL079937) provided funding for patient recruitment. The work was supported by the National Institute for Health Research Biomedical Research Centre (IS-BRC-1215-20012) at Great Ormond Street Hospital for Children NHS Foundation Trust and University College London.

ACKNOWLEDGMENTS

The authors would like to thank the patients and controls who participated in this research and their families. They would also like to thank the Great Ormond Street Hospital research radiographers for their efforts; Tina Banks, Jessica Cooper, Nichola Sellers, Paul Xavier, Bella Said, Jorvaar Gill, and Michelle Quigley.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2022.867329/full#supplementary-material

REFERENCES

1. DeBaun MR, Armstrong FD, McKinstry RC, Ware RE, Vichinsky E, Kirkham FJ. Silent cerebral infarcts: a review on a prevalent and progressive cause of neurologic injury in sickle cell anemia. *Blood.* (2012) 119:4587–96. doi: 10.1182/blood-2011-02-272682
2. Casella JF, King AA, Barton B, White DA, Noetzel MJ, Ichord RN, et al. Design of the silent cerebral infarct transfusion (SIT) trial. *Pediatr Hematol Oncol.* (2010) 27:69–89. doi: 10.3109/08880010903360367
3. DeBaun MR, Gordon M, McKinstry RC, Noetzel MJ, White DA, Sarnaik SA, et al. Controlled trial of transfusions for silent cerebral infarcts in sickle cell anemia. *N Engl J Med.* (2014) 371:699–710. doi: 10.1056/NEJMoa1401731
4. Guilliams KP, Fields ME, Ragan DK, Chen Y, Eldeniz C, Hulbert ML, et al. Large-vessel vasculopathy in children with sickle cell disease: a magnetic resonance imaging study of infarct topography and focal atrophy. *Pediatr Neurol.* (2017) 69:49–57. doi: 10.1016/j.pediatrneurol.2016.11.005
5. Kwiatkowski JL, Zimmerman RA, Pollock AN, Seto W, Smith-Whitley K, Shults J, et al. Silent infarcts in young children with sickle cell disease. *Br J Haematol.* (2009) 146:300–5. doi: 10.1111/j.1365-2141.2009.07753.x
6. Cancio MI, Helton KJ, Schreiber JE, Smeltzer MP, Kang G, Wang WC. Silent cerebral infarcts in very young children with sickle cell anaemia are associated with a higher risk of stroke. *Br J Haematol.* (2015) 171:120–9. doi: 10.1111/bjh.13525
7. Pegelow CH, Macklin EA, Moser FG, Wang WC, Bello JA, Miller ST, et al. Longitudinal changes in brain magnetic resonance imaging findings in children with sickle cell disease. *Blood.* (2002) 99:3014–18. doi: 10.1182/blood.V99.8.3014
8. Bernaudin F, Verlhac S, Arnaud C, Kamdem A, Vasile M, Kasbi F, et al. Chronic and acute anemia and extracranial internal carotid stenosis are risk factors for silent cerebral infarcts in sickle cell anemia. *Blood.* (2015) 125:1653–61. doi: 10.1182/blood-2014-09-599852
9. Kassim AA, Pruthi S, Day M, Rodeghier M, Gindville MC, Brodsky MA, et al. Silent cerebral infarcts and cerebral aneurysms are prevalent in adults with sickle cell anemia. *Blood.* (2016) 127:2038–40. doi: 10.1182/blood-2016-01-694562
10. Prussien KV, Jordan LC, DeBaun MR, Compas BE. Cognitive function in sickle cell disease across domains, cerebral infarct status, and the lifespan: a meta-analysis. *J Pediatr Psychol.* (2019) 44:948–58. doi: 10.1093/jpepsy/jsz031
11. Schatz J, Brown RT, Pascual JM, Hsu L, DeBaun MR. Poor school and cognitive functioning with silent cerebral infarcts and sickle cell disease. *Neurology.* (2001) 56:1109–11. doi: 10.1212/WNL.56.8.1109
12. DeBaun MR, Jordan LC, King AA, Schatz J, Vichinsky E, Fox CK, et al. American Society of Hematology 2020 guidelines for sickle cell disease: prevention, diagnosis, and treatment of cerebrovascular disease in children and adults. *Blood Adv.* (2020) 4:1554–88. doi: 10.1182/bloodadvances.2019001142
13. Farrell AT, Panepinto J, Carroll CP, Darbari DS, Desai AA, King AA, et al. End points for sickle cell disease clinical trials: patient-reported outcomes, pain, the brain. *Blood Adv.* (2019) 3:3982–4001. doi: 10.1182/bloodadvances.2019000882
14. Hijmans CT, Grootenhuis MA, Oosterlaan J, Heijboer H, Peters M, Fijnvandraat K. Neurocognitive deficits in children with sickle cell disease are associated with the severity of anemia. *Pediatr Blood Cancer.* (2011) 57:297–302. doi: 10.1002/pbc.22892
15. Choi S, O'Neil SH, Joshi AA Li J, Bush AM, Coates TD, et al. Anemia predicts lower white matter volume and cognitive performance in sickle and non-sickle cell anemia syndrome. *Am J Hematol.* (2019) 94:1055–65. doi: 10.1002/ajh.25570

16. Stotesbury H, Kirkham FJ, Kölbel M, Balfour P, Clayden JD, Sahota S, et al. White matter integrity and processing speed in sickle cell anemia. *Neurology.* (2018) 90:e2042–50. doi: 10.1212/WNL.0000000000005644
17. Vichinsky EP, Neumayr LD, Gold JI, Weiner MW, Rule RR, Truran D, et al. Neuropsychological dysfunction and neuroimaging abnormalities in neurologically intact adults with sickle cell anemia. *JAMA.* (2010) 303:1823–31. doi: 10.1001/jama.2010.562
18. Choudhury NA, DeBaun MR, Rodeghier M, King AA, Strouse JJ, McKinstry RC. Silent cerebral infarct definitions and full-scale IQ loss in children with sickle cell anemia. *Neurology.* (2018) 90:e239–46. doi: 10.1212/WNL.0000000000004832
19. Kugler S, Anderson B, Cross D, Sharif Z, Sano M, Haggerty R, et al. Abnormal cranial magnetic resonance imaging scans in sickle-cell disease. *Arch Neurol.* (1993) 50:629. doi: 10.1001/archneur.1993.00540060059019
20. Bernaudin F, Verlhac S, Fréard F, Roudot-Thoraval F, Benkerrou M, Thuret I, et al. Multicenter prospective study of children with sickle cell disease: radiographic and psychometric correlation. *J Child Neurol.* (2000) 15:333–43. doi: 10.1177/088307380001500510
21. Houwing ME, Grohssteiner RL, Dremmen MHG, Atiq F, Bramer WM, de Pagter APJ, et al. Silent cerebral infarcts in patients with sickle cell disease: a systematic review and meta-analysis. *BMC Med.* (2020) 18:393. doi: 10.1186/s12916-020-01864-8
22. van der Land V, Zwanenburg JJM, Fijnvandraat K, Biemond BJ, Hendrikse J, Mutsaerts JMM, et al. Cerebral lesions on 7 tesla MRI in patients with sickle cell anemia. *Cerebrovasc Dis.* (2015) 39:181–9. doi: 10.1159/000373917
23. Nelson MD, Wilson DA, Kisker CT, Evatt BL, Fenstermacher MJ, Lynn HS, et al. Incidence of focal white matter lesions in a population of hemophiliac children and their normal siblings. *Pediatr Radiol.* (2000) 30:705–9. doi: 10.1007/s002470000290
24. Brugulat-Serrat A, Salvadó G, Sudre CH, Grau-Rivera O, Suárez-Calvet M, Falcon C, et al. Patterns of white matter hyperintensities associated with cognition in middle-aged cognitively healthy individuals. *Brain Imaging Behav.* (2020) 14:2012–23. doi: 10.1007/s11682-019-00151-2
25. Biesbroek JM, Weaver NA, Biessels GJ. Lesion location and cognitive impact of cerebral small vessel disease. *Clin Sci.* (2017) 131:715–28. doi: 10.1042/CS20160452
26. Choi S, Bush AM, Borzage MT, Joshi AA, Mack WJ, Coates TD, et al. Hemoglobin and mean platelet volume predicts diffuse T1-MRI white matter volume decrease in sickle cell disease patients. *Neuroimage Clin.* (2017) 15:239–46. doi: 10.1016/j.nicl.2017.04.023
27. Schatz J, Craft S, Koby M, Siegel MJ, Resar L, Lee RR, et al. Neuropsychologic deficits in children with sickle cell disease and cerebral infarction: role of lesion site and volume. *Child Neuropsychol.* (1999) 5:92–103. doi: 10.1076/chin.5.2.92.3170
28. Schatz J, White DA, Moinuddin A, Armstrong M, DeBaun MR. Lesion burden and cognitive morbidity in children with sickle cell disease. *J Child Neurol.* (2002) 17:891–5. doi: 10.1177/08830738020170122401
29. van der Land V, Hijmans CT, de Ruiter M, Mutsaerts HJMM, Cnossen MH, Engelen M, et al. Volume of white matter hyperintensities is an independent predictor of intelligence quotient and processing speed in children with sickle cell disease. *Br J Haematol.* (2015) 168:553–6. doi: 10.1111/bjh.13179
30. Estcourt LJ, Fortin PM, Hopewell S, Trivella M, Doree C, Abboud MR. Interventions for preventing silent cerebral infarcts in people with sickle cell disease. *Cochrane Database Syst Rev.* (2017) 5:CD012389. doi: 10.1002/14651858.CD012389.pub2
31. Howard J, Slee AE, Skene S, Inusa B, Kawadler J, Downes M, et al. Overnight auto-adjusting continuous airway pressure + standard care compared with standard care alone in the prevention of morbidity in sickle cell disease phase II (POMS2b): study protocol for a randomised controlled trial. *Trials.* (2018) 19:55. doi: 10.1186/s13063-017-2419-0
32. Rosen CL, Debaun MR, Strunk RC, Redline S, Seicean S, Craven DI, et al. Obstructive sleep apnea and sickle cell anemia. *Pediatrics.* (2014) 134:273–81. doi: 10.1542/peds.2013-4223
33. Kölbel M, Kirkham FJ, Iles R, Stotesbury H, Halstead E, Brenchley C, et al. Exploring the relationship of sleep, cognition, and cortisol in sickle cell disease. *Comprehens Psychoneuroendocrinol.* (2022) 10:100128. doi: 10.1016/j.cpnec.2022.100128
34. Wechsler D, WASI -II. Wechsler abbreviated scale of intelligence - second edition. *J Psychoeduc Assess.* (2013) 31:337–41. doi: 10.1177/0734282912467756
35. Wechsler D. *The Wechsler Intelligence Scale for Children*. 4th ed. San Antonio, TX: The Psychological Corporation (2004).
36. Wechsler D. *Wechsler Adult Intelligence Scale*. 4th ed (WAIS-IV). San Antonio, CA: The Psychological Corporation (2008). p. 1–3.
37. DCLG. *The English Indices of Deprivation 2015* Statistical Release (2015).
38. Sudre CH, Gomez Anson B, Davagnanam I, Schmitt A, Mendelson AF, Prados F, et al. Bullseye's representation of cerebral white matter hyperintensities. *J Neuroradiol.* (2018) 45:114–22. doi: 10.1016/j.neurad.2017.10.001
39. Fox J, Weisberg S, Adler D, Bates D, Baud-Bovy G, Ellison S, et al. *car: Companion to Applied Regression*. *R Packag. Version 2.0-21, 1–157*. California; London; New Delhi; Singapore: SAGE publications (2014).
40. Peña EA, Slate EH. Global validation of linear model assumptions. *J Am Stat Assoc.* (2006) 101:341–54. doi: 10.1198/016214505000000637
41. Cohen J. *Statistical Power Analysis for the Behavioral Sciences. 2nd ed*. New York, *NY*: N*ew* Y*ork* Acad*emic* Press (1988).
42. King AA, Strouse JJ, Rodeghier MJ, Compas BE, Casella JF, McKinstry RC, et al. Parent education and biologic factors influence on cognition in sickle cell anemia. *Am J Hematol.* (2014) 89:162–7. doi: 10.1002/ajh.23604
43. Choi E, Branch C, Cui MH, Yazdanbakhsh K, Mohandas N, Billett HH, et al. No evidence for cell activation or brain vaso-occlusion with plerixafor mobilization in sickle cell mice. *Blood Cells Mol Dis.* (2016) 57:67–70. doi: 10.1016/j.bcmd.2015.12.008
44. Silva GS, Vicari P, Figueiredo MS, Carrete H Jr., Idagawa MH, Massaro AR, et al. Brain magnetic resonance imaging abnormalities in adult patients with sickle cell disease: correlation with transcranial Doppler findings. *Stroke.* (2009) 40:2408–12. doi: 10.1161/STROKEAHA.108.537415
45. Wardlaw JM, Smith EE, Biessels GJ, Cordonnier C, Fazekas F, Frayne R, et al. Neuroimaging standards for research into small vessel disease and its contribution to ageing and neurodegeneration. *Lancet Neurol.* (2013) 12:822–38. doi: 10.1016/S1474-4422(13)70124-8
46. Garnier-Crussard A, Cotton F, Desestret V, Krolak-Salmon P, Fort R. Silent cerebral infarcts in sickle cell disease: Really silent? *J Neuroradiol.* (2021) 48:471–2. doi: 10.1016/j.neurad.2021.03.002
47. Griffanti L, Zamboni G, Khan A, Li L, Bonifacio G, Sundaresan V, et al. BIANCA (Brain Intensity AbNormality Classification Algorithm): a new tool for automated segmentation of white matter hyperintensities. *Neuroimage.* (2016) 141:191–205. doi: 10.1016/j.neuroimage.2016.07.018
48. Farris N, Billett HH, Branch CA, Minniti C, Branch K, Acharya SA. The Association of white matter hyperintensity volume and sickle cell anemia. *Blood.* (2016) 128:1321. doi: 10.1182/blood.V128.22.1321.1321
49. Krejza J, Arkuszewski M, Radcliffe J, Flynn TB, Chen R, Kwiatkowski JL, et al. Association of pulsatility index in the middle cerebral artery with intelligence quotient in children with sickle cell disease. *Neuroradiol J.* (2012) 25:351–9. doi: 10.1177/197140091202500311
50. Chen R, Krejza J, Arkuszewski M, Zimmerman RA, Herskovits EH, Melhem ER. Brain morphometric analysis predicts decline of intelligence quotient in children with sickle cell disease: a preliminary study. *Adv Med Sci.* (2017) 62:151–7. doi: 10.1016/j.advms.2016.09.002
51. Downes M, Keenan L, Duane Y, Duffy K, Fortune G, Geoghegan R, et al. Executive function in children with sickle cell anemia on transfusion: NIH toolbox utility in the clinical context. *Clin Neuropsychol.* (2020) 1–16. doi: 10.1080/13854046.2020.1847325. [Epub ahead of print].
52. Hood AM, King AA, Fields ME, Ford AL, Guilliams KP, Hulbert ML, et al. Higher executive abilities following a blood transfusion in children and young adults with sickle cell disease. *Pediatr Blood Cancer.* (2019) 66:e27899. doi: 10.1002/pbc.27899
53. Farris N, Branch CA, Zimmerman ME, Suri AK, Billett HH. Lack of association of CNS lesion number with cognitive performance and cerebral blood flow in sickle cell disease. *Blood.* (2015) 126:984. doi: 10.1182/blood.V126.23.984.984
54. Sanger M, Jordan L, Pruthi S, Day M, Covert B, Merriweather B, et al. Cognitive deficits are associated with unemployment in adults with sickle cell anemia. *J Clin Exp Neuropsychol.* (2016) 38:661–71. doi: 10.1080/13803395.2016.1149153

55. Baldeweg T, Hogan AM, Saunders DE, Telfer P, Gadian DG, Vargha-Khadem F, et al. Detecting white matter injury in sickle cell disease using voxel-based morphometry. *Ann Neurol.* (2006) 59:662–72. doi: 10.1002/ana.20790
56. Wang W, Enos L, Gallagher D, Thompson R, Guarini L, Vichinsky E, et al. Neuropsychologic performance in school-aged children with sickle cell disease: a report from the Cooperative Study of Sickle Cell Disease. *J Pediatr.* (2001) 139:391–7. doi: 10.1067/mpd.2001.116935
57. Coloigner J, Kim Y, Bush A, Choi S, Balderrama MC, Coates TD, et al. Contrasting resting-state fMRI abnormalities from sickle and non-sickle anemia. *PLoS ONE.* (2017) 12:e0184860. doi: 10.1371/journal.pone.0184860
58. Václavu L, Petr J, Petersen ET, Mutsaerts HJMM, Majoie CBL, Wood JC, et al. Cerebral oxygen metabolism in adults with sickle cell disease. *Am J Hematol.* (2020) 95:401–12. doi: 10.1002/ajh.25727
59. Chai Y, Ji C, Coloigner J, Choi S, Balderrama M, Vu C, et al. Tract-specific analysis and neurocognitive functioning in sickle cell patients without history of overt stroke. *Brain Behav.* (2021) 11:e01978. doi: 10.1002/brb3.1978
60. Wang WC, Zou P, Hwang SN, Kang G, Ding J, Heitzer AM, et al. Effects of hydroxyurea on brain function in children with sickle cell anemia. *Pediatr Blood Cancer.* (2021) 68:e29254. doi: 10.1002/pbc.29254
61. Heitzer AM, Longoria J, Okhomina V, Wang WC, Raches D, Potter B, et al. Hydroxyurea treatment and neurocognitive functioning in sickle cell disease from school age to young adulthood. *Br J Haematol.* (2021) 195:256–66. doi: 10.1111/bjh.17687
62. Wang Y, Fellah S, Fields ME, Guilliams KP, Binkley MM, Eldeniz C, et al. Cerebral oxygen metabolic stress, microstructural injury, and infarction in adults with sickle cell disease. *Neurology*. (2021) 97:e902–12. doi: 10.1212/WNL.0000000000012404
63. Fields ME, Guilliams KP, Ragan DK, Binkley MM, Eldeniz C, Chen Y, et al. Regional oxygen extraction predicts border zone vulnerability to stroke in sickle cell disease. *Neurology*. (2018) 90:e1134–42. doi: 10.1212/WNL.0000000000005194
64. Ford AL, Ragan DK, Fellah S, Binkley MM, Fields ME, Guilliams KP, et al. Silent infarcts in sickle cell disease occur in the border zone region and are associated with low cerebral blood flow. *Blood.* (2018) 132:1714–23. doi: 10.1182/blood-2018-04-841247
65. Stotesbury H, Hales PW, Hood AM, Koelbel M, Kawadler JM, Saunders DE, et al. (2022). Individual watershed areas in sickle cell anemia: An arterial spin labeling study. *Front Physiol.* 13:865391. doi: 10.3389/fphys.2022.865391
66. Kim KW, MacFall JR, Payne ME. Classification of white matter lesions on magnetic resonance imaging in elderly persons. *Biol Psychiatry.* (2008) 64:273–80. doi: 10.1016/j.biopsych.2008.03.024
67. Stotesbury H, Kawadler JM, Hales PW, Saunders DE, Clark CA, Kirkham FJ. Vascular instability and neurological morbidity in sickle cell disease: an integrative framework. *Front Neurol.* (2019) 10:871. doi: 10.3389/fneur.2019.00871
68. Miao X, Choi S, Tamrazi B, Chai Y, Vu C, Coates TD, et al. Increased brain iron deposition in patients with sickle cell disease: an MRI, quantitative susceptibility mapping study. *Blood.* (2018) 132:1618–21. doi: 10.1182/blood-2018-04-840322
69. Kirkham FJ, Shmueli K. Brain iron in sickle cell disease? *Blood.* (2018) 132:1550–2. doi: 10.1182/blood-2018-08-867010
70. Kawadler JM, Kirkham FJ, Clayden JD, Hollocks MJ, Seymour EL, Edey R, et al. White matter damage relates to oxygen saturation in children with sickle cell anemia without silent cerebral infarcts. *Stroke.* (2015) 46:1793–9. doi: 10.1161/STROKEAHA.115.008721
71. Stotesbury H, Kawadler JM, Saunders DE, Kirkham FJ. MRI detection of brain abnormality in sickle cell disease. *Expert Rev Hematol.* (2021) 14:473–91. doi: 10.1080/17474086.2021.1893687
72. Stotesbury H, Hales PW, Koelbel M, Hood AM, Kawadler JM, Saunders DE, et al. Venous cerebral blood flow quantification and cognition in patients with sickle cell anemia. *J Cereb Blood Flow Metab.* (2022) 271678X211072391. doi: 10.1177/0271678X211072391. [Epub ahead of print].
73. Prussien KV, Compas BE, Siciliano RE, Ciriegio AE, Lee CA, Kassim AA, et al. Cerebral hemodynamics, and executive function in sickle cell, anemia. *Stroke.* (2021) 52:1830–4. doi: 10.1161/STROKEAHA.120.032741
74. Fields ME, Mirro AE, Guilliams KP, Binkley MM, Gil Diaz L, Tan J, et al. Functional connectivity decreases with metabolic stress in sickle cell disease. *Ann Neurol.* (2020) 88:995–1008. doi: 10.1002/ana.25891
75. Kawadler JM, Clayden JD, Kirkham FJ, Cox TC, Saunders DE, Clark CA. Subcortical and cerebellar volumetric deficits in paediatric sickle cell anaemia. *Br J Haematol.* (2013) 163:373–6. doi: 10.1111/bjh.12496
76. Santini T, Koo M, Farhat N, Campos VP, Alkhateeb S, Vieira MAC, et al. Analysis of hippocampal subfields in sickle cell disease using ultrahigh field MRI. *Neuroimage Clin.* (2021) 30:102655. doi: 10.1016/j.nicl.2021.102655
77. Dowling MM, Quinn CT, Plumb P, Rogers ZR, Rollins NK, Koral K, et al. Acute silent cerebral ischemia and infarction during acute anemia in children with and without sickle cell disease. *Blood.* (2012) 120:3891–7. doi: 10.1182/blood-2012-01-406314

Conflict of Interest: FK was grantholder for GN2509, V4615, PB-PG-1112-29099 and R01HL079937 and has received honoraria from Global Blood Therapeutics, Bluebird Bio, Novartis, BIAL, Shire and Johnson and Johnson. JH received research funding from Bluebird Bio, and payments in relation to work as an advisory board member from IMR, Novartis, Global Blood Therapeutics, Novo Nordisk, Forma therapeutics, Agios, Add Medica, and Terumo, and also received a travel grant from Novartis and payments relating to work as a panel speaker from Novartis and Global Blood Therapeutics.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

APPENDIX

TABLE A1 | Global regression models.

Global Models	**1) SCI**	**2) SCI* SCA**		**3) SCI + SCI No.**		**4) SCI * SCA + SCI No * SCA**				**5) SCI + SCI Vol**		**6) SCI *SCA + SCI Vol * SCA**			
Predictors	**SCI**	**SCI**	**SCI* SCA**	**SCI**	**SCI No.**	**SCI**	**SCI* SCA**	**SCI No.**	**SCI No. * SCA**	**SCI**	**SCI Vol**	**SCI**	**SCI *SCA**	**SCI Vol**	**SCI Vol *SCA**
FSIQ															
L-SCI *any SCI (all, n = 154)*	b = −0.59, *p* = 0.79, r = −0.02, CI = −5.0–3.8	b = −0.29, *p* = 0.90, r = −0.01, CI = −4.8–4.2	b = −0.54, *p* = 0.92, r = −0.01, CI = −10.7–9.7	b = 0.73, *p* = 0.82, r = 0.02, CI = −5.8–7.2	b = −1.04, *p* = 0.58, r = −0.04, CI = −4.8–2.7	b = 2.21, *p* = 0.52, r = 0.05, CI = −4.6–9.0	b = −7.85, *p* = 0.34, r = −0.08, CI = −24.0–8.3	b = −2.32, *p* = 0.29, r = −0.09, CI = −6.6–2.0	b = 6.83, *p* = 0.23, r = 0.10, CI = −4.3–18.0	b = 1.71, *p* = 0.69, r = 0.03, CI = −6.8–10.2	b = −0.59, *p* = 0.53, r = −0.05, CI = −2.4–1.3	b = 2.08, *p* = 0.64, r = 0.04, CI = −6.6–10.7	b = −5.15, *p* = 0.58, r = −0.04, CI = −23.7–13.4	b = −0.74, *p* = 0.47, r = −0.06, CI = −2.7–1.3	b = 1.61, *p* = 0.49, r = 0.05, CI = −3.0–6.3
>1 SCI per decade (all, n = 154)	b = 0.06, *p* = 0.98, r = 0.00, CI = −4.6–4.7	b = 0.09, *p* = 0.97, r = 0.00, CI = −4.7–4.8	b = 1.98, *p* = 0.72, r = 0.03, CI = −9.0–13.0	–	–	–	–	–	–	–	–	–	–	–	–
Any SCI (SCA only, n = 106)	b = −0.04, *p* = 0.99, r = −0.00, CI = −5.3–5.2	–	–	b = 1.01, *p* = 0.79, r = 0.02, CI = −6.6–8.7	b = −0.77, *p* = 0.71, r = −0.04, CI = −4.9–3.3	–	–	–	–	b = 2.50, *p* = 0.64, r = 0.04, CI = −8.0–13.0	b = −0.60, *p* = 0.58, r = −0.05, CI = −2.8–1.6	–	–	–	–
T-SCI *any SCI (all, n = 154)*	b = −0.07, *p* = 0.98, r = −0.00, CI = −4.8–4.6	b = −0.12, *p* = 0.96, r = −0.00, CI = −5.4–5.1	b = 3.53, *p* = 0.60, r = 0.04, CI = −9.6–16.6	b = 1.03, *p* = 0.76, r = 0.02, CI = −5.7–7.8	b = −0.96, *p* = 0.65, r = −0.04, CI = −5.2–3.3	b = 1.37, *p* = 0.73, r = 0.03, CI = −6.6–9.3	b = 2.58, *p* = 0.81, r = 0.02, CI = −18.7–23.8	b = −1.43, *p* = 0.64, r = −0.04, CI = −7.5–4.6	b = 1.34, *p* = 0.88, r = 0.01, CI = −16.2–18.9	–	b = −0.21, *p* = 0.67, r = −0.03, CI = −1.2–0.8	–	–	b = −0.25, *p* = 0.67, r = −0.03, CI = −1.4–0.9	b = 0.64, *p* = 0.68, r = 0.03, CI = −2.4–3.7
>1 SCI per decade (all, n = 154)	b = 0.34, *p* = 0.89, r = 0.01, CI = −4.7–5.3	b = 0.11, *p* = 0.97, r = 0.00, CI = −5.3–5.5	b = 3.84, *p* = 0.57, r = 0.05, CI = −9.4–17.0	–	–	–	–	–	–	–	–	–	–	–	–
Any SCI (SCA only, n = 106)	b = 1.27, *p* = 0.64, r = 0.04, CI = −4.0–6.6	–	–	b = 3.30, *p* = 0.39, r = 0.08, CI = −4.3–10.9	b = −1.68, *p* = 0.45, r = −0.07, CI = −6.1–2.8	–	–	–	–	–	b = −0.08, *p* = 0.89, r = −0.01, CI = −1.2–1.0	–	–	–	–
R-SCI *any SCI (all, n = 154)*	b = 0.28, *p* = 0.92, r = 0.01, CI = −5.3–5.9	–	–	b = −0.44, *p* = 0.90, r = −0.01, CI = −7.5–6.6	b = 1.29, *p* = 0.74, r = 0.03, CI = −6.3–8.9	–	–	–	–	b = −1.91, *p* = 0.65, r = −0.04, CI = −10.1–6.3	b = 1.21, *p* = 0.47, r = 0.06, CI = −2.1–4.5	–	–	–	–
>1 SCI per decade (all, n = 154)	b = 3.35, *p* = 0.41, r = 0.07, CI = −4.59–11.29	–	–			–	–	–	–	–	–	–	–	–	–

(Continued)

TABLE A1 | Continued

Global Models	**1) SCI**	**2) SCI* SCA**		**3) SCI + SCI No.**		**4) SCI * SCA + SCI No * SCA**				**5) SCI + SCI Vol**		**6) SCI *SCA + SCI Vol * SCA**			
Predictors	**SCI**	**SCI**	**SCI* SCA**	**SCI**	**SCI No.**	**SCI**	**SCI* SCA**	**SCI No.**	**SCI No. * SCA**	**SCI**	**SCI Vol**	**SCI**	**SCI *SCA**	**SCI Vol**	**SCI Vol *SCA**
Any SCI (SCA only, n = 106)	b = 0.58, *p* = 0.85, r = 0.02, CI = −5.5–6.6	–	–	b = 0.48, *p* = 0.90, r = 0.01, CI = −7.4–8.3	b = 0.17, *p* = 0.97, r = 0.00, CI = −7.8–8.2	–	–	–	–	b = −1.41, *p* = 0.75, r = −0.03, CI = −10.3–7.5	b = 1.04, *p* = 0.55, r = 0.06, CI = −2.4–4.5	–	–	–	–
WMI *L-SCI* *any SCI (all, n = 154)*	b = −0.22, *p* = 0.93, r = −0.01, CI = −5.1–4.61	b = 0.11, *p* = 0.97, r = 0.00, CI = −4.81–5.03	b = 3.14, *p* = 0.58, r = 0.04, CI = −7.94–14.21	b = 2.16, *p* = 0.55, r = 0.05, CI = −4.95–9.26	b = −1.86, *p* = 0.37, r = −0.07, CI = −5.93–2.21	b = 2.63, *p* = 0.49, r = 0.06, CI = −4.81–10.06	b = 4.27, *p* = 0.63, r = 0.04, CI = −13.3–21.81	b = −1.97, *p* = 0.40, r = −0.07, CI = −6.64–2.69	b = −0.50, *p* = 0.94, r = −0.01, CI = −12.6–11.63	b = 3.39, *p* = 0.47, r = 0.06, CI = −5.91–12.68	b = −0.92, *p* = 0.37, r = −0.07, CI = −2.95–1.11	b = 3.78, *p* = 0.43, r = 0.06, CI = −5.60–13.15	b = 9.34, *p* = 0.36, r = 0.07, CI = −10.74–29.42	b = −0.85, *p* = 0.44, r = −0.06, CI = −3.01–1.30	b = −1.51, *p* = 0.55, r = −0.05, CI = −6.54–3.52
>1 SCI per decade (all, n = 154)	b = −0.83, *p* = 0.75, r = −0.03, CI = −5.94–4.27	b = −0.64, *p* = 0.81, r = −0.02, CI = −5.81–4.52	b = 1.83, *p* = 0.76, r = 0.02, CI = 10.14–13.79	–	–	–	–	–	–	–	–	–	–	–	–
Any SCI (SCA only, n = 106)	b = 1.56, *p* = 0.59, r = 0.05, CI = −4.10–7.21	–	–	b = 5.14, *p* = 0.22, r = 0.12, CI = −3.11–13.39	b = −2.63, *p* = 0.24, r = −0.11, CI = −7.05–1.79	–	–	–	–	b = 8.37, *p* = 0.15, r = 0.14, CI = −3.0–19.7	b = −1.61, *p* = 0.17, r = −0.13, CI = −4.0–0.7	–	–	–	–
T-SCI *any SCI (all, n = 154)*	b = 0.46, *p* = 0.86, r = 0.01, CI = −4.68–5.59	b = 1.22, *p* = 0.67, r = 0.03, CI = −4.48–6.93	b = 1.56, *p* = 0.83, r = 0.02, CI = −12.68–15.79	b = 2.45, *p* = 0.51, r = 0.05, CI = −4.92–9.81	b = −1.74, *p* = 0.46, r = −0.06, CI = −6.34–2.87	b = 2.50, *p* = 0.57, r = 0.05, CI = −6.15–11.15	b = 5.52, *p* = 0.64, r = 0.04, CI = −17.5–28.58	b = −0.96, *p* = 0.77, r = −0.02, CI = −7.54–5.62	b = −3.60, *p* = 0.71, r = −0.03, CI = −22.6–15.40	–	b = −0.20, *p* = 0.72, r = −0.03, CI = −1.28–0.89	–	–	b = 0.01, *p* = 0.99, r = 0.00, CI = −1.26–1.28	b = −0.18, *p* = 0.91, r = −0.01, CI = −3.46–3.10
>1 SCI per decade (all, n = 154)	b = 0.09, *p* = 0.97, r = 0.00, CI = −5.37–5.55	b = 0.60, *p* = 0.84, r = 0.02, CI = −5.27–6.48	b = 0.70, *p* = 0.92, r = 0.01, CI = −13.66–15.06	–	–	–	–	–	–	–	–	–	–	–	–
Any SCI (SCA only, n = 106)	b = 2.08, *p* = 0.48, r = 0.07, CI = −3.67–7.83	–	–	b = 5.44, *p* = 0.19, r = 0.13, CI = −2.73–13.62	b = −2.79, *p* = 0.25, r = −0.11, CI = −7.60–2.03	–	–	–	–	–	b = −0.05, *p* = 0.93, r = −0.01, CI = −1.23–1.13	–	–	–	–

(Continued)

TABLE A1 | Continued

Global Models	1) SCI	2) SCI* SCA		3) SCI + SCI No.		4) SCI * SCA + SCI No * SCA				5) SCI + SCI Vol		6) SCI *SCA + SCI Vol * SCA			
Predictors	**SCI**	**SCI**	**SCI* SCA**	**SCI**	**SCI No.**	**SCI**	**SCI* SCA**	**SCI No.**	**SCI No. * SCA**	**SCI**	**SCI Vol**	**SCI**	**SCI *SCA**	**SCI Vol**	**SCI Vol *SCA**
R-SCI *any SCI (all, n = 154)*	b = 0.29, p = 0.93, r = 0.01, CI = −5.84–6.41	–	–	b = 1.82, p = 0.64, r = 0.04, CI = −5.86–9.51	b = −2.76, p = 0.51, r = −0.05, CI = −11.06–5.55	–	–	–	–	b = −1.45, p = 0.75, r = −0.03, CI = −10.41–7.51	b = 0.96, p = 0.60, r = 0.04, CI = −2.65–4.57	–	–	–	–
>1 SCI per decade (all, n = 154)	b = −1.30, p = 0.77, r = −0.02, CI = −10.01–7.41	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Any SCI (SCA only, n = 106)	b = 1.13, p = 0.74, r = 0.03, CI = −5.46–7.72	–	–	b = 3.76, p = 0.38, r = 0.08, CI = −4.74–12.25	b = −4.25, p = 0.33, r = −0.09, CI = −12.91–4.41	–	–	–	–	b = −0.53, p = 0.91, r = −0.01, CI = −10.25–9.18	b = 0.87, p = 0.64, r = 0.04, CI = −2.86–4.60	–	–	–	–
PSI															
L-SCI *Any SCI (all, n = 154)*	b = −1.67, p = 0.46, r = −0.06, CI = −6.09–2.75	b = −1.13, p = 0.62, r = −0.04, CI = −5.58–3.32	b = 2.30, p = 0.65, r = 0.03, CI = −7.72–12.32	b = −1.26, p = 0.70, r = −0.03, CI = −7.77–5.24	b = −0.32, p = 0.87, r = −0.01, CI = −4.05–3.41	b = 0.04, p = 0.99, r = 0.00, CI = −6.70–6.77	b = −1.14, p = 0.89, r = −0.01, CI = −17.0–14.75	b = −1.09, p = 0.61, r = −0.04, CI = −5.32–3.14	b = 3.21, p = 0.56, r = 0.04, CI = −7.78–14.21	b = 0.30, p = 0.94, r = 0.01, CI = −8.20–8.80	b = −0.50, p = 0.59, r = −0.04, CI = −2.36–1.35	b = 1.05, p = 0.81, r = 0.02, CI = −7.45–9.54	b = −0.99, p = 0.91, r = −0.01, CI = −19.2–17.22	b = −0.66, p = 0.51, r = −0.05, CI = −2.61–1.30	b = 1.19, p = 0.61, r = 0.04, CI = −3.37–5.76
>1 SCI per decade (all, n = 154)	b = −0.93, p = 0.69, r = −0.03, CI = −5.60–3.74	b = −0.83, p = 0.73, r = −0.03, CI = −5.50–3.83	b = 4.73, p = 0.39, r = 0.07, CI = −6.07–15.53	–	–	–	–	–	–	–	–	–	–	–	–
Any SCI (SCA only, n = 106)	b = −0.08, p = 0.97, r = −0.00, CI = −5.08–4.91	–	–	b = 0.52, p = 0.89, r = 0.01, CI = −6.82–7.86	b = −0.44, p = 0.82, r = −0.02, CI = −4.37–3.49	–	–	–	–	b = 2.20, p = 0.67, r = 0.04, CI = −7.92–12.32	b = −0.54, p = 0.61, r = −0.05, CI = −2.62–1.54	–	–	–	–
T-SCI *any SCI (all, n = 154)*	b = −0.94, p = 0.69, r = −0.03, CI = −5.63–3.75	b = −0.35, p = 0.89, r = −0.01, CI = −5.51–4.81	b = 4.40, p = 0.50, r = 0.05, CI = −8.47–17.27	b = 0.32, p = 0.93, r = 0.01, CI = −6.42–7.06	b = −1.10, p = 0.61, r = −0.04, CI = −5.31–3.11	b = 0.59, p = 0.88, r = 0.01, CI = −7.24–8.41	b = 7.05, p = 0.51, r = 0.05, CI = −13.8–27.92	b = −0.72, p = 0.81, r = −0.02, CI = −6.68–5.24	b = −2.38, p = 0.78, r = −0.02, CI = −19.6–14.82	–	b = −0.38, p = 0.45, r = −0.06, CI = −1.37–0.61	–	–	b = −0.25, p = 0.67, r = −0.03, CI = −1.40–0.90	b = 0.50, p = 0.74, r = 0.03, CI = −2.46–3.47

(Continued)

TABLE A1 | Continued

Global Models	1) SCI	2) SCI* SCA		3) SCI + SCI No.		4) SCI * SCA + SCI No * SCA				5) SCI + SCI Vol		6) SCI *SCA + SCI Vol * SCA			
Predictors	**SCI**	**SCI**	**SCI* SCA**	**SCI**	**SCI No.**	**SCI**	**SCI* SCA**	**SCI No.**	**SCI No. * SCA**	**SCI**	**SCI Vol**	**SCI**	**SCI *SCA**	**SCI Vol**	**SCI Vol *SCA**
>1 SCI per decade (all, n = 154)	b = −0.99, *p* = 0.69, r = −0.03, CI = −5.99–4.00	b = −0.75, *p* = 0.78, r = −0.02, CI = −6.05–4.56	b = 3.85, *p* = 0.56, r = 0.04, CI = −9.12–16.83	–	–	–	–	–	–	–	–	–	–	–	–
Any SCI (SCA only, n = 106)	b = 1.22, *p* = 0.63, r = 0.04, CI = −3.86–6.30	–	–	b = 3.40, *p* = 0.35, r = 0.09, CI = −3.84–10.64	b = −1.81, *p* = 0.40, r = −0.08, CI = −6.07–2.46	–	–	–	–	–	b = −0.11, *p* = 0.84, r = −0.02, CI = −1.15–0.94	–	–	–	–
***R-SCI** any SCI (all, n = 154)*	b = −2.56, *p* = 0.37, r = −0.07, CI = −8.15–3.03	–	–	b = −1.91, *p* = 0.59, r = −0.04, CI = −8.93–5.11	b = −1.17, *p* = 0.76, r = −0.02, CI = −8.76–6.41	–	–	–	–	b = −3.88, *p* = 0.35, r = −0.07, CI = −12.06–4.30	b = 0.73, *p* = 0.66, r = 0.03, CI = −2.56–4.02	–	–	–	–
>1 SCI per decade (all, n = 154)	b = −0.62, *p* = 0.88, r = −0.01, CI = −8.59–7.35	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Any SCI (SCA only, n = 106)	b = −1.88, *p* = 0.52, r = −0.06, CI = −7.69–3.92	–	–	b = −0.92, *p* = 0.81, r = −0.02, CI = −8.44–6.59	b = −1.55, *p* = 0.69, r = −0.04, CI = −9.21–6.11	–	–	–	–	b = −3.72, *p* = 0.39, r = −0.08, CI = −12.27–4.83	b = 0.96, *p* = 0.56, r = 0.05, CI = −2.32–4.24	–	–	–	–
CI *L-SCI any SCI (all, n = 154)*	OR = 1.00, *p* = 0.99, CI = 0.26–3.87	–	–	OR = 0.78, *p* = 0.81, CI = 0.10–6.19	OR = 1.20, *p* = 0.75, CI = 0.39–3.73	–	–	–	–	–	OR = 1.06, *p* = 0.69, CI = 0.81–1.38	–	–	–	–
>1 SCI per decade (all, n = 154)	OR = 1.49, *p* = 0.57, CI = 0.39–5.74	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Any SCI (SCA only, n = 106)	OR = 0.63, *p* = 0.55, CI = 0.14–2.87	–	–	OR = 0.45, *p* = 0.51, CI = 0.04–4.73	OR = 1.30, *p* = 0.70, CI = 0.35–4.80	–	–	–	–	–	OR = 0.98, *p* = 0.91, CI = 0.73–1.33	–	–	–	–

(Continued)

TABLE A1 | Continued

Global Models	1) SCI	2) SCI* SCA		3) SCI + SCI No.		4) SCI * SCA + SCI No * SCA				5) SCI + SCI Vol		6) SCI *SCA + SCI Vol * SCA			
Predictors	**SCI**	**SCI**	**SCI* SCA**	**SCI**	**SCI No.**	**SCI**	**SCI* SCA**	**SCI No.**	**SCI No. * SCA**	**SCI**	**SCI Vol**	**SCI**	**SCI *SCA**	**SCI Vol**	**SCI Vol *SCA**
T-SCI *any SCI (all, n = 154)*	OR = 1.28, $p = 0.72$, CI = 0.33–4.91	–	–	OR = 1.24, $p = 0.82$, CI = 0.20–7.87	OR = 1.03, $p = 0.96$, CI = 0.34–3.08	–	–	–	–	–	b = 1.08, $p = 0.57$, CI = 0.83–1.40	–	–	–	–
>1 SCI per decade (all, n = 154)	OR = 0.97, $p = 0.96$, CI = 0.23–4.00	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Any SCI (SCA only, n = 106)	OR = 0.68, $p = 0.63$, CI = 0.15–3.16	–	–	OR = 0.57, $p = 0.59$, CI = 0.08–4.40	OR = 1.17, $p = 0.79$, CI = 0.35–3.91	–	–	–	–	–	OR = 0.99, $p = 0.94$, CI = 0.73–1.34	–	–	–	–
R-SCI *any SCI (all, n = 154)*	OR = 2.02, $p = 0.33$, CI = 0.49–8.26	–	–	OR = 2.59, $p = 0.27$, CI = 0.48–14.17	OR = 0.59, $p = 0.63$, CI = 0.07–5.05	–	–	–	–	OR = 4.35, $p = 0.13$, CI = 0.65–29.16	OR = 0.58, $p = 0.30$, CI = 0.21–1.61	–	–	–	–
>1 SCI per decade (all, n = 154)	OR = 1.63, $p = 0.64$, CI = 0.21–12.61	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Any SCI (SCA only, n = 106)	OR = 1.15, $p = 0.86$, CI = 0.24–5.51	–	–	OR = 1.44, $p = 0.71$, CI = 0.21–9.70	OR = 0.62, $p = 0.69$, CI = 0.06–6.57	–	–	–	–	OR = 2.51, $p = 0.38$, CI = 0.32–19.69	OR = 0.58, $p = 0.32$, CI = 0.20–1.69	–	–	–	–

Values are regression coefficients (b), odds ratios (OR), probability values (p), semi-partial correlation coefficients (r), and 95% confidence intervals (CI) from global regression models. SCI, silent cerebral infarction; SCA, sickle cell anemia; No, number; Vol, volume; L-/T-/R-SCI, Liberal/Traditional/Restrictive silent cerebral infarction definitions.

frontiers | Frontiers in Neurology

ORIGINAL RESEARCH
published: 01 July 2022
doi: 10.3389/fneur.2022.869117

Quantifying the Cerebral Hemometabolic Response to Blood Transfusion in Pediatric Sickle Cell Disease With Diffuse Optical Spectroscopies

Seung Yup Lee[1,2†], *Rowan O. Brothers*[1†], *Katherine B. Turrentine*[1], *Ayesha Quadri*[1], *Eashani Sathialingam*[1], *Kyle R. Cowdrick*[1], *Scott Gillespie*[3], *Shasha Bai*[3], *Adam E. Goldman-Yassen*[4], *Clinton H. Joiner*[1,4,5], *R. Clark Brown*[4,5] *and Erin M. Buckley*[1,4,6*]

[1] Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, Atlanta, GA, United States, [2] Department of Electrical and Computer Engineering, Kennesaw State University, Marietta, GA, United States, [3] Pediatric Biostatistics Core, Emory University School of Medicine, Atlanta, GA, United States, [4] Department of Pediatrics, Emory University School of Medicine, Atlanta, GA, United States, [5] Aflac Cancer & Blood Disorders Center, Children's Healthcare of Atlanta, Atlanta, GA, United States, [6] Children's Research Scholar, Children's Healthcare of Atlanta, Atlanta, GA, United States

OPEN ACCESS

Edited by:
Fenella Jane Kirkham,
University College London,
United Kingdom

Reviewed by:
Ashwin B. Parthasarathy,
University of South Florida,
United States
Silvina Ferradal,
Indiana University, United States

***Correspondence:**
Erin M. Buckley
erin.buckley@emory.edu

[†]These authors share first authorship

Specialty section:
This article was submitted to Stroke, a section of the journal Frontiers in Neurology

Received: 03 February 2022
Accepted: 13 May 2022
Published: 01 July 2022

Citation:
Lee SY, Brothers RO, Turrentine KB, Quadri A, Sathialingam E, Cowdrick KR, Gillespie S, Bai S, Goldman-Yassen AE, Joiner CH, Brown RC and Buckley EM (2022) Quantifying the Cerebral Hemometabolic Response to Blood Transfusion in Pediatric Sickle Cell Disease With Diffuse Optical Spectroscopies. Front. Neurol. 13:869117. doi: 10.3389/fneur.2022.869117

Red blood cell transfusions are common in patients with sickle cell disease who are at increased risk of stroke. Unfortunately, transfusion thresholds needed to sufficiently dilute sickle red blood cells and adequately restore oxygen delivery to the brain are not well defined. Previous work has shown that transfusion is associated with a reduction in oxygen extraction fraction and cerebral blood flow, both of which are abnormally increased in sickle patients. These reductions are thought to alleviate hemometabolic stress by improving the brain's ability to respond to increased metabolic demand, thereby reducing susceptibility to ischemic injury. Monitoring the cerebral hemometabolic response to transfusion may enable individualized management of transfusion thresholds. Diffuse optical spectroscopies may present a low-cost, non-invasive means to monitor this response. In this study, children with SCD undergoing chronic transfusion therapy were recruited. Diffuse optical spectroscopies (namely, diffuse correlation spectroscopy combined with frequency domain near-infrared spectroscopy) were used to quantify oxygen extraction fraction (OEF), cerebral blood volume (CBV), an index of cerebral blood flow (CBF_i), and an index of cerebral oxygen metabolism ($CMRO_{2i}$) in the frontal cortex immediately before and after transfusion. A subset of patients receiving regular monthly transfusions were measured during a subsequent transfusion. Data was captured from 35 transfusions in 23 patients. Transfusion increased median blood hemoglobin levels (Hb) from 9.1 to 11.7 g/dL ($p < 0.001$) and decreased median sickle hemoglobin (HbS) from 30.9 to 21.7% ($p < 0.001$). Transfusion decreased OEF by median 5.9% ($p < 0.001$), CBFi by median 21.2% ($p = 0.020$), and CBV by median 18.2% ($p < 0.001$). $CMRO_{2i}$ did not statistically change from pre-transfusion levels ($p > 0.05$). Multivariable analysis revealed varying degrees of

associations between outcomes (i.e., OEF, CBF_i, CBV, and $CMRO_{2i}$), Hb, and demographics. OEF, CBF_i, and CBV were all negatively associated with Hb, while $CMRO_{2i}$ was only associated with age. These results demonstrate that diffuse optical spectroscopies are sensitive to the expected decreases of oxygen extraction, blood flow, and blood volume after transfusion. Diffuse optical spectroscopies may be a promising bedside tool for real-time monitoring and goal-directed therapy to reduce stroke risk for sickle cell disease.

Keywords: diffuse correlation spectroscopy (DCS), near-infrared spectroscopy, sickle cell disease (SCD), blood transfusion, cerebral hemometabolics, cerebral oxygen extraction fraction, frequency-domain near-infrared spectroscopy, cerebral blood flow

INTRODUCTION

Sickle cell disease (SCD) is an autosomal recessive blood disorder affecting millions of people worldwide, with ∼300,000 newborns diagnosed each year (1). In SCD, a single gene mutation causes production of abnormal hemoglobin, hemoglobin S (HbS) (1). In addition to impaired oxygen-carrying ability, HbS rapidly forms intracellular polymers in the deoxygenated state, distorting red blood cells (RBC) into a characteristic sickle shape (1). Sickling, decreased RBC deformability, and increased cell aggregation and hemolysis, result in increased blood viscosity and decreased microvascular perfusion (1–3). The culmination of impaired oxygen-carrying capacity and altered blood flow dramatically increases the incidence of vascular occlusion and stroke in patients with sickle cell disease (1).

Currently, routine screening with transcranial Doppler ultrasound (TCD) is employed to minimize stroke risk in children with sickle cell disease (4). Patients with persistently elevated TCD blood flow velocities of the large feeding arteries to the brain are placed on chronic transfusion therapy (CTT) to reverse metabolic stress caused by anemia and to prevent vaso-occlusion and stroke (5). CTT involves periodic RBC transfusion to dilute sickle red blood cells (sRBC) with the general goal of maintaining HbS <30% and hemoglobin (Hb) >10 g/dL (5). CTT has drastically reduced the incidence and severity of both overt stroke and silent infarction (92 and 58% relative risk reduction, respectively) (6, 7). However, recurrent infarctions occur in 23–45% of patients receiving CTT, despite successful maintenance of clinical thresholds for HbS and Hb (8–10), suggesting that current standardized CTT guidelines are insufficient for infarct prevention in a substantial fraction of patients.

Transfusion is associated with a reduction in oxygen extraction fraction (OEF) and cerebral blood flow (CBF), both of which are abnormally increased in SCD patients due to reduced arterial oxygen content (C_aO_2) (11, 12). These reductions are thought to alleviate hemometabolic stress by improving the brain's ability to respond to increased metabolic demand, thereby reducing susceptibility to ischemic injury (11, 12). Monitoring the cerebral hemometabolic response to transfusion may enable individualized management of transfusion thresholds, with the goal of sufficiently restoring cerebrovascular reserve by diluting or replacing sickle red blood cells. In patients for whom CTT does not adequately address metabolic needs, such monitoring may be used to identify those who would benefit from more aggressive therapies (e.g., bone marrow transplant) (12, 13).

Magnetic resonance imaging (MRI) provides excellent, high-resolution images of cerebral blood flow and oxygen extraction fraction that have been used in recent years to elucidate mechanisms underlying regional vulnerabilities to injury in patients with SCD (11, 12, 14). Indeed, recent work has demonstrated the influence of CTT on relieving hemometabolic stress in SCD patients (11, 12). However, MRI is prohibitively expensive for routine monitoring, requires sedation in children < 6 y, and quantitative differences exist across scanners and software platforms. Thus, its utility in individualizing CTT management in SCD patients is limited.

Diffuse optical spectroscopies (DOS) present a low-cost, non-invasive alternative to current neuroimaging techniques like MRI. By operating in the near-infrared, DOS techniques exploit the spectral window of low absorption that exists in tissue. Photons emitted from a source placed on the tissue surface propagate several centimeters (through the scalp and skull) before reemission at the tissue surface. The properties of the detected light can be related to hemodynamic properties of the interrogated tissue, including hemoglobin oxygen saturation, blood flow, blood volume, and oxygen metabolism. While limited in depth sensitivity to the superficial cortex, DOS offers numerous advantages to other neuroimaging techniques, including portability, ease of measurements, and cost. Indeed, a handful of studies have employed a type of DOS known as continuous-wave near-infrared spectroscopy (CW-NIRS) to demonstrate the technology is sensitive to expected increases in cerebral oxygen saturation caused by transfusion (15–18). However, to date, these studies have been limited to commercially available CW-NIRS systems. While useful as a trend monitor, CW-NIRS have been shown to produce substantial inaccuracies in estimations of absolute oxygen saturation (19, 20). Thus, although these studies have observed expected trends in hemoglobin oxygen saturation following transfusion, the magnitude of these increases is likely significantly underestimated (19, 20).

Herein we employ two DOS techniques known as frequency-domain NIRS (FDNIRS) and diffuse correlation spectroscopy (DCS) to monitor the effects of blood transfusion on the brain in a cohort of children with sickle cell disease receiving CTT.

FDNIRS is an alternative to CW-NIRS that has been shown to more accurately estimate hemoglobin oxygen saturation, while also providing a measurement of cerebral blood volume (CBV). DCS is a type of DOS used to measure cerebral blood flow (CBF). The combination of oxygen saturation from FDNIRS and CBF from DCS also enables us to estimate the cerebral metabolic rate of oxygen ($CMRO_2$) using Fick's law (21). We hypothesize that FDNIRS combined with DCS (FDNIRS/DCS) can non-invasively quantify changes in markers of cerebral hemometabolic stress at the bedside following transfusion. Specifically, we hypothesize that FDNIRS/DCS can detect expected decreases in CBF, CBV, and OEF post-transfusion, while $CMRO_2$ will remain unchanged. Further, we hypothesize that CBF, CBV, and OEF will be inversely associated with hemoglobin.

METHODS

Patient Population

Children ages 2 to 18 years old with SCD (HbSS or HbS thalassemia) receiving chronic transfusion therapy were recruited for this study. RBC transfusions were administered by simple infusion, partial manual exchange, or apheresis following our institution's standard practice guidelines for CTT. Volume of transfusion was calculated to target a post-transfusion venous hemoglobin (Hb) of ∼11 g/dL based on Hb levels measured between 1–72 h (mean 5 h) prior to transfusion. Patients were excluded on the basis of: significant illness within one month of participation, hypertension, neurologic disorder not related to SCD (e.g., seizures), Moyamoya or previous revascularization surgery, prior history of major head injury requiring a visit to an emergency department, and/or prior curative therapy. All patients and/or their legal guardians provided informed assent/consent. The study was approved by the Institutional Review Board of Emory University.

Clinical and Laboratory Data

Complete blood count [hematocrit and hemoglobin (Hb)] and hemoglobin electrophoresis (Hb S%, HbA%) were performed on a venous blood sample obtained < 72 h before transfusion. Measurements were repeated immediately after transfusion. Transcutaneous oxygen saturation (SpO_2, %) and heart rate (HR, bpm) were measured with pulse oximetry on the index finger immediately before and after transfusion. Non-invasive cuff blood pressure was also measured before and after transfusion. C_aO_2 (mL/dL) was estimated from SpO_2 and Hb, $C_aO_2 = 1.39 \times SpO_2 \times Hb$.

FDNIRS/DCS Experimental Protocol and Analysis

To assess the cerebral hemometabolic effects of transfusion, brief (∼15 min) FDNIRS/DCS measurements were obtained within 2 h prior to the start of transfusion and were repeated at the cessation of transfusion. Due to limitations in software acquisition capabilities with our current system, FDNIRS and DCS data sets were taken sequentially, not simultaneously. For each measurement, we manually held an optical sensor over the right and left frontal cortex for 3–5 s intervals. The sensor was repositioned three times per hemisphere per optical modality (FDNIRS, DCS). After confirming that there were no significant hemispheric differences, all repetitions were averaged to yield a global mean of each measured parameter.

In the following sections, we detail the instrumentation utilized as well as the data analysis pipeline for both FDNIRS and DCS.

Frequency-Domain Near-Infrared Spectroscopy (FDNIRS)

FDNIRS enables quantification of oxy- and deoxy-hemoglobin concentrations (HbO and HbR, respectively, μM) of the interrogated tissue. In this study, we employed a customized FDNIRS system (Imagent, ISS Inc.) with eight near-infrared laser diode sources (690, 730, 750, 775, 785, 800, 825, and 830 nm) modulated at 110 MHz and rapidly multiplexed at 20.8 Hz, along with four photomultiplier tube detectors with gain modulation of 110 MHz + 5 kHz for heterodyne detection at 5 kHz. The patient interface consisted of a 3D-printed rigid black sensor containing five 2.5 mm fiber bundles (50 μm multimode fibers, NA = 0.66, FTTIIG23767, FiberOptics Technology, Pomfret, Connecticut), one of which was used as the source, and the other four of which were used as detectors spaced 2.0, 2.5, 3.0, and 3.5 cm from the source.

For FDNIRS data acquisition, AC amplitude and phase data for each wavelength, λ, were acquired for 3-5s at 20Hz. Measured AC amplitude attenuation, AC(r, λ), and phase shift, θ(r, λ), at each separation, r, and wavelength, λ, were first averaged over the 3–5 s acquisition interval and discarded if the phase standard deviation exceeded 5° or if the AC coefficient of variation was $> 10\%$. Averaged data were then fit to the semi-infinite solution to the diffusion equation to extract wavelength-dependent reduced scattering and absorption coefficients ($\mu_s'(\lambda)$ and $\mu_a(\lambda)$, respectively) (22, 23). This approximation assumes a linear relationship between $\ln(AC(r, \lambda) \times r^2)$ and $\theta(r, \lambda)$ vs. r. To ensure the data fit this linear model, we calculated Pearson's correlation coefficient, R, for both of these relationships, and we discarded data for a given λ in which $R^2 < 0.97$. The entire dataset was discarded if < 5 wavelengths passed this linear fit criterion or if the slope of $\mu_s'(\lambda)$ vs. λ was > 0.

Estimates of HbO and HbR were obtained by fitting $\mu_a(\lambda)$ vs. λ to the hemoglobin spectrum (24). Next, HbO and HbR were used to derive total hemoglobin concentration (HbT = HbO + HbR), hemoglobin oxygen saturation ($SO_2 = HbO/HbT \times 100\%$), oxygen extraction fraction ($OEF = (SpO_2 - SO_2)/(\gamma \times SpO_2)$, and cerebral blood volume [$CBV = MW \times HbT/(D_{bt} \times Hb)$]. Here, γ is the fraction of blood volume within the probed venous compartment of the tissue (assumed to be 1 for all subjects for simplicity), MW = 64,500 g/mol is the molecular weight of hemoglobin, and D_{bt} is the brain tissue density (assumed to be 1.05 g/mL).

Additionally, because DCS data were obtained at 852 nm to quantify blood flow, we extrapolated FDNIRS-measured μ_s' and μ_a to 852 nm. To estimate $\mu_s'(852nm)$, a linear model was fit to $\mu_s'(\lambda)$ vs. λ and extrapolated to 852 nm. To estimate $\mu_a(852nm)$,

we used the following formula:

$$\mu_a\,(852nm) = \varepsilon_{HbO}\,(852nm) \times HbO + \varepsilon_{HbR}\,(852nm) \times HbR + 0.75 \times \mu_{a,water}\,(852nm) \quad (1)$$

where HbO and HbR were obtained from FDNIRS ε_{HbO} and ε_{HbR} are the extinction coefficients of oxy- and deoxyhemoglobin, and the factor of 0.75 is the assumed water content of the tissue.

Diffuse Correlation Spectroscopy

Diffuse correlation spectroscopy quantifies temporal fluctuations in reflected light intensity measured by a detector located some fixed distance away from a near-infrared light source placed on the tissue surface. These intensity fluctuations, which are caused by the motion of red blood cells, can be used estimate an index of blood flow in the underlying tissue. The DCS system employed herein consisted of an 852 nm long-coherence laser (iBeam Smart, TOPTICA Photonics, Farmington, New York), two four-channel single photon counting modules (SPCMQA4C-IO, Perkin-Elmer, Quebec, Canada), an eight-channel hardware correlator (Flex05-8ch, New Jersey), and a counter/timer data acquisition board (PCIe-6612, National Instruments) (25). Detected photon counts were used to estimate an intensity autocorrelation function, $g_2(\tau)$, either *via* the hardware correlator or with a custom software correlator. For the first 26 transfusion events, we used the hardware correlator; for the remaining transfusion events we transitioned to the software correlator to increase versatility of data acquisition [e.g., acquisition rate, temporal resolution of $g_2(\tau)$]. *In vitro* phantom validations were used to confirm that the hardware correlator and the software correlator yielded the same flow index, as demonstrated by Wang *et al.* (25).

The patient interface for DCS measurements consisted of a black sensor containing one 800 μm multimode source fiber (FT800EMT, Thorlabs) and seven single-mode detector fibers (780-HP, Thorlabs) bundled together and spaced 2.5 cm from the source. Compliance with the American National Standards Institute (ANSI) maximum permissible exposure of skin to laser radiation (< 4 mW/mm^2 at 852 nm) was ensured by utilizing a 5mm right-angle prism (MRA03-E03, Thorlabs) to couple light from the source fiber to the tissue surface, thereby yielding a spot size radius >2 mm, and by adjusting the laser power so that the output at the tissue surface was <50 mW.

For DCS data acquisition, hardware correlator data was acquired at 1Hz. Software correlator data was acquired at 20 Hz and downsampled by averaging to 1 Hz prior to analysis for consistency. Thus, each measurement repetition consisted of 3–5 data points. Data points with intensity $<$ 6 kHz were excluded from analysis to avoid $g_2(\tau)$ with poor signal-to-noise ratio (SNR).

To estimate CBF_i, the measured intensity autocorrelation functions, $g_2(\tau)$, were first averaged across the 7 detector channels to improve SNR. Averaged curves were fit to the semi-infinite solution of the correlation diffusion equation to derive CBF_i (24). When FDNIRS data was available, measured μ_a and μ_s' at 852 nm were incorporated into the fit for CBF_i. When unavailable (as was the case in 2 measurements), μ_a and μ_s' were assumed to be 0.16 cm^{-1} and 8.4 cm^{-1}, respectively (26). Fits were discarded if $\epsilon > 14\%$ where $\epsilon = \sum_i (g_{2,\,fit}(\tau_i) - g_{2,\,meas}(\tau_i))/g_{2,\,fit}(\tau_i) \times 100\%$. As a final quality control step, repetitions with $<$ 3 data points were discarded, and entire measurement sessions were discarded if $<$ 2/6 repetitions failed to meet quality control criteria.

To estimate $CMRO_{2i}$, we employed a derivative of Fick's law that relates $CMRO_{2i}$ to measurable quantities by

TABLE 1 | Patient characteristics ($n = 35$).

Age (y)	13.9 (10.7, 15.9)
Height (cm)	158.0 (147.8, 161.9)
Weight (kg)	55.7 (42.4, 69.6)
Type of transfusion, no. (%)	
Simple	30 (85.7 %)
Partial exchange	4 (11.4 %)
Apheresis	1 (2.8 %)
Hydroxyurea, no. (%)	8 (22.9 %)
Duration on CTT, (mo)	16.1 (8.3, 21.3)

Data are reported as median (interquartile range) unless stated otherwise.

TABLE 2 | Effects of transfusion on hemoglobin and patient vitals.

	Pre-transfusion	Post-transfusion	Difference in Post- and Pre-transfusion	*p*
Hb (g/dL)	9.1 (8.3, 10.0), 35	11.7 (11.3, 12.3), 31	2.9 (1.8, 3.3), 31	**< 0.001**
HbA (%)	61.9 (51.1, 71.2), 34	74.2 (65.8, 81.8), 28	10.5 (7.9, 17.2), 27	**< 0.001**
HbS (%)	30.9 (22.5, 39.7), 34	21.7 (15.3, 27.2), 28	−8.0 (−14.3, −6.8), 27	**< 0.001**
C_aO_2 (mL/dL)	12.4 (11.6, 13.8), 35	16.3 (15.5, 17.1), 31	4.0 (2.5, 4.6), 31	**< 0.001**
SpO_2 (%)	100 (99, 100), 35	100 (99, 100), 35	0.0 (0.0, 0.8), 35	0.75
HR (bpm)	91.0 (79.3, 98.5), 35	82.0 (73.3, 90.9), 35	−8.0 (−15.0, −2.3), 35	**< 0.001**
MAP (mmHg)	80.3 (76.4, 86.9), 35	80.0 (74.3, 85.4), 35	−3.0 (−7.7, 5.3), 35	0.29

Data are reported as median (interquartile range), count of non-missing values. p-values were obtained from a two-sided Wilcoxon signed-rank test. Hb, hemoglobin; HbA, hemoglobin A; HbS, hemoglobin S; CaO_2, arterial oxygen concentration; SpO_2, transcutaneous oxygen saturation; HR, heart rate; MAP, mean arterial blood pressure. The bold values indicate the value of $p < 0.05$ which are statistically significant.

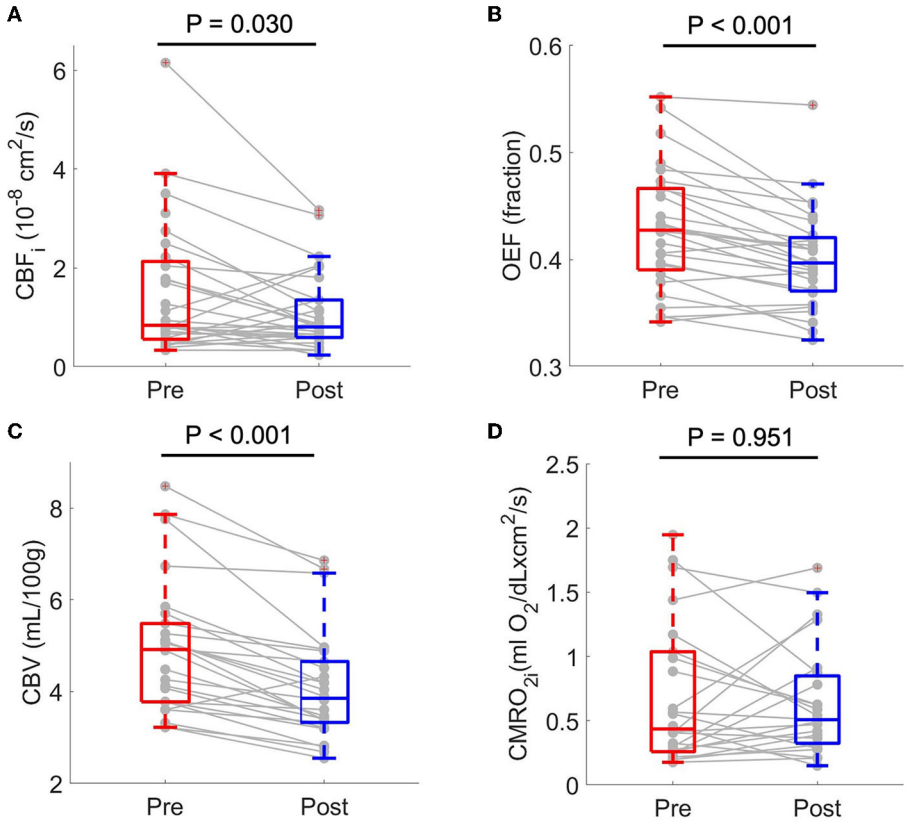


FIGURE 1 | Boxplots of paired pre- and post-transfusion cerebral blood flow index, CBF_i **(A)** oxygen extraction fraction, OEF **(B)** cerebral blood volume, CBV **(C)** and cerebral metabolic rate of oxygen index, $CMRO_{2i}$ **(D)**. For each boxplot, the central line denotes the median and the bottom and top edges of the box indicate the 25th and 75th percentiles, respectively. The whiskers extend to the most extreme data points not considered outliers. Individual connected dots represent each pre- and post-transfusion matched pair. *p*-values were obtained from two-sided paired Wilcoxon signed rank tests. $CMRO_{2i}$ scaled by a factor of 10^7.

TABLE 3 | Effects of transfusion on blood flow, oxygen extraction, blood volume, and oxygen metabolism.

	Pre-transfusion	Post-transfusion	Relative change (%)	*p*
CBF_i (10^{-8} cm^2/s)	0.84 (0.56, 2.13), 32	0.82 (0.60, 1.35), 30	−21.5 (−46.5, 16.1), 29	**0.030**
OEF (fraction)	0.43 (0.40, 0.47), 32	0.40 (0.37, 0.42), 30	−5.9 (−10.1, −2.9), 28	**<0.001**
CBV (mL/100g)	4.7 (3.8, 5.4), 32	3.9 (3.3, 4.6), 28	−18.2 (−22.8, −10.2), 26	**<0.001**
$CMRO_{2i}$	0.46 (0.28, 1.15), 31	0.53 (0.34, 0.90), 24	13.8 (−36.3, 82.3), 23	0.951

Data are reported as median (interquartile range), count of non-missing values. p-values were obtained from a two-sided Wilcoxon signed-rank test. CBFi, cerebral blood flow index; OEF, oxygen extraction fraction; CBV, cerebral blood volume; $CMRO_{2i}$, cerebral metabolic rate of oxygen, in units of 10^{-7} mL O_2/dL × cm^2/s. The bold values indicate the value of p < 0.05 which are statistically significant.

assuming a compartmentalized model of the vasculature (24, 27):

$$CMRO_{2i} = OEF \times CBF_i \times C_aO_2 \quad (2)$$

Statistical Analysis

Primary analyses were performed at the transfusion level, with a total of 35 transfusions. Summary statistics are expressed as median (interquartile range: 1st quartile, 3rd quartile) for continuous measurements and count (percentage) for categorical measurements. Due to the relatively small sample size, non-parametric Wilcoxon signed-rank test for paired data was used to compare pre- and post-transfusion measures. Linear mixed-effects regression models with random-intercept at the transfusion level were used to examine the association of each outcome measure (OEF, CBF_i, CBV, and $CMRO_{2i}$) with demographical (sex and age) and laboratory data (Hb, HbA and HbS). These models were two-level and accounted for repeated measures (pre- and post-transfusion measurements) nested within the same transfusion event. Selection of predictors in the final multivariable model was performed by backward selection using marginally significant predictors from bivariable results

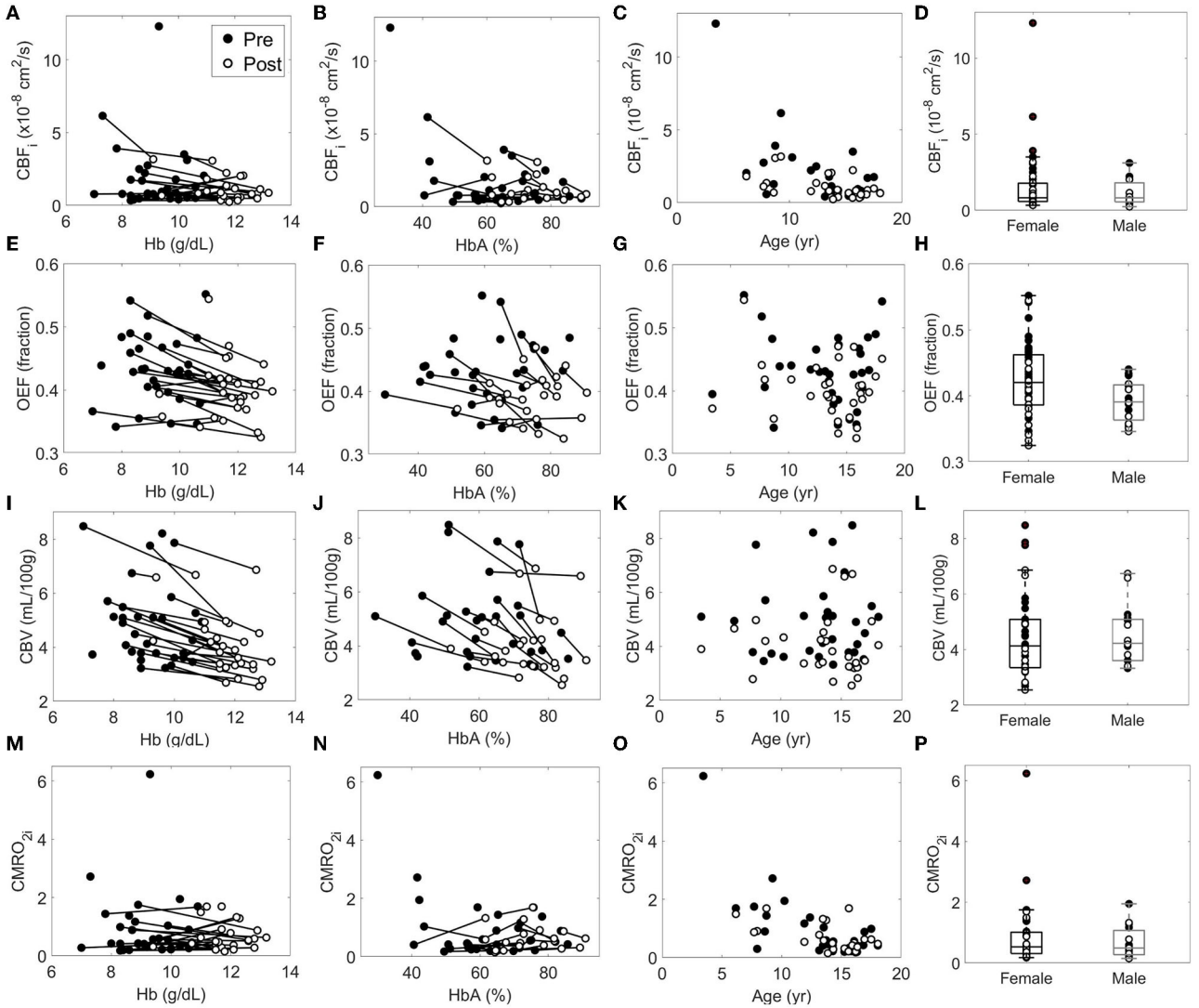


FIGURE 2 | Relationship between each cerebral hemometabolic parameter (**A–D**: CBF_i, **E–H**: OEF, **I–L**: CBV and **M–P**: $CMRO_{2i}$) and hemoglobin (Hb), hemoglobin A (HbA), age, and sex. In each subplot, solid circles represent pre-transfusion data and hollow circles represent post-transfusion data. Solid lines connect pairs of pre- and post-transfusion data from a single measurement session; solid lines were not included for age and gender panels to aide in visualization, as neither age nor sex changed during pre-post transfusion. $CMRO_{2i}$, cerebral metabolic rate of oxygen, in units of 10^{-7} mL O_2/dL × cm^2/s.

with a threshold of p-value < 0.1. We used variance inflation factors to assess collinearity among predictors included in multivariable models and found a certain degree of collinearity among Hb, HbA, and HbS. In these scenarios, Hb was chosen as the representative predictor, since Hb is generally accepted as the clinical parameter to manage RBC transfusion volume. When the slope estimate of a certain predictor was a small decimal number with too many trailing zeros, the predictor was linearly scaled by a simple conversion (e.g., 0.1×HbS) to produce an easily readable and interpretable slope estimate. As a secondary analysis, a three-level linear mixed-effect model was fit for the 10 patients who had longitudinal measurements from repeated transfusions, where pre- and post-transfusion were nested within each transfusion event, and multiple transfusions were nested within the patient. Due to the limited sample size, results from this analysis are for preliminary information only and should be6g interpreted with care. Further, ordinary least square regression was used to examine the association of the relative change in each outcome measure (rOEF, $rCBF_i$, rCBV, and $rCMRO_{2i}$) with demographical data (sex and age) and changes in laboratory data from pre- to post-transfusion (dHb, dHbA and dHbS). Relative changes in outcome measures were defined as the percent change in outcome measure from pre- to post-transfusion (e.g., rOEF = (OEF_{post} –

TABLE 4 | Bivariable and multivariable analysis of factors influencing cerebral hemometabolic parameters.

	Bivariable		Multivariable			
	Est (95% CI)	***p***	**Est (95% CI)**[a]	***p***	**Est (95% CI)**[b]	***p***
CBF$_i$						
Age (y)	−0.35 (−0.49, −0.21)	**<0.001**	−0.35 (−0.49, −0.22)	**<0.001**	−0.31 (−0.45, −0.18)	**<0.001**
Sex	0.17 (−1.49, 1.82)	0.847	-		-	
Hb	−0.13 (−0.26, 0.00)	0.053	−0.14 (−0.27, −0.01)	**0.040**	-	
HbA% (×0.1)	−0.36 (−0.67, −0.09)	**0.011**	-		−0.29 (−0.56, −0.04)	**0.028**
HbS% (×0.1)	0.38 (0.08, 0.72)	**0.016**	-		-	
OEF						
Age (y, ×0.01)	−0.17 (−0.65, 0.31)	0.493	-		-	
Sex	0.04 (0.00, 0.07)	0.059	0.03 (−0.01, 0.07)	0.107	0.04 (0.00, 0.08)	**0.045**
Hb (×0.1)	−0.11 (−0.14, −0.08)	**<0.001**	−0.11 (−0.14, −0.07)	**<0.001**	-	
HbA% (×0.01)	−0.11 (−0.20, −0.02)	**0.008**	-		−0.12 (−0.20, −0.03)	**0.005**
HbS% (×0.01)	0.11 (0.00, 0.21)	**0.024**	-		-	
CBV						
Age (y, ×0.1)	−0.15 (−1.45, 1.14)	0.818	-		-	
Sex	0.03 (−1.03, 1.08)	0.961	-		-	
Hb	−0.33 (−0.41, −0.25)	**<0.001**	-		-	
HbA% (×0.1)	−0.49 (−0.70, −0.27)	**<0.001**	-		-	
HbS%(×0.1)	0.51 (0.23, 0.76)	**0.002**	-		-	
CMRO$_{2i}$						
Age (y)	−0.21 (−0.29, −0.14)	**<0.001**	-		-	
Sex	0.27 (−0.64, 1.17)	0.570	-		-	
Hb (×0.1)	−0.10 (−0.74, 0.51)	0.745	-		-	
HbA% (×0.01)	−0.21 (−2.00, 1.11)	0.741	-		-	
HbS% (×0.01)	0.06 (−1.37, 1.88)	0.933	-		-	

CBFi, cerebral blood flow index; Hb, hemoglobin; HbA%, percent hemoglobin A out of total hemoglobin; HbS%, percent hemoglobin S out of total hemoglobin; OEF, oxygen extraction fraction; CBV, cerebral blood volume; CMRO$_{2i}$, cerebral metabolic rate of oxygen. Hb variables (Hb, HbA %, HbS % were not modeled together due to multicollinearity; [a]Multivariable models considering Hb with other predictors; [b]Multivariable models considering HbA with other predictors. The bold values indicate the value of $p < 0.05$ which are statistically significant.

OEF_{pre})/OEF_{pre} × 100%); changes in laboratory data were defined as the difference in laboratory data from pre- to post- transfusion (e.g., dHb = Hb_{post} − Hb_{pre}). All statistical analyses were performed with CRAN R (version 3.4.1, R Foundation). Significance was assessed at the 0.05 level.

RESULTS

Patient Characteristics

A total of 23 children with HbSS on chronic transfusion therapy were enrolled. Patients were mostly female (17/23, 73.9%). Of these 23 patients, 8 (34.8%) were monitored on 2 separate transfusions, and 2 (8.7%) were monitored on 3 separate transfusions. Each follow-up measurement was performed > 6 months after the prior transfusion. In total, we measured 35 transfusion events. Of these 35 transfusions, 30 (85.7%) were simple, 4 (11.4%) were partial exchange, and one (2.9%) was apheresis (**Table 1**). At the time of transfusion, patients had a median (IQR) age of 13.9 (10.7, 15.9) years, and 23% were reported to be receiving hydroxyurea therapy.

Effect of Transfusion on Hb and Patient Vitals

Tranfusion significantly increased Hb by median 2.9 (IQR: 1.8, 3.3) g/dL ($p < 0.001$), HbA by median 10.5 (7.9, 17.2) % ($p < 0.001$), and decreased HbS by median 8.0 (14.3, 6.8) % ($p < 0.001$, **Table 2**). Heart rate decreased significantly by median 8.0 (15.0, 2.3) bpm ($p < 0.001$) after transfusion, consistent with the known adaptive response from improved oxygen delivery and cardiac output. SpO_2 and MAP remained statistically unchanged.

Effect of Transfusion on Cerebral Hemometabolics

Out of 70 FDNIRS/DCS measurements, 62 FDNIRS (89%) and 62 DCS (89%) measurements passed quality control criteria to be included in analysis. Transfusion was associated with significant decreases in CBF_i, OEF, and CBV. CBF_i decreased by median 21.5 (46.1, −16.1) % ($p = 0.030$), OEF decreased by median 5.9 (10.1, 2.9) % ($p < 0.001$), and CBV decreased by median 18.2 (22.8, 10.2) % ($p < 0.001$). $CMRO_{2i}$ did not statistically change in response to transfusion ($p > 0.05$, **Figure 1**, **Table 3**). An outlier in both CBF_i and $CMRO_{2i}$ made pre-/post- transfusion trends difficult to visualize in the subplots of

TABLE 5 | Bivariable analysis of factors influencing changes in cerebral hemometabolic parameters.

	Bivariable	
	Est (95% CI)	***p***
rCBF$_i$		
Age (y)	−3.13 (−8.97, 2.71)	0.598
Sex	−75.2 (−101.09, −49.31)	**0.007**
dHb (g/dL)	−8.34 (−21.23, 4.55)	0.523
dHbA%	2.74 (0.15, 5.33)	0.303
dHbS%	−2.29 (−5.01, 0.42)	0.408
rOEF		
Age (y)	−0.54 (−0.83, −0.25)	0.074
Sex	−3.64 (−5.95, −1.33)	0.127
dHb (g/dL)	−2.65 (−3.60, −1.70)	**0.010**
dHbA%	0.12 (−0.11, 0.25)	0.589
dHbS%	−0.13 (−0.37, 0.11)	0.571
rCBV		
Age (y)	0.27 (−0.36, 0.90)	0.671
Sex	−10.03 (−14.64, −5.39)	**0.040**
dHb (g/dL)	−0.73 (−0.96, −0.57)	**<0.001**
dHbA%	0.23 (−0.12, 0.58)	0.510
dHbS%	−0.38 (−0.74, −0.02)	0.305
rCMRO$_{2i}$		
Age (y)	−3.13 (−8.97, 2.71)	0.598
Sex	−73.36 (−111.26, −35.46)	0.067
dHb (g/dL)	−6.66 (−23.91, 10.59)	0.703
dHbA%	3.65 (0.04, 7.26)	0.325
dHbS%	−3.33 (−7.07, 0.40)	0.384

rCBFi, relative % change in cerebral blood flow index; dHb, delta hemoglobin; dHbA%, delta percent hemoglobin A out of total hemoglobin; dHbS%, delta percent hemoglobin S out of total hemoglobin; rOEF, relative % change in oxygen extraction fraction; rCBV, relative % change in cerebral blood volume; rCMRO$_{2i}$, relative % change in cerebral metabolic rate of oxygen. The bold values indicate the value of p < 0.05 which are statistically significant.

Figure 2. **Supplementary Figure 1** shows this same data with outliers removed. Note, these outliers were included in all analyses; secondary analysis performed by removing outliers did not change results.

Bivariable relationships between predictors (Hb, HbA, HbS, age, and sex) and cerebral hemometabolic outcomes (CBF_i, OEF, CBV, and $CMRO_{2i}$) are visualized in **Figure 2**, and linear regression models for bivariable and multivariable relationships are presented in **Table 4**. Bivariably, CBF_i was inversely associated with age ($p < 0.001$), HbA ($p = 0.011$), and HbS ($p = 0.016$). A marginally significant inverse association between CBF_i and Hb was also observed ($p = 0.053$). In multivariable models, age and Hb, as well as age and HbA, remained significant independent predictors of CBF_i. For OEF, significant inverse associations were observed with Hb ($p < 0.001$) and HbA ($p = 0.008$); additionally, OEF was significantly positively associated with HbS ($p = 0.024$). OEF was marginally significantly higher in females than in males ($p = 0.059$). In multivariable analysis, Hb, as well as sex and HbA, remained significant independent predictors of OEF (Hb, HbS and HbA were not modeled together due to multicollinearity). For CBV, bivariable analysis revealed significant inverse associations with Hb ($p < 0.001$) and HbA ($p < 0.001$) and a positive association with HbS ($p = 0.002$). For $CMRO_{2i}$, age was the only significant correlate ($p < 0.001$). While the current analysis employed 2-level hierarchical models at the transfusion-level, nesting pre-post observations within transfusions, we also performed a sensitivity analysis wherein repeated transfusions were also nested within patients, resulting in 3-level hierarchical models. The only meaningful change in the results from **Table 4** observed in this 3-level analysis was a significant inverse association between HbA and $CMRO_{2i}$ ($p = 0.023$, **Supplementary Table 1**).

Finally, bivariable models were used to investigate the relationship between predictor variables and relative changes in each cerebral hemometabolic outcome from pre- to post-transfusion (**Table 5**, **Figures 3A-D**). Relative change in OEF was inversely associated with dHb ($p = 0.010$, **Figure 3B**). Similarly, relative change in CBV was also inversely associated with dHb ($p < 0.001$, **Figure 3C**). Relative change in CBF_i and CBV were significantly higher in males versus females ($p = 0.007$ and 0.040, respectively).

DISCUSSION

Herein, we demonstrate the feasibility of using diffuse optical spectroscopies, specifically FDNIRS and DCS, as a low-cost and non-invasive means to evaluate changes in oxygen extraction fraction, cerebral blood flow, blood volume, and oxygen metabolism after transfusion in children with sickle cell disease. We found that FDNIRS/DCS measurements of OEF, CBF_i, and CBV were significantly lower post-transfusion than pre-transfusion, while no significant changes were observed in $CMRO_{2i}$ (**Figure 1**). These observations are consistent with prior neuroimaging studies using MRI and positron emission tomography that demonstrate significant whole-brain decreases in OEF, CBF, and CBV following transfusion (11, 12). Further, OEF, CBF_i, and CBV were significantly inversely associated with hemoglobin concentration, and these associations persisted after accounting for the influence of age and sex (**Table 4**). While our changes in OEF largely agree with previous reports (11, 12), the decreases in CBF_i that we measured were larger than expected (26). We hypothesize that the origin of these differences is due to the confounding influence of hematocrit (28) and work is ongoing to develop correction factors that account for this influence.

As expected, when regressed against all cerebral hemometabolic outcome variables, age was significantly inversely correlated with CBF_i (**Figure 2C**, bivariable and multivariable analyses, **Table 4**) and $CMRO_{2i}$ (**Figure 2O**, bivariable analysis, **Table 4**). However, the magnitude of the decreases in CBF_i and $CMRO_{2i}$ seen with age are large compared to previous reports (29–33). These exaggerated changes may be due to age-related changes that alter extracerebral signal contributions to the measured optical signal, e.g.,

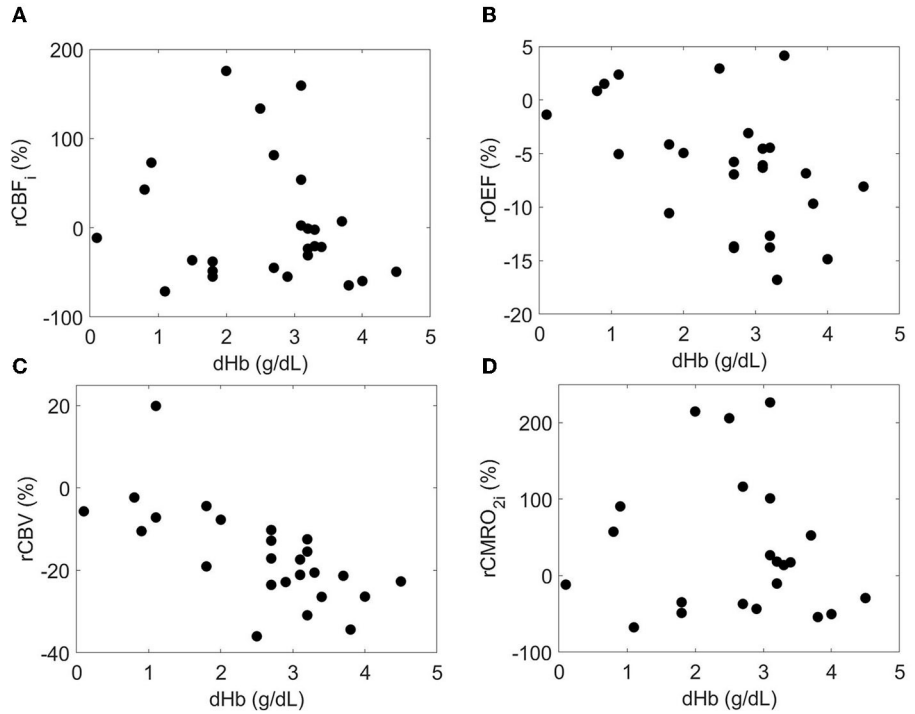


FIGURE 3 | Relationship between the relative each cerebral hemometabolic parameter due to transfusion (**A**: $rCBF_i$, **B**: rOEF, **C**: rCBV and **D**: $rCMRO_{2i}$) and the change in hemoglobin (dHb).

increases in scalp/skull thickness, vessel density, and possibly increased concentration of hemoglobin production in skull bone marrow (34).

We note that we found sex was a significant predictor of OEF after adjusting for HbA (**Table 4**), with females having a higher OEF than males, and that sex was significantly associated with relative changes in both CBF and CBV due to transfusion with males having a higher relative change than females (**Table 5**). However, these correlations have not been reported in previous studies of children with sickle cell disease (11, 12, 35) and may be an artifact of the relatively small number of males in our cohort.

Finally, we note several limitations of this study. First, hemispheric data was averaged, resulting in a single, whole-brain value for each cerebral hemometabolic marker. While this approach was justified given that no significant differences were observed between hemispheres for the cohort on a whole, marked hemispheric asymmetry was observed in a small subset of patients. Future work with an expanded cohort will explore the complex relationship between vasculopathy and asymmetrical response to transfusion. Second, for subject convenience, post-transfusion measurements were performed immediately after transfusion, which likely missed post-transfusion equilibrium. While previous studies have shown that the most significant changes in cerebral tissue oxygen saturation occur within the first 30 min to 1 h of transfusion start (17, 36), measuring prior to equilibrium could potentially blunt changes in measured cerebral hemometabolic markers (17). Third, our optical measurements are limited in sensitivity to the frontal cortex. While data from MRI suggest the effects of transfusion are global and uniform within gray matter (35), we are unable to directly comment on the whole-brain hemometabolic response to transfusion. Fourth, 11% of data was discarded due to motion artifact and/or poor SNR. With improvements in real-time analysis to ensure adequate data quality at the bedside, we anticipate that this discard rate can be dramatically reduced. Fifth, both our FDNIRS and DCS analyses make the first-order assumption that the head is a homogenous medium of uniform optical properties/flow dynamics. This oversimplification neglects the influence of extracerebral layers (skull, scalp, cerebrospinal fluid), which can be appreciable (37) and, in the case of this cohort, may be variable given the range of ages (2–18y). While the consistency of our results with those of other neuroimaging modalities suggest that this influence is not insurmountable, minimizing these contributions either through advanced analytical modeling (37–39) or through hardware approaches that isolate photons that have traveled deeper in tissue (40, 41) will be an important future step toward the clinical translation of NIRS/DCS. Finally, when estimating OEF with FDNIRS, we assumed that the fraction of our signal that arises from the venous compartment is the same across our subjects (and equal to 1, although the exact magnitude of this fractional value is less important since we assumed it was the same for everyone). Inter-participant variations in this assumed value could lead to errors in the estimation of OEF.

CONCLUSION

In summary, we demonstrate that diffuse optical spectroscopies (namely, FDNIRS combined with DCS) can be used to detect

expected decreases in OEF, CBV, and CBF_i that occur in sickle cell patients in response to blood transfusion. These results suggest that FDNIRS/DCS could be useful as a low-cost, non-invasive screening tool to enable individualized management of transfusion thresholds, and to identify patients who might benefit from a more aggressive therapy such as stem cell transplant.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The study was approved by the Institutional Review Board of Emory University. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

EB, RCB, and CJ conceived and designed the study. SYL, ROB, KT, ES, KC, AG-Y, and AQ collected data. SYL and ROB analyzed data. SG and SB helped with statistical analysis. SYL, ROB, and EB drafted the manuscript. SYL, ROB, SG, SB, AQ, CJ, RCB, and EB edited the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This project was supported by National Institute of Health R01HL152322 (EB), R21HL138062 (EB), F31HL154703 (ES), and American Heart Association 19POST34380337 (SYL). This work was supported by the National Science Foundation Graduate Research Fellowship Program under Grant No. 1937971 (ROB).

ACKNOWLEDGMENTS

We thank the Aflac Clinical Research Office, especially Amanda Watt, Morgan Hathaway, Lauren Trotter, and Nwanna Ifendu, as well as the clinical staff at the Aflac transfusion clinic for their valuable support.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2022.869117/full#supplementary-material

REFERENCES

1. Kato GJ, Piel FB, Reid CD, Gaston MH, Ohene-Frempong K, Krishnamurti L, et al. Sickle cell disease. *Nat Rev Dis Primers.* (2018) 4:1–22. doi: 10.1038/nrdp.2018.10
2. Schmalzer EA, Lee Jo, Brown Ak, Usami S, Chien S Viscosity of mixtures of sickle and normal red cells at varying hematocrit levels. *Transfusion.* (1987) 27:228–33. doi: 10.1046/j.1537-2995.1987.27387235626.x
3. Alexy T, Pais E, Armstrong JK, Meiselman HJ, Johnson CS, Fisher TC. Rheologic behavior of sickle and normal red blood cell mixtures in sickle plasma: implications for transfusion therapy. *Transfusion.* (2006) 46:912–8. doi: 10.1111/j.1537-2995.2006.00823.x
4. Adams RJ, McKie VC, Brambilla D, Carl E, Gallagher D, Nichols FT, et al. Stroke prevention trial in sickle cell anemia. *Control Clin Trials.* (1998) 19:110–29. doi: 10.1016/S0197-2456(97)00099-8
5. DeBaun MR, Jordan LC, King AA, Schatz J, Vichinsky E, Fox CK, et al. American Society of Hematology 2020 guidelines for sickle cell disease: prevention, diagnosis, and treatment of cerebrovascular disease in children and adults. *Blood Adv.* (2020) 4:1554–88. doi: 10.1182/bloodadvances.2019001142
6. Adams RJ, McKie VC, Hsu L, Files B, Vichinsky E, Pegelow C, et al. Prevention of a first stroke by transfusions in children with sickle cell anemia and abnormal results on transcranial doppler ultrasonography. *New England J Med.* (1998) 339:5–11. doi: 10.1056/NEJM199807023390102
7. DeBaun MR, Gordon M, McKinstry RC, Noetzel MJ, White DA, Sarnaik SA, et al. controlled trial of transfusions for silent cerebral infarcts in sickle cell anemia. *New England J Med.* (2014) 371:699–710. doi: 10.1056/NEJMoa1401731
8. Scothorn DJ, Price C, Schwartz D, Terrill C, Buchanan GR, Shurney W, et al. Risk of recurrent stroke in children with sickle cell disease receiving blood transfusion therapy for at least five years after initial stroke. *J Pediatr.* (2002) 140:348–54. doi: 10.1067/mpd.2002.122498
9. Hulbert ML, Scothorn DJ, Panepinto JA, Scott JP, Buchanan GR, Sarnaik S, et al. Exchange blood transfusion compared with simple transfusion for first overt stroke is associated with a lower risk of subsequent stroke: a retrospective cohort study of 137 children with sickle cell anemia. *J Pediatr.* (2006) 149:710–2. doi: 10.1016/j.jpeds.2006.06.037
10. Hulbert ML, McKinstry RC, Lacey JL, Moran CJ, Panepinto JA, Thompson AA, et al. Silent cerebral infarcts occur despite regular blood transfusion therapy after first strokes in children with sickle cell disease. *Blood.* (2011) 117:772–9. doi: 10.1182/blood-2010-01-261123
11. Guilliams KP, Fields ME, Ragan DK, Eldeniz C, Binkley MM, Chen Y, et al. Red cell exchange transfusions lower cerebral blood flow and oxygen extraction fraction in pediatric sickle cell anemia. *Blood.* (2018) 131:1012–21. doi: 10.1182/blood-2017-06-789842
12. Juttukonda MR, Lee CA, Patel NJ, Davis LT, Waddle SL, Gindville MC, et al. Differential cerebral hemometabolic responses to blood transfusions in adults and children with sickle cell anemia: hemodynamics of transfusions in SCA. *J Magn Reson Imaging.* (2019) 49:466–77. doi: 10.1002/jmri.26213
13. Jordan LC, Kassim AA, Donahue MJ, Juttukonda MR, Pruthi S, Davis LT, et al. Silent infarct is a risk factor for infarct recurrence in adults with sickle cell anemia. *Neurology.* (2018) 91:e781–4. doi: 10.1212/WNL.0000000000006047
14. Jordan LC, Gindville MC, Scott AO, Juttukonda MR, Strother MK, Kassim AA, et al. Non-invasive imaging of oxygen extraction fraction in adults with sickle cell anaemia. *Brain.* (2016) 139:738–50. doi: 10.1093/brain/awv397
15. Nahavandi M, Tavakkoli F, Hasan SP, Wyche MQ, Castro O. Cerebral oximetry in patients with sickle cell disease. *Eur J Clin Invest.* (2004) 34:143–8. doi: 10.1111/j.1365-2362.2004.01307.x
16. Raj A, Bertolone SJ, Mangold S. Edmonds HL. Assessment of cerebral tissue oxygenation in patients with sickle cell disease: effect of transfusion therapy. *J Pediatric Hematol Oncol.* (2004) 26:279–83. doi: 10.1097/00043426-200405000-00004
17. Quinn CT, Dowling MM. Cerebral tissue hemoglobin saturation in children with sickle cell disease. *Pediatr Blood Cancer.* (2012) 59:881–7. doi: 10.1002/pbc.24227
18. Dhabangi A, Ainomugisha B, Cserti-Gazdewich C, Stowell C, Dzik WS. Cerebral oxygenation during transfusion for profound

anemia. *Transfusion.* (2014) 54:2802–2802. doi: 10.1111/trf.12872
19. Kleiser S, Nasseri N, Andresen B, Greisen G, Wolf M. Comparison of tissue oximeters on a liquid phantom with adjustable optical properties. *Biomed Opt Express.* (2016) 7:2973–92. doi: 10.1364/BOE.7.002973
20. Kleiser S, Ostojic D, Andresen B, Nasseri N, Isler H, Scholkmann F, et al. Comparison of tissue oximeters on a liquid phantom with adjustable optical properties: an extension. *Biomed Opt Express.* (2017) 9:86–101. doi: 10.1364/BOE.9.000086
21. Jain V, Buckley EM, Licht DJ, Lynch JM, Schwab PJ, Naim MY, et al. Cerebral oxygen metabolism in neonates with congenital heart disease quantified by MRI and optics. *J Cerebral Blood Flow Metabolism.* (2014) 34:380–8. doi: 10.1038/jcbfm.2013.214
22. Fantini S, Franceschini MA, Fishkin JB, Barbieri B, Gratton E. Quantitative determination of the absorption spectra of chromophores in strongly scattering media: a light-emitting-diode based technique. *Appl Opt, AO.* (1994) 33:5204–13. doi: 10.1364/AO.33.005204
23. Fantini S, Franceschinifantini MA, Maier JS, Walker SA, Barbieri B, Gratton E. Frequency-domain multichannel optical-detector for noninvasive tissue spectroscopy and oximetry. *Optic Eng.* (1995) 34:32–42.
24. Durduran T, Choe R, Baker WB, Yodh AG. Diffuse optics for tissue monitoring and tomography. *Rep Progress Physics.* (2010) 73:43. doi: 10.1088/0034-4885/73/7/076701
25. Wang D, Parthasarathy AB, Baker WB, Gannon K, Kavuri V, Ko T, et al. Fast blood flow monitoring in deep tissues with real-time software correlators. *Biomed Opt Express.* (2016) 7:776–97. doi: 10.1364/BOE.7.000776
26. Lee SY, Cowdrick KR, Sanders B, Sathialingam E, McCracken CE, Lam WA, et al. Noninvasive optical assessment of resting-state cerebral blood flow in children with sickle cell disease. *Neurophoton.* (2019) 6:1. doi: 10.1117/1.NPh.6.3.035006
27. Kety SS, Schmidt CF. The effects of altered arterial tensions of carbon dioxide and oxygen on cerebral blood flow and cerebral oxygen consumption of normal young men. *J Clinic Investigation.* (1948) 27:484–92.
28. Sathialingam E, Williams EK, Lee SY, McCracken CE, Lam WA, Buckley EM. Hematocrit significantly confounds diffuse correlation spectroscopy measurements of blood flow. *Biomed Opt Express.* (2020) 11:4786. doi: 10.1364/BOE.397613
29. Wu C, Honarmand AR, Schnell S, Kuhn R, Schoeneman SE, Ansari SA, Carr J, Markl M, Shaibani A. Age-related changes of normal cerebral and cardiac blood flow in children and adults aged 7 months to 61 years. *J Am Heart Association* 5:e002657. doi: 10.1161/JAHA.115.002657
30. Zhang N, Gordon ML, Ma Y, Chi B, Gomar JJ, Peng S, et al. The age-related perfusion pattern measured with arterial spin labeling mri in healthy subjects. *Front Aging Neurosci.* (2018) 10:214. doi: 10.3389/fnagi.2018.00214
31. Moses P, Hernandez LM, Orient E. Age-related differences in cerebral blood flow underlie the BOLD fMRI signal in childhood. *Front Psychol.* (2014) 5:300. doi: 10.3389/fpsyg.2014.00300
32. Lu H, Xu F, Rodrigue KM, Kennedy KM, Cheng Y, Flicker B, et al. Alterations in cerebral metabolic rate and blood supply across the adult lifespan. *Cerebral Cortex.* (2011) 21:1426–34. doi: 10.1093/cercor/bhq224
33. Takahashi T, Shirane R, Sato S, Yoshimoto T. Developmental Changes of Cerebral Blood Flow and Oxygen Metabolism in Children. *Am J Neuroradiol.* (1999) 20:917–22.
34. Kosaraju V, Harwani A, Partovi S, Bhojwani N, Garg V, Ayyappan S, et al. Imaging of musculoskeletal manifestations in sickle cell disease patients. *BJR.* (2017) 90:20160130. doi: 10.1259/bjr.20160130
35. Fields ME, Guilliams KP, Ragan DK, Binkley MM, Eldeniz C, Chen Y, et al. Regional oxygen extraction predicts border zone vulnerability to stroke in sickle cell disease. *Neurology.* (2018) 90:e1134–42. doi: 10.1212/WNL.0000000000005194
36. Dhabangi A, Ainomugisha B, Cserti-Gazdewich C, Ddungu H, Kyeyune D, Musisi E, et al. Cerebral oximetry in ugandan children with severe anemia: clinical categories and response to transfusion. *JAMA Pediatr.* (2016) 170:995–1002. doi: 10.1001/jamapediatrics.2016.1254
37. Dehaes M, Grant PE, Sliva DD, Roche-Labarbe N, Pienaar R, Boas DA, et al. Assessment of the frequency-domain multi-distance method to evaluate the brain optical properties: monte carlo simulations from neonate to adult. *Biomed Opt Express.* (2011) 2:552. doi: 10.1364/BOE.2.000552
38. Kienle A, Patterson MS, Dögnitz N, Bays R, Wagnières G, van den Bergh H. Noninvasive determination of the optical properties of two-layered turbid media. *Appl Opt.* (1998) 37:779. doi: 10.1364/AO.37.000779
39. Martelli F, Sassaroli A, Bianco SD, Zaccanti G. Solution of the time-dependent diffusion equation for a three-layer medium: application to study photon migration through a simplified adult head model. *Phys Med Biol.* (2007) 52:2827–43. doi: 10.1088/0031-9155/52/10/013
40. Selb J, Ogden TM, Dubb J, Fang Q, Boas DA. Comparison of a layered slab and an atlas head model for Monte Carlo fitting of time-domain near-infrared spectroscopy data of the adult head. *J Biomed Opt.* (2014) 19:016010. doi: 10.1117/1.JBO.19.1.016010
41. Borycki D, Kholiqov O, Chong SP, Srinivasan VJ. Interferometric Near-Infrared Spectroscopy (iNIRS) for determination of optical and dynamical properties of turbid media. *Opt Express.* (2016) 24:329. doi: 10.1364/OE.24.000329

Author Disclaimer: Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation.

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers | Frontiers in Neurology

BRIEF RESEARCH REPORT
published: 07 July 2022
doi: 10.3389/fneur.2022.867437

Sluggish Cognitive Tempo in Pediatric Sickle Cell Disease

Steven J. Hardy[1,2]*, *Sydney Forman*[1], *Kristina K. Hardy*[2,3] *and Jeffrey Schatz*[4]

[1] *Divisions of Hematology and Oncology, Children's National Hospital, Washington, DC, United States,* [2] *Department of Psychiatry and Behavioral Sciences, The George Washington University School of Medicine and Health Sciences, Washington, DC, United States,* [3] *Division of Neuropsychology, Children's National Hospital, Washington, DC, United States,* [4] *Department of Psychology, University of South Carolina, Columbia, SC, United States*

Background: Sickle cell disease (SCD) imparts risk for a range of neurodevelopmental and neurocognitive disorders. Sluggish cognitive tempo (SCT) is a distinct syndrome that often co-occurs with attention-deficit/hyperactivity disorder (ADHD) but has not been described in SCD. We investigated the reliability and validity of a SCT measure in SCD and examined associations with biopsychosocial risk factors and functional outcomes.

Materials and Methods: Caregivers ($n = 85$) of children with SCD ages 7-16 reported on socio-demographics and the Kiddie-Sluggish Cognitive Tempo (K-SCT) measure, Behavior Rating Inventory of Executive Function, and Conners 3. Disease-related characteristics were extracted from health records.

Results: The K-SCT demonstrated excellent internal consistency ($\alpha = 0.92$) and test-retest reliability ($r = 0.82$, $p < 0.001$). K-SCT scores were correlated with ADHD-Inattention ($r = 0.64$, $p < 0.001$) and ADHD-Hyperactive/Impulsive ($r = 0.46$, $p < 0.001$) scores, as well as functional outcomes, including learning problems ($r = 0.69$, $p < 0.001$). In multivariate analyses controlling for ADHD symptoms, SCT accounted for unique variance in learning ($b = 9.67$, $p < 0.01$) and executive functioning ($b = 5.93$, $p < 0.01$). Nearly all participants (93%) with elevated levels of co-occurring SCT and ADHD-Inattention symptoms had significant learning problems.

Conclusion: The K-SCT is a reliable and valid measure of SCT in SCD. SCT symptoms are associated with learning difficulties even after controlling for ADHD symptoms. Further research is needed to understand the biopsychosocial factors that lead to SCT symptoms in SCD and examine long-term implications of SCT.

Keywords: sickle cell, cognitive, sluggish cognitive tempo, ADHD, attention, learning

OPEN ACCESS

Edited by:
Fenella Jane Kirkham,
University College London,
United Kingdom

Reviewed by:
Euclides José de Mendonça Filho,
McGill University, Canada
Eboni Lance,
Kennedy Krieger Institute,
United States

***Correspondence:**
Steven J. Hardy
sjhardy@childrensnational.org

Specialty section:
This article was submitted to Stroke, a section of the journal Frontiers in Neurology

Received: *01 February 2022*
Accepted: *13 June 2022*
Published: *07 July 2022*

Citation:
Hardy SJ, Forman S, Hardy KK and Schatz J (2022) Sluggish Cognitive Tempo in Pediatric Sickle Cell Disease. Front. Neurol. 13:867437. doi: 10.3389/fneur.2022.867437

INTRODUCTION

Sickle cell disease (SCD) is an inherited autosomal recessive disorder characterized by a genetic mutation that produces sickle β-globin, causing hemoglobin molecules to polymerize when deoxygenated and leading to hemolysis, inflammation, and vaso-occlusion (1). Millions of people live with SCD globally, primarily in sub-Saharan Africa and Southeast Asian countries, though the exact prevalence is unknown, due in part to the paucity of newborn screening programs (2, 3). SCD is the most common genetic disorder in the United States, affecting nearly 100,000 people, of whom 90% identify as Black (4). Early concerns of SCD center on an increased risk for life-threatening

infection and although pain is often described as the hallmark symptom, SCD has the potential to cause myriad acute complications and chronic organ damage (1). The pathophysiology underlying clinical manifestations of SCD is complex and influenced by environmental factors; consequently, there is notable variability in symptom presentation and course (5, 6).

Neurocognitive and neurodevelopmental deficits are a common complication of SCD. Slowed processing speed, attention difficulties, and executive dysfunction are often identified as salient neurocognitive symptoms beginning in the preschool period, often continuing into adulthood (7). Neurodevelopmental syndromes have also been linked to disease severity in SCD with as many as half of all preschool-age children showing evidence of developmental delay with elevated rates of neurodevelopmental issues persisting into adolescence (8–11).

A key disease-specific contributor to these deficits are cerebrovascular complications that interfere with oxygen delivery to the brain. Cerebrovascular effects of SCD can begin in the preschool period and increase steadily in prevalence into adulthood with half or more of all adult patients showing cerebrovascular disease (7, 8, 12). These disruptions to oxygen delivery are believed to underlie the development of silent cerebral infarction, the most common form of acquired brain injury documented in SCD, and changes in white matter tissue that are linked to cognitive deficits (13–15). In contrast to cognitive deficits demonstrated on formal cognitive testing, neurodevelopmental syndromes are based largely on behavioral symptoms and have received less attention as indicators of neurologic sequalae of SCD. The purpose of the present study is to investigate distinct patterns of neurodevelopmental symptoms that may result from the heterogeneity in brain impacts observed in SCD.

Although there are common underlying cerebrovascular mechanisms that affect brain health in SCD, less attention has been paid to the variability in impact among individuals. For example, cerebral infarction from overt stroke often occurs in a variety of regions supplied by the anterior and middle cerebral arteries with lesion locations differing across patients (16). Silent cerebral infarction often involves patchy lesions in the border zone regions between these arterial distributions (13, 16). Thus, the brain effects of SCD occur in a wide range of areas that could disrupt functional connections in the frontal, parietal, and temporal lobes as well as subcortical nuclei such as the caudate and putamen. The effects on deep white matter regions surrounding the lateral ventricles (both anteriorly and posteriorly) also mean small differences in the location of lesions could have implications for the white matter pathways affected.

Despite these individual differences in the functional brain systems most affected, there has been a strong tendency to study the cognitive and behavioral effects of the disease in a unidimensional manner, such as identifying the most common neurocognitive or behavioral sequalae averaged across a group. This approach is an important starting point to understand the most salient and common deficits in a condition but can lead to an underappreciation of heterogeneity. In the present study, we use recent conceptualizations of dimensions of attention-deficit/hyperactivity disorder (ADHD) as a framework for understanding different presentations of neurodevelopmental effects of SCD. More specifically, we examined the extent of symptoms reflecting inattention, hyperactivity and impulsivity, and sluggish cognitive tempo (SCT) to understand if these represent distinct dimensions of SCD-related behavioral complications potentially stemming from variability in underlying brain effects.

In current diagnostic approaches, there are three subtypes of ADHD which reflect two dimensions of symptoms: inattentive symptoms and hyperactive-impulsive symptoms with the third category representing a combined presentation with both dimensions present (17). The predominantly inattentive subtype of ADHD (ADHD-I) has typically been noted as the most common behavioral phenotype observed in youth with acquired brain insults (18, 19). Between 7 and 25% of youth with SCD have been diagnosed with ADHD; though, reports may underestimate true prevalence due to complexities associated with diagnosing ADHD in SCD and the significant barriers to formal evaluation (9, 20, 21).

The dimension of SCT was originally developed within the literature on ADHD as a possible phenotype that may better identify youth with predominantly inattentive symptoms but was later identified as a distinct phenotype from inattentive symptoms that often co-occurs with ADHD-I (22). SCT as a syndrome is marked by pronounced symptoms of daydreaming, drowsiness, being easily confused, difficulty initiating and sustaining effort, low activity level and passivity as compared with inattentive symptoms, such as a short attention span, inattentiveness to what others say, and distractibility. Since the original development of the SCT concept there has been growing support for SCT as either an additional variant of ADHD that is distinct from the predominantly inattentive subtype or perhaps a separate disorder with high comorbidity with ADHD (22, 23).

Cognitive neuroscience methods have been used to identify likely underlying neural systems for SCT that differ from those observed in ADHD, whereas more traditional clinical neuropsychological tools have typically not identified distinct features of SCT (23). Elevated SCT symptoms have been associated with deficits in arousal (both at rest and the regulation of arousal in response to environmental stimuli) and selective attention (specifically, in the engagement and shifting of attention), implicating the autonomic nervous system, thalamic, and posterior parietal systems that underlie these functions (24–27). This contrasts with studies of classic ADHD presentations, which are noted to show deficits in executive function and reward sensitivity with critical regions for these systems in prefrontal and superior parietal brain regions (23, 27, 28). In addition to having different neurocognitive profiles, SCT symptoms have been noted to confer additional functional impacts on academic performance not captured by classic ADHD symptoms, are more likely to be comorbid with internalizing disorders, and are associated with lower family socioeconomic status (23, 29, 30).

Prior research on SCT has examined this construct within people that have ADHD and in typically developing individuals without major health conditions but no studies, to our knowledge, have described SCT in SCD. Therefore, we conducted

a study with the following objectives: (1) Determine the reliability and validity of the Kiddie-Sluggish Cognitive Tempo (K-SCT) measure in a sample of youth with SCD; (2) Describe biopsychosocial correlates of SCT symptoms in pediatric SCD; and (3) Examine the unique contribution of SCT symptoms to functional difficulties (e.g., learning problems, mood concerns) and the synergistic influence of SCT and ADHD symptoms on functional outcomes in SCD. We hypothesized that the K-SCT would demonstrate acceptable reliability and validity. It was expected that participants with lower socioeconomic status (e.g., lower parent educational attainment, lower family income) and greater SCD-related neurological risk (e.g., HbSS or HbSβ^0 thalassemia genotypes, overt stroke, silent cerebral infarcts) would be rated as having more SCT symptoms. We also hypothesized that higher SCT symptoms would be significantly correlated with greater functional impairment (e.g., learning problems, mood concerns) and that SCT symptoms would uniquely account for variance in parent-reported learning problems beyond the influence of inattentive ADHD symptoms. Also consistent with prior studies, we expected that participants with both high SCT symptoms and high inattentive ADHD symptoms would exhibit the greatest degree of functional impairment (23, 29, 30).

MATERIALS AND METHODS

Participants

Children and adolescents with SCD and their caregivers were recruited to participate in a larger clinical trial evaluating a computerized cognitive rehabilitation intervention. Patients were deemed eligible if they were between ages 7–16 years, were fluent in English, were accompanied by a parent or legal guardian, and had reliable access to electricity so they would be able to charge a mobile device at home if they screened into the intervention phase of the trial. Exclusion criteria included having a physical limitation that interfered with computer use (including severe intellectual disability) and recently starting a stimulant medication (<30 days). If patients had recently started a stimulant, they were still invited to enroll in the study after taking the medication for at least 30 days, at which point they would meet eligibility criteria.

Procedures

Study procedures were reviewed and approved by the Institutional Review Board prior to data collection. Eligible patients were approached during routine hematology clinic visits and recruited to participate. After obtaining informed consent, participants completed a brief neurocognitive screening assessment to identify participants with working memory difficulties who would be eligible to be randomized to either a digital cognitive training intervention arm or an inactive waitlist arm. Data for the present analyses were obtained from all participants at baseline regardless of whether they exhibited working memory difficulties. Data were also extracted from participants in the waitlist arm 5–8 weeks after the baseline assessment, though these data were only used to examine test-retest reliability of the SCT measure. The current study focused on caregiver ratings of behavior and learning completed as part of a baseline assessment. Participants were provided with a $20 gift card and either parking validation or a $10 public transportation pass following each assessment.

Measures

Socio-Demographic, Disease, and Treatment Characteristics

Primary caregivers reported on socio-demographic characteristics including participant age and sex, annual household gross income, and caregiver educational attainment. A medical chart review was conducted to determine SCD genotype (HbSS/HbSβ^0 thalassemia or HbSC/HbSβ^+ thalassemia), current treatments (e.g., hydroxyurea, chronic blood transfusion therapy), hemoglobin, and stroke history.

Sluggish Cognitive Tempo

The Kiddie-Sluggish Cognitive Tempo measure was completed by caregivers to report on symptoms of SCT (31). This 15-item measure produces a total score and three subscales that aim to quantify: Daydreaming (6 items), Working Memory Slips (5 items), and Sleepy/Tired (4 items). Caregivers answer each item by rating how often their child exhibits certain behaviors as Never or Rarely, Sometimes, Often, or Very Often. The 15-item version of the K-SCT was reduced from a larger item pool based on an exploratory factor analysis that demonstrated these items had high loadings on primary factors of SCT and low cross-loading on measures of ADHD-I symptoms. The K-SCT has been previously shown to have good psychometric properties including strong reliability and validity among children with ADHD (31).

Related Constructs and Functional Outcomes

The Conners 3rd Edition Parent-report (Conners 3) was used to measure ADHD symptoms and associated cognitive and behavioral problems (32). Caregivers completed the 99-item Conners 3 by reporting observations of cognitive and behavioral symptoms using a Likert-type scale with four options: 0 (not at all), 1 (just a little true), 2 (pretty much true), and 3 (very much true). The measure consists of several subscales but for the focus of the current study, analyses focused on the following domains: Executive Functioning, Learning Problems, Aggression, Peer Relations, ADHD Predominantly Hyperactive/Impulsive, and ADHD Predominantly Inattentive. The Conners 3 has good internal consistency, with a Cronbach's alpha of 0.91. Previous reports suggest there is no evidence of race or sex bias, and the demographics of the standardization sample reflected the demographic makeup of the 2,000 U.S. Census (33).

The Behavior Rating Inventory of Executive Function (BRIEF) was also administered to assesses caregiver observations of functional impairment across metacognitive and behavioral regulation domains (34). The 86-item BRIEF asks caregivers to choose one of three options (never, sometimes, often) in response to the listed statements. Two primary index scores are produced: The Metacognition Index (MI) and the Behavioral Regulation Index (BRI). The MI score reflects abilities to monitor behavior and performance, organize materials, plan and organize tasks, engage working memory, and initiate tasks. The BRI score

represents functioning related to inhibiting behavior, shifting tasks, and controlling emotions. The BRIEF has good internal consistency, with alphas ranging from 0.80 to 0.89. Demographic characteristics of the standardization sample reflected the census distribution, and analyses found that race and ethnicity do not significantly affect BRIEF scores (34).

Data Analysis

Descriptive analyses were conducted to examine socio-demographic and medical characteristics of the study sample. Unless otherwise noted, data used in analyses were collected at baseline. Cronbach's alpha was calculated to evaluate internal consistency of the K-SCT total score as well as each of the subscales. Paired samples correlations were used to determine test-retest reliability on the K-SCT total score from baseline to 5 to 8-week follow-up. In order to evaluate for convergent evidence of the K-SCT, we examined Pearson correlation coefficients among the K-SCT total score and the Conners 3 ADHD Primarily Inattentive symptom scale and the Conners 3 ADHD Primarily Hyperactive/Impulsive symptom scale. A similar procedure using Pearson's *r* was followed to evaluate test-criterion evidence between K-SCT scores and concurrently measured functional outcomes (e.g., metacognitive functioning, behavioral regulation, learning problems, aggression, and peer relations). Analyses to determine associations between the K-SCT and socio-demographic and disease-related risk factors varied according to the risk variable type (e.g., Pearson's *r* for continuous variables, independent samples *t*-tests for dichotomous variables).

Linear regression analyses were conducted to assess the variance in functional outcomes attributable to SCT after controlling for the influence of ADHD-I symptoms and conceptually relevant covariates. To explore the implications of having a single elevation of domain symptoms (i.e., either high SCT or ADHD-I) compared to a mixed presentation of symptoms (i.e., high SCT *and* ADHD-I), variables were transformed to signal the presence or absence of significant symptoms. Specifically, K-SCT scores were dichotomized at the 75th percentile, as no established cutoffs exist, and Conners ADHD-I scores were dichotomized at a *T* score of 65 (i.e., $\geq$1 *SD* above the mean). Participants were assigned to one grouping based on the presence or absence of elevated ADHD-I and SCT scores: (1) normal ADHD-I and SCT symptoms; (2) elevated ADHD-I with normal SCT symptoms; (3) normal ADHD-I symptoms with elevated SCT symptoms; and (4) elevated ADHD-I and SCT symptoms.

RESULTS

Eighty-five caregivers completed the K-SCT at baseline to report on SCT symptoms in their children. Descriptive results for the sample are presented in **Table 1**. Five caregivers elected not to report gross family income; these cases were excluded from regression analyses where income was used as a covariate. A review of medical records found that a clinical MRI had been obtained for 53 participants (62%) an average of 1.25 years (*SD* = 1.73) prior to study enrollment. The K-SCT exhibited excellent internal consistency, with a Cronbach's alpha of 0.92 for the 15-item total score. Internal consistency ranged from good to excellent for the three subscales, with a Cronbach's alpha of 0.94 for the Daydreaming subscale, 0.87 for the Working Memory Slips subscale, and.81 for the Sleepy/Tired subscale. Test-retest statistics comparing baseline to 5 to 8-week follow-up ($n = 17$) were also acceptable, as paired samples correlations were 0.82 ($p < 0.001$) for the total score, 0.69 ($p = 0.002$) for the Daydreaming subscale, 0.82 ($p < 0.001$) for Working Memory Slips subscale, and 0.77 ($p < 0.001$) for the Sleepy/Tired subscale.

TABLE 1 | Descriptive characteristics of the study sample.

	n (%)	Range	Mean (SD)
Socio-demographic variables			
Age		7–16	10.45 (2.97)
Sex (female)	49 (58%)		
Caregiver education (no college degree)	47 (55%)		
Annual family income (<$50,000)	42 (53%)		
Medical variables			
Genotype (HbSS/HbSβ^0 thalassemia)	64 (75%)		
Taking hydroxyurea	46 (54%)		
Chronic blood transfusion therapy	23 (27%)		
Hemoglobin		6.90–14.20	9.40 (1.46)
Overt stroke	7 (8%)		
MRI documented in medical record	53 (62%)		
MRI-confirmed silent infarct	18 (34%)		
Caregiver-reported behavioral functioning			
K-SCT total		0–2.60	0.60 (0.50)
K-SCT daydreaming		0–3.00	0.51 (0.65)
K-SCT working memory slips		0–2.60	0.60 (0.56)
K-SCT sleepy/tired		0–2.75	0.74 (0.61)
Conners-3 ADHD primarily inattentive		36–90	57.64 (14.52)
Conners-3 ADHD primarily hyperactive/impulsive		38–90	52.22 (10.94)

n = 85. K-SCT possible scores range from 0 to 3. Higher K-SCT scores reflect greater sluggish cognitive tempo symptoms. Conners 3 subscale scores represent T scores where the population mean is 50 and the standard deviation is 10. Higher Conners 3 scores reflect greater difficulties.

Results also provided convergent evidence of the K-SCT in youth with SCD (see **Table 2**). The baseline K-SCT Total score was significantly correlated with the Conners 3 ADHD Primarily Inattentive Presentation domain score ($r = 0.64$, $p < 0.001$) and the ADHD Primarily Hyperactive/Impulsive Presentation domain score ($r = 0.46$, $p < 0.001$), such that participants who were rated has having more SCT symptoms were also described as having more ADHD Inattentive and ADHD Hyperactive/Impulsive symptoms.

SCT was unrelated to parent educational attainment and family income. Also contrary to hypotheses, SCT did not vary significantly by genotype, hemoglobin, or overt stroke. Although SCT also did not vary by silent infarct history, the sample size for this analysis was reduced as the variable was restricted to only those cases where a clinical MRI had been previously

TABLE 2 | Correlations between Kiddie-Sluggish Cognitive Tempo scores, related constructs, and socio-demographic and disease characteristics.

	K-SCT total score	Conners-3 ADHD inattentive presentation	Conners-3 ADHD hyperactive/impulsive presentation
Socio-demographics			
Age	−0.04	0.06	0.10
Sex	0.01	−0.06	−0.19
Parent education	0.04	0.12	0.05
Family income	−0.03	0.10	0.02
Medical variables			
HbSS or HbSβ^0 thalassemia	−0.19	−0.29*	−0.21
Overt stroke	−0.17	−0.08	0.05
Silent infarct	−0.03	−0.08	0.06
Hemoglobin	0.02	0.11	0.08
Kiddie-Sluggish Cognitive Tempo			
K-SCT total	–	0.64**	0.46**
K-SCT daydreaming	0.90**	0.58**	0.47**
K-SCT working memory slips	0.87**	0.62**	0.44**
K-SCT sleepy/tired	0.64**	0.32**	0.16
Conners 3			
ADHD inattentive	0.64**	–	0.52**
ADHD hyperactive/impulsive	0.46**	0.52**	–
Learning problems	0.69**	0.79**	0.42**
Executive functioning	0.55**	0.92**	0.39**
Defiance/aggression	0.23*	0.31**	0.53**
Peer relations	0.23*	0.18	0.25*
BRIEF			
Metacognition index (MI)	0.66**	0.83**	0.51**
Behavioral regulation index (BRI)	0.47**	0.51**	0.71**

*n = 85. **Indicates p < 0.01. *Indicates p < 0.05. "Silent infarct" reflects only silent infarcts confirmed by MRI. A chart review found a clinical MRI had been completed for 53 participants.*

conducted ($n = 53$; 62%), of which 34% ($n = 18$) revealed evidence of silent infarct. Results supported hypotheses regarding the associations between SCT and functional outcomes. Greater SCT symptoms as measured by the K-SCT total score were significantly associated with greater caregiver-rated learning problems, metacognitive and behavior regulation difficulties, defiance/aggression, and peer problems (**Table 2**).

Based on prior literature suggesting conceptual overlap between SCT and ADHD Inattentive symptoms, we examined whether there was a unique influence of SCT on caregiver-rated learning problems, metacognitive functioning, and behavior regulation when controlling for ADHD Inattentive symptoms (**Table 3**). Linear regressions demonstrated that models incorporating socio-demographic variables, SCD characteristics, ADHD Inattentive and ADHD Hyperactive/Impulsive symptoms, and SCT symptoms as independent variables significantly predicted caregiver-rated learning problems ($R^2 = 0.75$, $p < 0.001$), metacognitive functioning ($R^2 = 0.75$, $p < 0.001$), and behavior regulation ($R^2 = 0.52$, $p < 0.001$). Even after controlling for ADHD symptoms, higher SCT symptoms significantly predicted learning problems ($\beta = 0.35$, $p < 0.001$). For every 1-point increase in the K-SCT total score, there was a T-score increase of 9.67 on the Conners 3 Learning Problems subscale. Similarly, after controlling for ADHD symptoms, greater SCT symptoms predicted more difficulties with metacognitive functioning ($\beta = 0.25$, $p < 0.01$). Each 1-point increase on the K-SCT Total score was associated with a T-score increase of 5.93 on the BRIEF MI. SCT did not significantly predict caregiver-rated behavior regulation.

Participants were grouped based on the presence of significant SCT symptoms ($\geq$75th percentile; $n = 21$; 25%) and ADHD-I symptoms (T score $\geq$ 65; $n = 26$; 31%) to better characterize the functional effect of SCT, ADHD-I, and the combination of SCT and ADHD-I symptoms on learning problems (see **Figure 1**). Conners 3 Learning Problems subscale means and standard deviations are available in **Supplementary Table 1**. Most participants were categorized as having normal levels of both ADHD-I and SCT symptoms ($n = 53$; 62%). The next largest group had both clinically elevated ADHD-I and SCT symptoms ($n = 15$; 18%), followed by elevated ADHD-I symptoms with normal SCT symptoms ($n = 11$; 13%), and normal ADHD-I symptoms with elevated SCT symptoms ($n = 6$; 7%). A majority of participants with clinically elevated ADHD-I symptoms were rated as having significant learning problems, regardless of their degree of SCT symptoms (21 of 26 participants; 81%). Similarly, most participants with elevated SCT symptoms had significant learning problems, regardless of ADHD-I symptoms (15 of 21 participants; 71%). While most participants with elevated

TABLE 3 | Multiple regression models predicting functional outcomes.

	b	SE	β	*p*	R^2
Dependent variable: Conners-3 learning problems					
(Constant)	19.33	7.65			0.75**
Age	0.48	0.30	0.10	0.11	
Severe SCD genotype	0.63	2.25	0.02	0.78	
Stroke or silent infarct	0.82	2.00	0.03	0.68	
Parent education	−1.01	0.78	−0.09	0.20	
Family income	−0.38	0.51	−0.06	0.45	
Conners-3 ADHD inattentive	0.58**	0.08	0.59	<0.01	
Conners-3 ADHD hyperactive/impulsive	0.04	0.10	0.03	0.72	
K-SCT total	9.67**	2.23	0.35	<0.01	
Dependent variable: BRIEF metacognition index					
(Constant)	14.30	6.07			0.75**
Age	0.66*	0.25	0.16	0.01	
Severe SCD genotype	1.32	1.88	0.05	0.49	
Stroke or silent infarct	−0.37	1.67	−0.01	0.83	
Parent education	−0.65	0.65	−0.07	0.32	
Family income	0.80	0.42	0.14	0.06	
Conners-3 ADHD inattentive	0.52**	0.07	0.63	<0.01	
Conners-3 ADHD hyperactive/impulsive	0.06	0.08	0.05	0.45	
K-SCT total	5.93**	1.86	0.25	<0.01	
Dependent variable: BRIEF behavioral regulation index					
(Constant)	6.93	7.51			0.52**
Age	0.57	0.61	0.16	0.07	
Severe SCD genotype	3.31	2.33	0.14	0.16	
Stroke or silent infarct	−0.34	2.07	−0.02	0.87	
Parent education	0.15	0.80	0.02	0.86	
Family income	−0.35	0.52	−0.07	0.51	
Conners-3 ADHD Inattentive	0.10	0.08	0.14	0.23	
Conners-3 ADHD Hyperactive/Impulsive	0.57**	0.10	0.55	<0.01	
K-SCT Total	3.03	2.30	0.14	0.19	

**Indicates $p < 0.05$; **Indicates $p < 0.01$.*

ADHD-I symptoms and normal SCT symptoms were rated as having significant learning problems (7 of 11 participants; 64%), nearly all of those with both elevated ADHD-I and SCT symptoms had learning problems (14 of 15 participants; 93%).

DISCUSSION

The current study characterized SCT symptoms in children and adolescents with SCD by reporting on the reliability and validity of a parent proxy-report measure of SCT symptoms, examining associations between SCT and socio-demographic and disease-related risk factors, and describing the effect of SCT symptoms and combined ADHD-I and SCT symptoms on functional outcomes. Reliability and validity analyses supported the K-SCT as a reliable measurement tool with evidence of convergent and test-criterion validity. Findings also revealed that, contrary to hypotheses based on the broader ADHD literature, we were not able to identify risk factors for SCT symptoms among youth with SCD: SCT symptoms did not vary by any socio-demographic factors or SCD-related risk variables that were measured. Data did, however, support the clinical significance of SCT symptoms: Higher SCT symptoms were associated with functional outcomes even after controlling for ADHD-I symptoms. In addition, the combined presentation of SCT and ADHD-I symptoms conferred significant risk for learning problems.

In order for novel phenomena to be studied, rigorous methods of measurement are needed. The K-SCT has been used to study SCT in typically developing children and those with other neurodevelopmental diagnoses but to our knowledge, its psychometric properties had not been evaluated in SCD. Results supported the 15-item K-SCT as a reliable and valid instrument for assessing SCT symptoms in children ages 7–16 with SCD. Data suggested that the K-SCT has excellent internal consistency and produces reliable scores across a 5 to 8-week period. Moreover, results provided convergent evidence for the K-SCT as the K-SCT total score was significantly correlated with the Conners 3 ADHD Primarily Inattentive and ADHD Primarily Hyperactive/Impulsive subscales. Many associations followed expected patterns, with K-SCT scores correlating stronger with

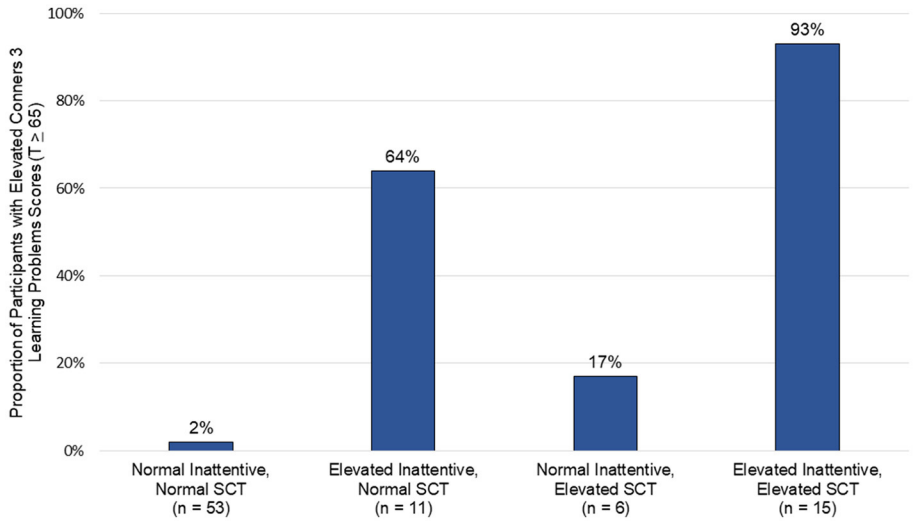


FIGURE 1 | Clusters of inattentive and sluggish cognitive tempo symptoms and their association with learning problems.

symptoms of inattention than symptoms hyperactivity and impulsivity. These findings provide initial support for the reliability and validity of the K-SCT and suggest that it could be used to further investigate SCT in pediatric SCD.

Based on previous studies suggesting associations between socio-demographic risk factors and neurocognitive outcomes in typically developing children and in youth with SCD, we expected that demographic characteristics and measures of socioeconomic status would be related to SCT symptoms. However, K-SCT scores did not vary by age, sex, parental education, or family income. These expectations were derived primarily from prior studies of youth with ADHD, and it may be that associations do not generalize across all populations at risk for SCT symptoms, particularly among more diverse groups exposed to a multitude of neurologic risks. It is also possible that community-level factors unique to the region where patients were recruited (e.g., proximity to a large children's hospital with a comprehensive SCD care clinic and dedicated social workers, high quality schools and other community resources, access to early intervention services) helped buffer the effect of broad proxy measures of socioeconomic status.

Our findings may also reflect nuances of measuring socioeconomic risk and associated outcomes. Prior studies point to variability in relationships between socioeconomic variables and neurocognitive outcomes that depend in part on the domain examined and how socioeconomic risk is operationalized. Strong evidence supports a connection between socioeconomic status (parent educational attainment, in particular) and global intellectual functioning in SCD (35), whereas others have reported no association between socioeconomic status and certain neurocognitive abilities (36). Researchers have been attuned to the complexity of measuring socioeconomic and social-environmental risk factors, recognizing that parent educational attainment and income are important predictors that lead to downstream risks (e.g., parenting stress) known to more directly influence neurodevelopmental and behavioral outcomes.

Variability in defining and measuring risk inevitably leads to conflicting findings in the literature, especially when studying heterogenous groups such as youth with SCD. For instance, Yarboi and colleagues found that maternal experiences of financial stress predicted performance on measures of verbal intelligence but not visual-spatial functioning and non-verbal reasoning in school-age children with SCD (37). Similarly, Bills et al. reported that, in a sample of young children with SCD, traditional measures of socioeconomic status were not uniformly correlated with neurocognitive performance (36). Moreover, they found that parent and family functioning (e.g., positive parent-child interactions, caregiver warmth and support) predicted phonological processing and ADHD symptoms over and above socioeconomic status (36). Future research should incorporate an intentional and multimodal approach to measuring social-environmental risk factors and evaluate effects on neurodevelopmental outcomes prospectively to better explicate these complicated relationships.

Results also suggested that disease-related variables were not associated with SCT. A limitation of this study was that neuroimaging data were not consistently collected across participants. Prior research also suggests a more specific pattern of neurocognitive deficits (e.g., specific to arousal regulation) may be important for SCT symptoms, rather than simply examining the presence/absence of neurologic injury. The available neuroimaging data identified relatively few participants with silent infarcts and overt strokes in our sample and data regarding infarct location and size that could have helped to clarify potential associations between

neural injury and SCT were not available. Thus, there were multiple methodological factors that could account for these null findings. Future research should consider including more refined measures of structural brain effects and cognitive neuroscience methods that could identify those children with specific neurocognitive deficits associated with SCT in prior research. It may also be useful for future studies to incorporate prospective neuroimaging, which would enable characterization of pathways that give rise to distinct neurocognitive phenotypes over time and could determine effects of disease-modifying therapies on the prevention or slowing of neurocognitive decline.

The most salient aspect of the current findings was the influence of SCT on functional outcomes. High SCT was associated with more problems with learning, metacognitive functioning, executive abilities, behavioral regulation, defiance and aggression, and peer relationships. These findings suggest that SCT symptoms significantly contribute to learning problems and negatively influence cognitive, behavioral, and social outcomes in youth with SCD. Furthermore, regression models controlling for ADHD-I symptoms appeared to suggest that SCT symptoms represent a unique risk to functional outcomes including learning problems and metacognitive functioning difficulties.

Additional evidence is needed to more fully understand the extent to which ADHD-I and SCT represent overlapping or distinct neurodevelopmental presentations. The influence of SCT was especially prominent among children who had elevated ADHD-I symptoms, as they were rated as having the greatest difficulties with learning, metacognition, and behavioral regulation. Ninety-three percent of participants with this combined presentation of high ADHD-I and SCT symptoms demonstrated clinically significant learning problems. Therefore, elevated SCT symptoms appear to be not only independently related to real-world neurocognitive, behavioral, and social challenges, but also contribute to a synergistic effect on functional outcomes when occurring in the setting of significant ADHD-I symptoms.

This finding has implications for the treatment of ADHD in SCD and efforts to mitigate behavioral and learning difficulties, as SCT has been shown to be associated with non-response to stimulant medication for children with ADHD (38). Although there is no indication that evidence-based treatments for ADHD will not be similarly effective in youth with SCD with ADHD, studies have also demonstrated the availability of safe and efficacious treatments for attention and working memory in SCD (39, 40). Optimizing available treatments for neurodevelopmental syndromes in SCD is a critical need, given the reportedly low rates of engagement in behavioral (∼50%) and pharmacologic treatments (21–63%) for ADHD (9, 21). Tailoring interventions to specific neurodevelopmental phenotypes (e.g., ADHD-I and SCT symptoms) has the potential to enhance efficacy and could affect interest in treatment; though, facilitators and barriers to accessing care need further investigation.

SCT is a neurodevelopmental syndrome that has been recently described and may be underappreciated due to confusing its symptoms with those of ADHD-I. Additional research on SCT is needed to advance our understanding of the ways SCD negatively affects everyday functioning, including the high rate of learning difficulties observed in SCD. SCT symptoms may be particularly important for their negative synergy with ADHD-I symptoms in producing learning difficulties; however, larger samples are needed to conduct analyses that can better disentangle complex cognitive phenotypes and overlapping symptom clusters (e.g., latent profile analysis). More research is also needed to understand the long-term functional implications of SCT symptoms and the specific etiological factors in SCD that may increase risk for SCT. In particular, there are specific functional neural systems that have been found to be disrupted among children with SCT symptoms and future research should consider examining the integrity of these neurocognitive systems in relation to SCT symptoms in SCD to better identify how to prevent these difficulties.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Children's National Institutional Review Board. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

SH, KH, and JS contributed to conception and design of the study. SH performed the statistical analysis. SH and JS wrote the first draft of the manuscript. SF and KH wrote sections of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

FUNDING

This work was supported by the Doris Duke Charitable Foundation (Grant #2013141 to SH and KH) and the National Institutes of Health (Grant #K23HL14166-01A1 to SH).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2022.867437/full#supplementary-material

REFERENCES

1. Kato GJ, Piel FB, Reid CD, Gaston MH, Ohene-Frempong K, Krishnamurti L, et al. Sickle cell disease. *Nat Rev Dis Primer.* (2018) 4:18010. doi: 10.1038/nrdp.2018.10
2. Wastnedge E, Waters D, Patel S, Morrison K, Goh MY, Adeloye D, et al. The global burden of sickle cell disease in children under five years of age: a systematic review and meta-analysis. *J Glob Health.* (2018) 8:021103. doi: 10.7189/jogh.08.021103
3. Piel FB, Patil AP, Howes RE, Nyangiri OA, Gething PW, Dewi M, et al. Global epidemiology of sickle haemoglobin in neonates: a contemporary geostatistical model-based map and population estimates. *Lancet Lond Engl.* (2013) 381:142–51. doi: 10.1016/S0140-6736(12)61229-X
4. Brousseau DC, Panepinto JA, Nimmer M, Hoffmann RG. The number of people with sickle-cell disease in the United States: national and state estimates. *Am J Hematol.* (2010) 85:77–8. doi: 10.1002/ajh.21570
5. Weatherall MW, Higgs DR, Weiss H, Weatherall DJ, Serjeant GR. Phenotype/genotype relationships in sickle cell disease: a pilot twin study. *Clin Lab Haematol.* (2005) 27:384–90. doi: 10.1111/j.1365-2257.2005.00731.x
6. Rees DC, Williams TN, Gladwin MT. Sickle-cell disease. *Lancet Lond Engl.* (2010) 376:2018–31. doi: 10.1016/S0140-6736(10)61029-X
7. Prussien KV, Jordan LC, DeBaun MR, Compas BE. Cognitive function in sickle cell disease across domains, cerebral infarct status, and the lifespan: a meta-analysis. *J Pediatr Psychol.* (2019) 44:948–58. doi: 10.1093/jpepsy/jsz031
8. Schatz J, Schlenz A, Reinman L, Smith K, Roberts CW. Developmental screening in pediatric sickle cell disease: disease-related risk and screening outcomes in 4 year olds. *J Dev Behav Pediatr JDBP.* (2017) 38:654–62. doi: 10.1097/DBP.0000000000000486
9. Lance EI, Cannon AD, Shapiro BK, Lee LC, Johnston MV, Casella JF. Co-occurrence of neurodevelopmental disorders in pediatric sickle cell disease. *J Dev Behav Pediatr JDBP.* (2021) 42:463–71. doi: 10.1097/DBP.0000000000000914
10. Hoyt CR, Varughese TE, Erickson J, Haffner N, Luo L, L'Hotta AJ, et al. Developmental delay in infants and toddlers with sickle cell disease: a systematic review. *Dev Med Child Neurol.* (2022) 64:168–75. doi: 10.1111/dmcn.15048
11. Heitzer AM, Cohen DL, Okhomina VI, Trpchevska A, Potter B, Longoria J, et al. Neurocognitive functioning in preschool children with sickle cell disease. *Pediatr Blood Cancer.* (2022) 69:e29531. doi: 10.1002/pbc.29531
12. Kassim AA, Pruthi S, Day M, Rodeghier M, Gindville MC, Brodsky MA, et al. Silent cerebral infarcts and cerebral aneurysms are prevalent in adults with sickle cell anemia. *Blood.* (2016) 127:2038–40. doi: 10.1182/blood-2016-01-694562
13. Ford AL, Ragan DK, Fellah S, Binkley MM, Fields ME, Guilliams KP, et al. Silent infarcts in sickle cell disease occur in the border zone region and are associated with low cerebral blood flow. *Blood.* (2018) 132:1714–23. doi: 10.1182/blood-2018-04-841247
14. Scantlebury N, Mabbott D, Janzen L, Rockel C, Widjaja E, Jones G, et al. White matter integrity and core cognitive function in children diagnosed with sickle cell disease. *J Pediatr Hematol Oncol.* (2011) 33:163–71. doi: 10.1097/MPH.0b013e3182036f33
15. Stotesbury H, Kirkham FJ, Kölbel M, Balfour P, Clayden JD, Sahota S, et al. White matter integrity and processing speed in sickle cell anemia. *Neurology.* (2018) 90:e2042–50. doi: 10.1212/WNL.0000000000005644
16. Pegelow CH, Macklin EA, Moser FG, Wang WC, Bello JA, Miller ST, et al. Longitudinal changes in brain magnetic resonance imaging findings in children with sickle cell disease. *Blood.* (2002) 99:3014–8. doi: 10.1182/blood.V99.8.3014
17. Epstein JN, Loren REA. Changes in the definition of ADHD in DSM-5: subtle but important. *Neuropsychiatry.* (2013) 3:455–8. doi: 10.2217/npy.13.59
18. Arpino C, Compagnone E, Montanaro ML, Cacciatore D, De Luca A, Cerulli A, et al. Preterm birth and neurodevelopmental outcome: a review. *Childs Nerv Syst ChNS Off J Int Soc Pediatr Neurosurg.* (2010) 26:1139–49. doi: 10.1007/s00381-010-1125-y
19. Levin H, Hanten G, Max J, Li X, Swank P, Ewing-Cobbs L, et al. Symptoms of attention-deficit/hyperactivity disorder following traumatic brain injury in children. *J Dev Behav Pediatr JDBP.* (2007) 28:108–18. doi: 10.1097/01.DBP.0000267559.26576.cd
20. Lance EI, Comi AM, Johnston MV, Casella JF, Shapiro BK. Risk factors for attention and behavioral issues in pediatric sickle cell disease. *Clin Pediatr.* (2015) 54:1087–93. doi: 10.1177/0009922815594356
21. Acquazzino MA, Miller M, Myrvik M, Newby R, Scott JP. Attention deficit hyperactivity disorder in children with sickle cell disease referred for an evaluation. *J Pediatr Hematol Oncol.* (2017) 39:350–4. doi: 10.1097/MPH.0000000000000847
22. Becker SP, Marshall SA, McBurnett K. Sluggish cognitive tempo in abnormal child psychology: an historical overview and introduction to the special section. *J Abnorm Child Psychol.* (2014) 42:1–6. doi: 10.1007/s10802-013-9825-x
23. Barkley RA. Distinguishing sluggish cognitive tempo from ADHD in children and adolescents: executive functioning, impairment, and comorbidity. *J Clin Child Adolesc Psychol.* (2013) 42:161–73. doi: 10.1080/15374416.2012.734259
24. Baytunca MB, Inci SB, Ipci M, Kardas B, Bolat GU, Ercan ES. The neurocognitive nature of children with ADHD comorbid sluggish cognitive tempo: might SCT be a disorder of vigilance? *Psychiatry Res.* (2018) 270:967–73. doi: 10.1016/j.psychres.2018.03.038
25. Huang-Pollock CL, Nigg JT, Carr TH. Deficient attention is hard to find: applying the perceptual load model of selective attention to attention deficit hyperactivity disorder subtypes. *J Child Psychol Psychiatry.* (2005) 46:1211–8. doi: 10.1111/j.1469-7610.2005.00410.x
26. Yung TWK, Lai CYY, Chan JYC, Ng SSM, Chan CCH. Neurophysiological correlates of sluggish cognitive tempo (SCT) symptoms in school-aged children. *Eur Child Adolesc Psychiatry.* (2020) 29:315–26. doi: 10.1007/s00787-019-01353-1
27. Kim K, Kim HJ. Normal executive attention but abnormal orienting attention in individuals with sluggish cognitive tempo. *Int J Clin Health Psychol IJCHP.* (2021) 21:100199. doi: 10.1016/j.ijchp.2020.08.003
28. Pievsky MA, McGrath RE. The neurocognitive profile of attention-deficit/hyperactivity disorder: a review of meta-analyses. *Arch Clin Neuropsychol Off J Natl Acad Neuropsychol.* (2018) 33:143–57. doi: 10.1093/arclin/acx055
29. Tamm L, Garner AA, Loren REA, Epstein JN, Vaughn AJ, Ciesielski HA, et al. Slow sluggish cognitive tempo symptoms are associated with poorer academic performance in children with ADHD. *Psychiatry Res.* (2016) 242:251–9. doi: 10.1016/j.psychres.2016.05.054
30. Lee S, Burns GL, Snell J, McBurnett K. Validity of the sluggish cognitive tempo symptom dimension in children: sluggish cognitive tempo and ADHD-inattention as distinct symptom dimensions. *J Abnorm Child Psychol.* (2014) 42:7–19. doi: 10.1007/s10802-013-9714-3
31. McBurnett K, Villodas M, Burns GL, Hinshaw SP, Beaulieu A, Pfiffner LJ. Structure and validity of sluggish cognitive tempo using an expanded item pool in children with attention-deficit/hyperactivity disorder. *J Abnorm Child Psychol.* (2014) 42:37–48. doi: 10.1007/s10802-013-9801-5
32. Conners K. *Conners 3rd Ed.* Toronto: Multi-Health Systems (2008).
33. Kao GS, Thomas HM. Test review: C. *J Psychoeduc Assess.* (2010) 28:598–602. doi: 10.1177/0734282909360011
34. Gioia GA, Isquith PK, Guy SC, Kenworthy L. Behavior rating inventory of executive function. *Child Neuropsychol.* (2000) 6:235–8. doi: 10.1076/chin.6.3.235.3152
35. King AA, Strouse JJ, Rodeghier MJ, Compas BE, Casella JF, McKinstry RC, et al. Parent education and biologic factors influence on cognition in sickle cell anemia. *Am J Hematol.* (2014) 89:162–7. doi: 10.1002/ajh.23604
36. Bills SE, Schatz J, Hardy SJ, Reinman L. Social-environmental factors and cognitive and behavioral functioning in pediatric sickle cell disease. *Child Neuropsychol J Norm Abnorm Dev Child Adolesc.* (2020) 26:83–99. doi: 10.1080/09297049.2019.1577371
37. Yarboi J, Compas BE, Brody GH, White D, Rees Patterson J, Ziara K, et al. Association of social-environmental factors with cognitive function in children with sickle cell disease. *Child Neuropsychol J Norm Abnorm Dev Child Adolesc.* (2017) 23:343–60. doi: 10.1080/09297049.2015.1111318
38. Froehlich TE, Becker SP, Nick TG, Brinkman WB, Stein MA, Peugh J, et al. Sluggish cognitive tempo as a possible predictor of methylphenidate response in children with ADHD: a randomized controlled trial. *J Clin Psychiatry.* (2018) 79:17m11553. doi: 10.4088/JCP.17m11553

39. Hardy SJ, Bills SE, Meier ER, Schatz JC, Keridan KJ, Wise S, et al. A randomized controlled trial of working memory training in pediatric sickle cell disease. *J Pediatr Psychol.* (2021) 46:1001–14. doi: 10.1093/jpepsy/jsab030
40. Daly B, Kral MC, Brown RT, Elkin D, Madan-Swain A, Mitchell M, et al. Ameliorating attention problems in children with sickle cell disease: a pilot study of methylphenidate. *J Dev Behav Pediatr JDBP.* (2012) 33:244–51. doi: 10.1097/DBP.0b013e31824ba1b5

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers | Frontiers in Neurology

TYPE Original Research
PUBLISHED 26 July 2022
DOI 10.3389/fneur.2022.894742

OPEN ACCESS

EDITED BY
Fenella Jane Kirkham,
University College London,
United Kingdom

REVIEWED BY
Tales Santini,
Western University, Canada
Melanie Fields,
Washington University in St. Louis,
United States

*CORRESPONDENCE
John C. Wood
jwood@chla.usc.edu

SPECIALTY SECTION
This article was submitted to Stroke,
a section of the journal
Frontiers in Neurology

RECEIVED 12 March 2022
ACCEPTED 29 June 2022
PUBLISHED 26 July 2022

CITATION
González-Zacarías C, Choi S, Vu C, Xu B, Shen J, Joshi AA, Leahy RM and Wood JC (2022) Chronic anemia: The effects on the connectivity of white matter. *Front. Neurol.* 13:894742. doi: 10.3389/fneur.2022.894742

Chronic anemia: The effects on the connectivity of white matter

Clio González-Zacarías[1,2,3], Soyoung Choi[1,2,3], Chau Vu[3,4], Botian Xu[3,4], Jian Shen[3,4], Anand A. Joshi[2], Richard M. Leahy[2,4] and John C. Wood[3,4]*

[1]Neuroscience Graduate Program, University of Southern California, Los Angeles, CA, United States, [2]Signal and Image Processing Institute, University of Southern California, Los Angeles, CA, United States, [3]Department of Pediatrics and Radiology, Children's Hospital Los Angeles, Los Angeles, CA, United States, [4]Biomedical Engineering, University of Southern California, Los Angeles, CA, United States

Chronic anemia is commonly observed in patients with hemoglobinopathies, mainly represented by disorders of altered hemoglobin (Hb) structure (sickle cell disease, SCD) and impaired Hb synthesis (e.g. thalassemia syndromes, non-SCD anemia). Both hemoglobinopathies have been associated with white matter (WM) alterations. Novel structural MRI research in our laboratory demonstrated that WM volume was diffusely lower in deep, watershed areas proportional to anemia severity. Furthermore, diffusion tensor imaging analysis has provided evidence that WM microstructure is disrupted proportionally to Hb level and oxygen saturation. SCD patients have been widely studied and demonstrate lower fractional anisotropy (FA) in the corticospinal tract and cerebellum across the internal capsule and corpus callosum. In the present study, we compared 19 SCD and 15 non-SCD anemia patients with a wide range of Hb values allowing the characterization of the effects of chronic anemia in isolation of sickle Hb. We performed a tensor analysis to quantify FA changes in WM connectivity in chronic anemic patients. We calculated the volumetric mean of FA along the pathway of tracks connecting two regions of interest defined by BrainSuite's BCI-DNI atlas. In general, we found lower FA values in anemic patients; indicating the loss of coherence in the main diffusion direction that potentially indicates WM injury. We saw a positive correlation between FA and hemoglobin in these same regions, suggesting that decreased WM microstructural integrity FA is highly driven by chronic hypoxia. The only connection that did not follow this pattern was the connectivity within the left middle-inferior temporal gyrus. Interestingly, more reductions in FA were observed in non-SCD patients (mainly along with intrahemispheric WM bundles and watershed areas) than the SCD patients (mainly interhemispheric).

KEYWORDS

chronic anemia, diffusion MRI, tensor analysis, sickle cell disease (SCD), thalassemia, white matter (WM)

Introduction

Chronic anemia (CA) is a condition in which the number of erythrocytes or hemoglobin (Hb) concentration is lower than expected and incapable of meeting physiological needs (1). Worldwide, the prevalence of anemia is very high, affecting around 1.93 billion people and causing more significant disability than asthma, diabetes, and cardiovascular disease combined (2). Tissue oxygen consumption is heterogeneous and organ-specific. The brain is one of the organs with higher metabolic demand that receives preferential blood flow under acute circumstances (3). As a result, neurons are specifically sensitive to hypoxia (4). CA causes reduced oxygenation in the brain, leading to hypoxia, neuroinflammation, and white matter (WM) remodeling (5).

CA is a standard clinical feature seen in patients with hemoglobinopathies (6), mainly represented by qualitative disorders in Hb structure (e.g., sickle cell disease, SCD) and quantitative disorders of Hb synthesis (e.g., thalassemia syndromes, non-SCD) (7). Hemoglobinopathies have been associated with gray matter (GM) (8–11) and WM alterations (12–17), cerebral vasculopathies (18–20), and changes in cerebral blood flow (21–26), thus serving as a model for the cerebral changes caused by CA (15, 17).

Studying SCD and non-SCD patients with a wide range of Hb values and genetic predisposition to anemia simultaneously allows the characterization of the effects of CA in isolation from sickle Hb (17, 22). In this context, novel structural magnetic resonance imaging (MRI) research done in our laboratory, where SCD and non-SCD patients were simultaneously analyzed, demonstrated that WM volume was diffusely lower in deep, watershed areas proportional to anemia severity regardless of Hb genotype (15). This relationship between anemia and WM volume was confirmed in a repeated analysis with a restricted population consisting of patients with beta-thalassemia (27).

We hypothesized that CA causes similar damaging effects and changes in structural connectivity of WM in patients with hemoglobinopathies. Furthermore, the damage is driven by hyperemia and not by the intrinsic pathophysiology of these hemoglobinopathies. To characterize alterations in the WM, we performed a diffusion tensor imaging (DTI) analysis and quantified the average fractional anisotropy (FA, overall directionality of water diffusion) along the pathways connecting every pair of regions of interest (ROIs) defined by an anatomical atlas. This approach covers all the WM bundles in the brain and not merely the main association fascicles (28).

Diffusion MRI (dMRI) is used to study in-vivo WM microstructure and allows quantitative characterization of healthy and diseased tissue. The most widely used dMRI technique is DTI, despite various limitations. Its derived metrics like FA are potential biomarkers of brain abnormalities in patients with neurodegenerative diseases. For example, DTI analysis already provided evidence of the relationship between WM microstructure and markers of anemia severity, such as oxygen saturation level and Hb value (13). Additionally, SCD patients have shown lower FA in the corticospinal tract and cerebellum (13), across the internal capsule (14), the corpus callosum (14, 16, 29, 30), and in the deep WM (30). Decrement of FA was also observed in major WM tracts in CA patients, regardless of the anemia subtype, and correlated significantly with the neurocognitive decline observed in the CA population (29).

Materials and methods

Participation criteria

All participants in this study were part of a larger project on Sickle Cell Disease at Children's Hospital Los Angeles that its Institutional Review Board approved. Each participant was recruited with informed consent. We collected MRI data and blood tests on patients, including non-SCD, SCD, and healthy controls matched by sex and age. The accepted age range was between 10 to 50 years old. Eligibility criteria included patients with SCD diagnosis (Hb SS, Hb SC, Hb Sβ^0, and Hb Sβ^+genotypes), patients with chronic anemia diagnosis (beta-thalassemia major, beta-thalassemia intermedia, hemoglobin H-constant spring, congenital dyserythropoietic anemia, spherocytosis anemia, and autoimmune hemolytic anemia) and healthy controls (mainly recruited from family members of SCD patients to match race and ethnicity between groups). The exclusion criteria disqualified those patients with previous overt stroke, acute chest syndrome, pain crisis hospitalization (within one month), and pregnant candidates. Similarly, individuals with prior history of neurologic insults, developmental delay, or chronic medical conditions that require regular medical care or medications and pregnant candidates would be ineligible as healthy controls.

We followed the standard guidelines and regulations for MRI safety and exclusion criteria. On the same day, each participant completed the MRI examination without sedating medications, and we collected vital signs and blood samples.

Laboratory markers

To account for the similarities and differences in CA pathophysiology, all participants enrolled in our study underwent a thorough examination of their blood samples. Complete blood count, reticulocyte total, and quantitative Hb electrophoresis percentages of Hb S, Hb A, Hb A2, hemoglobin F, etc.) were analyzed in the clinical laboratory. Additional

TABLE 1 Demographics.

	CTL	non-SCD	SCD	CTL vs. non-SCD†	CTL vs. SCD†	non-SCD vs. SCD†
N	23	15	19	-	-	-
Sex (F:M)	14:9	8:7	10:9	-	-	-
Age	21.3 ± 5.9	22.4 ± 4.8	21.8 ± 8.3	0.88	0.97	0.96
Transfused	0	9	5	-	-	-
Hemoglobin (g/dL)	13.2 ± 1.2	10.3 ± 1.7	10.2 ± 2.1	≤0.01	≤0.01	0.96
Hematocrit (%)	39.8 ± 3.6	32.2 ± 5.82	28.7 ± 5.3	≤0.01	≤0.01	0.10
White blood cell count (x10^3)	5.6 ± 1.7	7.0 ± 3.1	9.3 ± 4.3	0.33	≤0.01	0.11
Reticulocytes (%)	1.2 ± 0.5	3.1 ± 3.1	7.8 ± 3.5	0.07	≤0.01	≤0.01
Plasma-free hemoglobin	6.7 ± 5.2	22.3 ± 23.8	21.8 ± 19.6	0.02	0.02	0.99
Lactose dehydrogenase	519.5 ± 75.2	632.2 ± 361.5	1008.5 ± 573.7	0.64	≤0.01	≤0.01
Absolute neutrophil count	3.2 ± 1.6	4.2 ± 1.8	5.4 ± 3.6	0.41	≤0.01	0.37
Heart rate (min^{-1})	70.5 ± 20.4	79.0 ± 13.4	81.7 ± 13.9	0.28	0.08	0.88
Systolic blood preassure (mmHg)	116.1 ± 9.2	113.9 ± 9.1	115.5 ± 12.6	0.79	0.98	0.89
Dyastolic blood preassure (mmHg)	66.1 ± 9.5	60.3 ± 9.3	63.7 ± 7.3	0.13	0.65	0.51
O_2 saturation (%)	99.5 ± 0.9	98.3 ± 2.9	97.8 ± 1.6	0.17	≤0.01	0.66
Hemoglobin A (%)	82.2 ± 17.9	90.7 ± 7.9	23.4 ± 32.4	-	-	-
Hemoglobin F (%)	0.7 ± 2.4	2.9 ± 4.1	8.8 ± 9.5	-	-	-
Hemoglobin S (%)	14.1 ± 18.1	0.0 ± 0.0	50.9 ± 26.0	-	-	-

Mean and standard deviation of demographic information and selected complete blood count measurements.
CTL, control; non-SCD, non sickle cell disease; SCD, sickle cell disease.
†Group comparison using one-way analysis of variance (ANOVA) result with Tukey-Kramer test for multiple comparisons. Statistically significant values ($p \leq 0.05$) are color-coded as follows: green color denotes the comparison between CTL and non-SCD groups, red compares CTL and SCD, and blue color non-SCD with SCD patients.

surrogates for hemolysis like lactate dehydrogenase (LDH) and plasma-free Hb levels were also quantified (Table 1).

Image acquisition

The MRI data were acquired on a 3T Philips Achieva scanner using an 8-channel head coil for each participant. The structural 3D T1-weighted (T1-w) sequence specification was TR/TE = 8.3/3.8 ms, SENSE = 2, and isotropic voxel size of 1 mm^3. In addition, a single-shell dMRI sequence was acquired with TR/TE = 6,700/86 ms; isotropic voxel size of 2.5 mm^3; 30 diffusion-encoding directions at b-value = 1,000 s/mm^2 and one b-value = 0 s/mm^2 using a single-shot echo-planar imaging sequence.

Post-processing

The Brain extraction and parcellation from the T1-weighted (T1-w) images were processed with BrainSuite (brainsuite.org, v19b). Specifically, BrainSuite's Cortical Surface Extraction (CSE) tool was used to perform skull stripping (31), tissue classification, including partial volume fraction of voxels identified as WM, GM, and CSF (32), topological corrections (33), and delineation of the inner/outer cortex. In addition, BrainSuite's Surface Volume Registration (SVReg) tool (34, 35) performed anatomical co-registration to the BCI-DNI anatomical atlas (35), and brain segmentation.

The dMRI data were corrected for localized geometric distortions to enable accurate multi-modal analysis. Each subject's motion and eddy current-induced distortions were corrected with FSL's eddy module (36, 37). Using BrainSuite's Diffusion Pipeline (BDP), we registered the dMRI to the T1-w data, followed by susceptibility-distortion correction based on the inverse contrast normalization (38).

Diffusion modeling

Using the well-known tensor equation, we calculated the fractional anisotropy maps in BDP. To render more accurate tractography in the WM, we also computed in BDP the orientation density functions (ODFs). In particular, the ensemble average propagator response function optimized (ERFO) uses machine learning and linear estimation theory to optimize ODF accuracy for arbitrary q-space sampling schemes. It has shown advantages over other methods (39). Furthermore, ERFO can model single-shell (and multi-shell) data and has the capacity of rendering crossing fibers with the most negligible false positives (40).

Whole-brain deterministic fiber tracking, based on quantitative anisotropy (41), and visualization were

performed with the DSI Studio tractography package (http://dsi-studio.labsolver.org). The sections of tracks entering cortical GM or subcortical regions were excluded to avoid partial volume effects. Afterward, detail connectivity analysis of fiber bundles connecting two ROIs (previously labeled on the T1-w structural images) was implemented with the TractConnect Matlab package (https://neuroimage.usc.edu/neuro/Resources/TractConnect). Specifically, TractConnect uses filtered tracks connecting two ROIs to define a volumetric white matter surface (WMS) and projects it into the FA maps (Figure 1). Then FA values within WMSs were averaged.

For each individual, the average FA values were used as the elements of the connectivity matrix. 88 ROIs from the BCI-DNI atlas were used: 66 cortical regions, 14 subcortical, corpora quadrigemina, mammillary bodies, brainstem, and cerebellum.

Overall, this modeling method offers higher sensitivity and specificity to detect not only regional differences in WM microstructure (like voxel-wise analysis would do) but along the connectivity pathways, and it is robust to some of the commonly criticized featured of DTI ((42, 43)): the inability to render crossing fibers and to define connectivity between ROIs by streamlining counting. The first was overcome by using ERFO to model diffusion ODFs and the latter by defining the WMSs and characterizing these "connections" with the mean FA value.

Statistical analysis

For each element of the connectivity matrix (upper triangular part), Figure 1, the FA differences between groups were modeled using multiple linear regression analysis after controlling for logarithm of age (log-age), sex, and group. The logarithm of age was used because brain maturational effects are nonlinear with age in adolescents and young adults (44, 45). Finally, the results were also corrected for multiple comparisons using the False Discovery Rate (FDR) to adjust the correspondent p-values (46) with a 20% acceptance rate.

A similar analysis was performed using a permutation analysis using Manly's method (47, 48), which was also FDR corrected (49) with the same threshold. Given the complexity of the data, it was not possible to guarantee all the assumptions of linear modeling. Consequently, we also chose to model the WMSs using nonparametric permutation analysis. Overlapping between the two methods provided an additional confidence level in our results.

For completeness, we tested the possible contribution of changes in FA caused by monthly transfusions and LDH values in patients. For this, we only ran a multiple linear regression analysis controlling also for log-age, sex, and group. All the statistical analyses were calculated using the R statistical package (50).

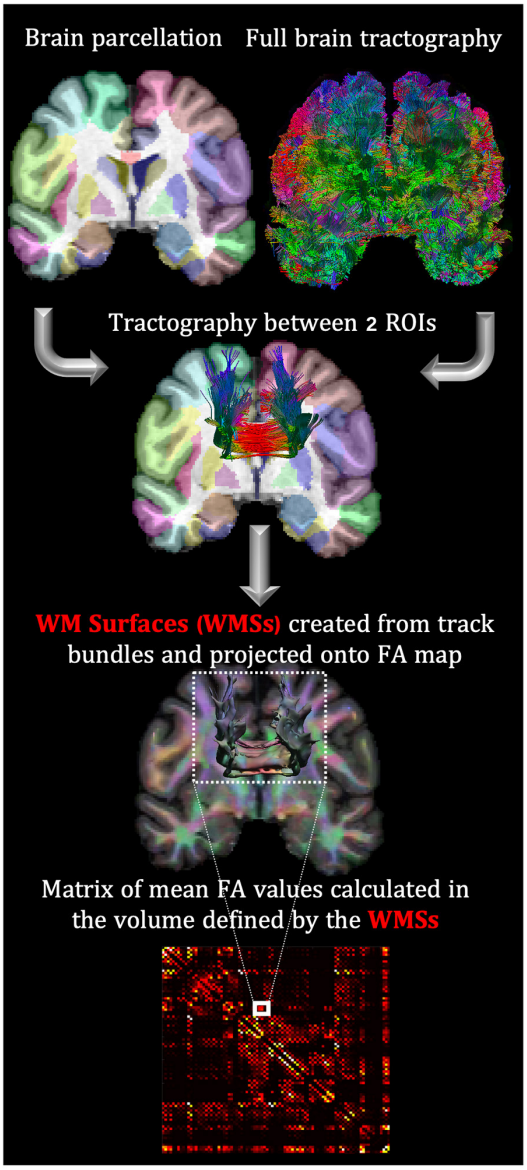


FIGURE 1
FA analysis in WMS based on the coregistration of the parceled T1-w with dMRI maps. This coregistration allows mapping the connectivity tracks between two ROIs to the FA map. The exact process is repeated to create a connectivity matrix for each subject.

Results

Demographics

In this analysis we considered 19 clinically asymptomatic SCD patients (age = 22.4 ± 7.8 years; Hb = 10.1 ± 2.1 g/dL; F = 9 patients), 15 non-SCD anemic patients (age = 22.4 ± 4.8 years; Hb = 10 ± 2.8 g/dL; F = 8 patients) and 23 control subjects (age = 21.3 ± 6 years; Hb = 13.3 ± 1.2 g/dL; F = 14 individuals).

The age range for all the participants was 11.2 to 35.8 years. All demographics are reported in Table 1.

The breakdown of the race (and ethnicity) for control subjects was 17 African-American (non-Hispanic) and 5 White (Hispanic) individuals. SCD patients included 17 African-American (non-Hispanic) and 2 White (Hispanic) patients. The non-SCD group consisted of 8 Asian (non-Hispanic), 5 White (non-Hispanic), and 2 White (Hispanic) patients.

For the SCD group, the genotypes were 12 Hb SS and 7 Hb SC patients. Because of the specific matches between control and SCD, 9 of the control subjects were identified with sickle cell trait having hemoglobin AS (Hb AS). Previous work in our laboratory suggests that the Hb AS subtype does not alter normal cerebral blood flow (CBF) regulation and balance of oxygen supply and demand Field (22), making Hb AS carriers good candidates for control subjects.

The specific anemias in the non-SCD group consisted of 7 patients with beta-thalassemia major, 3 beta-thalassemia intermedia, 2 hemoglobin H-constant spring, 1 congenital dyserythropoietic anemia, 1 spherocytosis anemia and 1 autoimmune hemolytic anemia.

Of the CA patients, 8 non-SCD (7 beta-thalassemia major and 1 congenital dyserythropoietic anemia) and 5 SCD (Hb SS patients) were on monthly transfusions. The rest of the non-transfused SS patients were prescribed hydroxyurea and had a mean hemoglobin F fraction of 18%. One patient with SC was also taking hydroxyurea. At Children's Hospital Los Angeles, it is recommended to treat all pediatric patients, of all SCD genotypes, nine months and older with hydroxyurea unless they have been placed on chronic transfusion (51, 52), as indicated by NIH guidelines (53). Furthermore, as of 2,000, all SCD patients at our facility have received access to the transcranial Doppler screening (51–54).

Laboratory comparisons

Laboratory and clinical markers are shown in Table 1. Hemoglobin ($p = 0.96$) and hematocrit ($p = 0.10$) levels were not statistically different between CA groups, but both had statistically significant lower values compared with healthy control (non-SCD, SCD: $p \leq 0.01$). Furthermore, the SCD population showed significantly higher levels of reticulocytes ($p \leq 0.01$) and LDH ($p \leq 0.01$) compared to both non-SCD and control, suggesting increased intravascular hemolysis. However, plasma-free Hb was not different between CA types ($p = 0.99$). SCD patients had mildly increased white cell counts with respect to control subjects ($p \leq 0.01$) but not relative to the non-SCD anemic patients ($p = 0.11$). In the case of Hb electrophoresis, Hb S was highest for SCD patients. Still, our control also exhibited a smaller percentage of Hb S because of the inclusion of sickle trait subjects. SCD patients demonstrated the highest hemoglobin F (Hb F) concentration, with intermediate levels observed in non-SCD patients. Most control subjects had no Hb F, but one subject had 11.7% Hb F.

White matter connectivity

Overall, no statistically significant WMSs were found when comparing SCD with non-SCD patients. 10 WMSs in CTL vs. non-SCD and 5 WMSs in CTL vs. SCD analysis showed significant differences in both multiple linear regression and permutation analysis (Table 2). *FA indicates the group mean FA after controlling for log-age and sex using the multiple linear regression. The point-biserial correlation coefficient, $r_{*FA,Gr}$, shows the direction and strength of the relationship between *FA and the status of being anemic or not. A negative $r_{*FA,Gr}$ value depicts higher *FA in control individuals than CA patients. All WMSs reported in Table 2 showed this behavior except for one (left middle and inferior temporal gyrus) in healthy controls vs. non-SCD comparison. The *FA unpaired two-samples t-test statistic is also displayed for completeness, which agrees with the multiple linear regression analysis.

When Hb was included in the mathematical models as a covariate, all the *FA differences reported in Table 2 were no longer statistically significant. This suggests that many of the effects reported are driven by the Hb differences between healthy controls and CA patients. To further study the relationship between *FA and Hb, we calculated the Pearson correlation coefficient, $r_{*FA,Hb}$, and their respective p-value, $p_{*FA,Hg}$, for the WMSs reported in Table 2. *FA was significantly correlated with Hb levels in 8 out of the 10 WMSs in the population consisting of control and non-SCD analysis and in 3 out of the 5 WMSs in the control and SCD population. Consequently, by calculating $r^2_{*FA,Hb}$, the proportion of variance in *FA explained by Hb, we observed that in control and non-SCD for the WMSs reported in Table 2, Hb accounts for up to 26% (right thalamus and right amygdala) of the variance in *FA, and up to 21% (left thalamus and left parahippocampal gyrus) in the case of controls with SCD patients.

The spatial locations of the WMSs listed in Table 2 are 3D-rendered in the left and right hemispheres of a representative subject (Figure 2). In these same WMSs, we saw a positive correlation of *FA with Hb. Significant results were bilateral and generally symmetrical across hemispheres. Interestingly, more WMSs survived for the non-SCD (mainly intrahemispheric and along with watershed areas) than for the SCD (mainly interhemispheric) group compared with healthy controls.

Table 3 shows the results of the multiple linear regression when adding in the model transfusion status. For non-SCD patients, 4 out of 10 WMSs displayed in Table 2 were still statistically significant, and for SCD patients, all the WMSs reported in Table 2 still appeared. When including LDH as a marker of hemolysis in the mathematical model, 6 out of 10

TABLE 2 Results for *FA.

ROI-1	ROI-2	CTL *FA	CA *FA	$r_{*FA,Hg}$ †	T-statictic†	$r_{*FA,Hg}$ ¥	$p_{*FA,Hg}$ ¥
CTL vs. non-SCD							
R. caudate nucleus	R. middle frontal gyrus	0.37	−0.57	−0.46	$t(36) = 3.1\ p \leq 0.01$	0.29	≤0.01
R. thalamus	R. middle frontal gyrus	0.49	−0.70	−0.59	$t(30) = 3.9\ p \leq 0.01$	0.41	≤0.01
R. thalamus	R. amygdala	0.47	−0.65	−0.55	$t(34) = 3.8\ p \leq 0.01$	0.51	≤0.01
L. thalamus	L. gyrus rectus	0.40	−0.63	−0.50	$t(34) = 3.4\ p \leq 0.01$	0.37	≤0.01
L. thalamus	L. parahipppocampal gyrus	0.40	−0.56	−0.48	$t(34) = 3.2\ p \leq 0.01$	0.32	0.06
R. superior frontal gyrus	R. cingulate gyrus	0.46	−0.73	−0.58	$t(34) = 4.2\ p \leq 0.01$	0.41	≤0.01
R. transvers frontal gyrus	R. subcallosal gyrus	0.49	−0.68	−0.59	$t(31) = 4.0\ p \leq 0.01$	0.37	0.03
R. cingulate gyrus	L. cingulate gyrus	0.52	−0.72	−0.61	$t(34) = 4.5\ p \leq 0.01$	0.53	≤0.01
L. cingulate gyrus	L. pre-cuneus	0.45	−0.82	−0.61	$t(32) = 4.3\ p \leq 0.01$	0.39	0.02
L. middle temporal gyrus	L. inferior temporal gyrus	−0.40	0.62	0.50	$t(34) = 3.3\ p \leq 0.01$	−0.25	≤0.01
CTL vs. SCD							
L. thalamus	L. parahipppocampal gyrus	0.50	−0.55	−0.54	$t(38) = 3.9\ p \leq 0.01$	0.46	≤0.01
R. gyrus rectus	L. middle orbito-frontal gyrus	0.63	−0.73	−0.68	$t(37) = 5.6\ p \leq 0.01$	0.35	0.03
R. middle orbito-frontal gyrus	L. middle orbito-frontal gyrus	0.48	−0.59	−0.54	$t(36) = 3.8\ p \leq 0.01$	0.28	0.09
L. middle orbito-frontal gyrus	R. subcallosal gyrus	0.54	−0.63	−0.58	$t(39) = 4.5\ p \leq 0.01$	0.42	≤0.01
L. middle orbito-frontal gyrus	L. subcallosal gyrus	0.58	−0.71	−0.64	$t(38) = 5.1\ p \leq 0.01$	0.27	0.1

Connectivity between ROI-1 and ROI-2 that was statistically significant after FDR correction in multilinear and permutation models controlling for the group, sex, and age (log-transformed). The upper and lower sections of the table show the statistics for FA when comparing healthy controls (CTL) with non-SCD and SCD patients. No conections were statistically significat when comparing non-SCD with SCD patients.

*Mean group FA controlled for sex and age (log-transformed) along the volumetric white matter surface connecting these ROIs. Standardized (unitless) values are shown.

†Point-biserial correlation coefficient and results of the unpaired two-samples t-test on the *FA values between groups.

¥Pearson correlation of *FA with Hb and the correspondent p-value is also displayed.

WMSs were statistically significant for non-SCD and 4 of 5 WMSs for SCD patients.

Discussion

We observed potential microstructural differences along WMSs in both groups of patients with CA with and without SCD compared to controls. These results predominantly showed lower FA values in CA patients, indicating the loss of coherence in the main diffusion direction, which could indicate WM injury. Lower FA was highly associated with decreasing Hb levels revealing that the decreased microstructural integrity found in CA patients is highly driven by chronic hypoxia.

Previous work in CA has shown that the whole brain increases cerebral blood flow (CBF) to compensate for the loss of oxygen-carrying capacity (21, 24, 55–57). This offset in CBF preserves total resting oxygen delivery to the whole brain (21, 22, 55, 58), such that the correspondent oxygen extraction fraction (OEF) from the cerebral cortex seems to be normal or even reduced (26, 59–62). Although resting oxygen delivery is preserved in the cortex, the cerebral vascular reserve is diminished, proportional to the resting hyperemia (23, 25), potentially leaving the brain vulnerable to acute insults such as nighttime hypoxia, acute anemia, and fever. However, while there have been some reports of reduced cortical and subcortical GM volumes (9, 63, 64) in patients not receiving chronic transfusion or hydroxyurea treatment, total GM volume appears preserved (65) in clinically asymptomatic SCD patients (adolescents and young adults) treated with either therapy suggesting that cortical volume loss may not manifest until later in life.

However, regions of deep WM do not seem to have the same metabolic stability during periods of ischemic stress. OEF is increased in the deep watershed areas (56), colocalizing with WM injury patterns (18, 58, 66). Chai et al. (58) showed that independently of disease state, CBF and oxygen delivery to regions of deep WM and border zone regions are considerably smaller than those measured elsewhere within the WM and GM. Furthermore, Wang et al. (30) showed that elevated CBF can be associated with normal-appearing (i.e., infarct-free) WM disruption. Inadequate resting oxygen delivery in the WM is further compounded by blunted cerebrovascular reserve (25).

Thus, chronic hypoperfusion plays a role in the development of the entire WM damage phenotype, including hyperintensities on the T2 FLAIR (17, 18, 58, 66, 67), reduced WM volume (15, 27, 68) or changes in diffusion metrics (12–14, 16, 29, 30).

Based on this consideration, the damage observed in non-SCD patients compared with controls followed an intuitive pattern located primarily in the frontal-parietal WM watershed areas (Table 2). Watershed areas are regions in the brain that sit

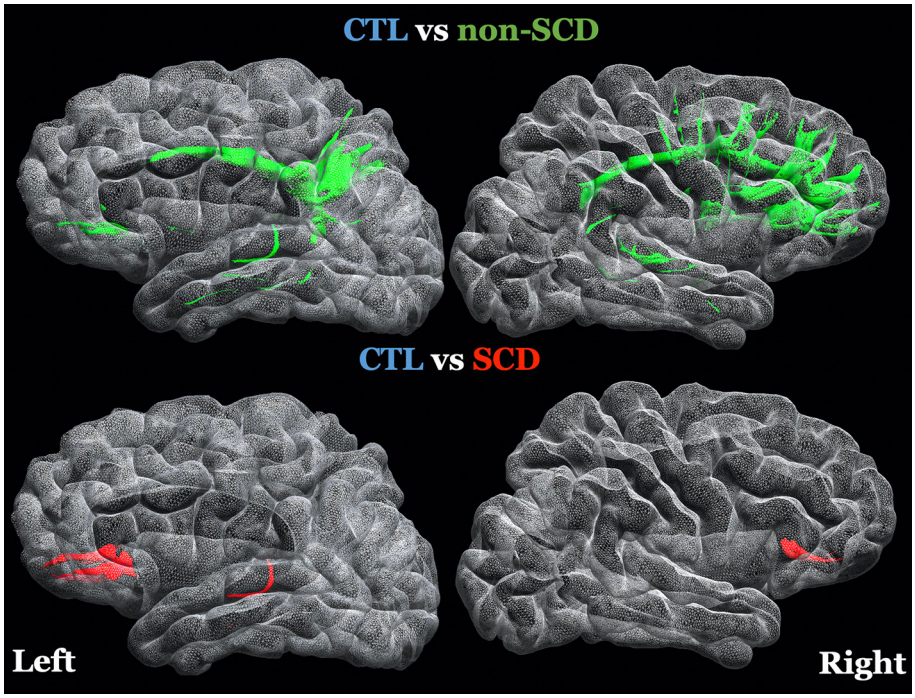


FIGURE 2
3D-rendering of left and right hemispheres of a representative subject, the white matter surfaces (WMSs) where FA was controlled for age (log transformed), sex and group and it was statistically significant in both statistical models (linear regression and permutation analysis) after FDR correction. The specific regions of interest are listed on Table 2. Top Row: green WMSs, comparison of healthy controls (CTL) with non-sickle cell anemia (non-SCD). Bottom row: red WMSs, comparison of CTL with sickle cell anemia (SCD).

in-between major cerebral arterial territories and are the most susceptible to hypoxic-ischemic damage when a supply-demand mismatch occurs in the cerebrovascular supply (18, 58, 69).

While most of the affected connections were unilateral, WMSs observed with lower FA in non-SCD patients appeared similarly distributed between the two hemispheres (Figure 2). These results are consistent with the spatial patterns of lower WM volume associated with the severity of anemia diffusely across frontal, parietal lobes, and temporal lobes especially in these watershed areas (15, 68).

Given that most of the WMSs survived when controlling for a hemolysis marker (Table 3), the results are also aligned to a model of global hypoxia that will usually cause diffuse, bilateral brain injury as seen in patients in drowning accidents, cardiac arrest, or bilateral carotid stenosis, in contrast to more localized and asymmetric injury patterns caused by embolic stroke (70). Therefore, we suggest that the injury pattern in WM microstructure of non-SCD patients can indicate global chronic hypoxia driven by anemia's effect on the brain's hemodynamics. A possible explanation is that the vascular architecture providing blood perfusion to WM areas is the long-penetrating medullary arteries with poor collateralization. Consequently, WM is especially vulnerable to hyperintensities development under focal ischemic events or periods of acute stress (71).

For SCD patients and healthy controls, three out of five connections crossed to the contralateral side. Interhemispheric involvement is consistent with previous results from our laboratory showing lower FA in the corpus callosum in CA patients (higher burden on SCD) (29). There are similar observations on SCD in studies performed in Tanzania (16), the United Kingdom (14), and the United States (30). Kawadler et al. (13) also showed associations between microstructural properties in the corpus callosum with daytime oxygen saturation and Hb levels in SCD patients, indicating that hypoxia related at least in part to low hemoglobin in SCD patients drives the WM injury patterns.

Previous DTI studies in SCD patients have also reported widespread FA decrease in the WM (12–14, 30). Surprisingly, we did not observe this extent of systematic FA derangements. This difference possibly reflects the variability in disease expression in our SCD cohort compared to previous reports; 7 subjects had SC genotype, and 5 of the 12 SS patients were receiving chronic transfusions. While SC and chronically transfused patients develop WM hyperintensities, the phenotype of their WM disease is less severe than nontransfused SS and Sβ^0 (72), and may even result from different mechanisms. Furthermore, the mean hemoglobin F fractions among the nontransfused SS patients was 18%, suggesting good response to hydroxyurea. Our

TABLE 3 Results of FA when controlling for transfusion status and lactose dehydrogenase (LDH).

ROI-1	ROI-2	T-statictic	
		Transfusion♦	LDH◊
CTL vs non-SCD			
R. caudate nucleus	R. middle frontal gyrus	-	-
R. thalamus	R. middle frontal gyrus	$t(30) = 3.0\ p \leq 0.01$	$t(30) = 3.4\ p \leq 0.01$
R. thalamus	R. amygdala	-	$t(34) = 3.5\ p \leq 0.01$
L. thalamus	L. gyrus rectus	-	-
L. thalamus	L. parahipppocampal gyrus	-	-
R. superior frontal gyrus	R. cingulate gyrus	$t(34) = 2.9\ p \leq 0.01$	$t(34) = 3.5\ p \leq 0.01$
R. transvers frontal gyrus	R. subcallosal gyrus	-	$t(31) = 4.1\ p \leq 0.01$
R. cingulate gyrus	L. cingulate gyrus	$t(34) = 2.7\ p \leq 0.01$	$t(34) = 4.0\ p \leq 0.01$
L. cingulate gyrus	L. pre-cuneus	$t(32) = 2.3\ p \leq 0.01$	$t(32) = 3.8\ p \leq 0.01$
L. middle temporal gyrus	L. inferior temporal gyrus	-	$t(34) = -3.2\ p \leq 0.01$
CTL vs SCD			
L. thalamus	L. parahipppocampal gyrus	$t(38) = 3.9\ p \leq 0.01$	-
R. gyrus rectus	L. middle orbito-frontal gyrus	$t(37) = 5.6\ p \leq 0.01$	$t(37) = 4.1\ p \leq 0.01$
R. middle orbito-frontal gyrus	L. middle orbito-frontal gyrus	$t(36) = 3.8\ p \leq 0.01$	$t(36) = 2.8\ p \leq 0.01$
L. middle orbito-frontal gyrus	R. subcallosal gyrus	$t(39) = 4.5\ p \leq 0.01$	$t(39) = 3.2\ p \leq 0.01$
L. middle orbito-frontal gyrus	L. subcallosal gyrus	$t(38) = 5.1\ p \leq 0.01$	$t(38) = 4.1\ p \leq 0.01$

Connectivity between ROI-1 and ROI-2 that was statistically significant after FDR correction in the multilinear model. For consistency same ROIs are displayed as in Table 1. The upper and lower sections of the table show the statistics for FA when comparing healthy controls (CTL) with non-SCD and SCD patients. Similar to Table 2, no conections were statistically significat when comparing non-SCD with SCD patients.

♦Unpaired two-sample t-test on FA values controlled for the group, sex, age (log-transformed), and transfusion status.

◊Unpaired two-sample t-test on FA values controlled for the group, sex, age (log-transformed), and LDH values.

non-SCD and SCD cohorts were matched for hemoglobin level, so one could reasonably have expected a similar spectrum of disease. Nevertheless, this is a cross-sectional study of young adults, and there is a possibility that exposure to severely reduced arterial oxygen content prior to treatment irreversibly affected brain microstructure during brain development in transfusion-dependent non-SCD patients.

Additionally, Table 3 shows almost no contribution from monthly transfusions or LDH in the WMSs found in SCD patients. Possibly, the distribution of WMS in SCD and non-SCD looks substantially different due to uncontrolled confounders, such as chronic pain reported extensively as a burden for SCD patients (73, 74).

In childhood, SCD patients might have an intermittent pain phenotype. Around 50% of the cases evolve as a chronic pain syndrome in adulthood, with periods of lower and higher pain correlated with the ongoing vaso-occlusion (75). Table 2 shows a significant involvement of the orbito-frontal gyrus, which has been implicated in the modulation of chronic pain (76–78) and pain-related emotions (79). Furthermore, functional imaging studies have shown that regions like the thalamus and the parahippocampal gyrus, also depicted in Table 2, belong to the functional pain network (80, 81). In particular, the thalamus has been identified as a central region that processes pain (82). Anemia, by itself, is a robust biomarker of disease severity in SCD, so it is not surprising that hemoglobin levels correlate with FA in pain circuits.

Several research groups have also shown neurocognitive decline in patients with CA, suggesting a possible and early involvement of the brain even in the absence of overt strokes (83, 84). Many significant WMSs were in the prefrontal cortex (Figure 2), where WM abnormality has been associated with negative effects on neurocognitive function in CA patients (14, 15, 29). Specifically, Chai et al. reported that lower FA in the corpus callosum was associated with lower scores across nine neurocognitive measures. At the same time, Stotesbury et al. (14) found that white matter microstructural properties were associated with processing speed, where FA was the strongest predictor. Additional work in our laboratory has previously demonstrated that lower WM volume predicted low matrix reasoning scores, a measure of executive function, in CA patients and identified changes in resting-state fMRI activity in the orbitofrontal and subcallosal gyri (11). Altogether, microstructural injury patterns indicated in CA patients driven by low Hb levels may have cognitive consequences.

Limitations

The small sample size limited our study. All participants were part of a larger project on Sickle Cell Disease at Children's Hospital Los Angeles that involved various MRI protocols (75 minutes of total scan time) and was not limited to the dMRI sequence (4 minutes). Our current approach allowed us to include all WM tracks, but it required to have high-quality structural and diffusion data, limiting us to a smaller sample size than described in Chai et al. (29). While significant sex differences have been previously indicated in the study of CA, we could not fully resolve sex disease-specific differences. The use of data from multiple cohorts of CA, whose intrinsic pathophysiology is different, weakens statistical power in the short term but opens the possibility to differentiate and characterize the unique damage induced by individual hemoglobinopathies. In addition, the control subjects were mainly recruited from family members of SCD patients, and they do not necessarily represent the non-SCD population. Consequently, the statistical differences that we found in non-SCD patients (even when controlling for log-age and sex) could be affected by random effects.

Although our contemporaneous hematological investigations are a strength of this study, we did not have previous hemoglobin or any oxygen saturation values. The use of chronic blood transfusion therapy in some of our patients potentially represents a limitation on our findings because no single hemoglobin level characterizes the hypoxic exposure. Furthermore, SCD patients are often placed on chronic transfusions later in life (than non-SCD patients) and their current hemoglobin values do not reflect their lifetime hypoxic exposure. Chronic transfusion is also a complicated therapeutic yielding improvement in erythrocyte deformability and oxygen-carrying capacity but increased viscosity in the microcirculation can potentially worsen the blood flow and oxygen delivery (85). Given our sample size, it would be tough to accurately separate the rheologic and oxygen-carrying capacity effects of red blood cells. Furthermore, the inclusion of transfusion status in the model weakens the statistical power by adding an additional degree of freedom. In addition, we were not able to assess any additional effect of low oxygen saturation on arterial oxygen content and therefore hypoxic exposure.

The constraints associated with using single-shell diffusion images and simple tensor modeling are well documented in the literature, and urge caution to draw firm conclusions from a single tensor metric like FA. This work tried to address some of these limitations by using ERFO ODFs to correctly render crossing fibers and creating WMSs to avoid characterizing connectivity by streamlining counting. However, we recognize that the information provided by FA is limited and that other methods like diffusion kurtosis imaging and neurite orientation dispersion have proven to be more robust to some of the pitfalls of DTI and could provide a more biological explanation of our current observations.

Conclusion

To characterize the effects of CA in white matter, mean FA along the WMSs (surface connecting two ROIs) of chronic anemic patients with sickle and non-sickle anemias were compared with healthy controls. This grouping allowed the isolation of sickle hemoglobin effects in our analysis. Both CA cohorts showed localized FA differences along the WMSs of patients compared with controls but did not show differences between them. However, non-SCD patients manifested bigger systematic FA derangements in the watershed areas that were bilateral and spatially symmetrical. These results suggested that the broad spectrum of variability in disease expression in our sickle cell anemia cohort and uncontrolled confounders of mesostructure integrity affected our ability to detect widespread WM abnormalities as proposed in the literature. Nevertheless, finding interhemispheric WMSs affected in SCD aligns with previous literature reports showing decreased FA in the corpus callosum in CA patients. Recognizing both the differences and the similitudes between CA patients and the affliction that anemia causes in white matter may help develop earlier and more generalized interventions to help overcome the anemia burden.

Data availability statement

The datasets presented in this article are not readily available because the complete data set from this trial will be made available upon reasonable request and with an approved data sharing agreement. Requests to access the datasets should be directed to JW, JWood@chla.usc.edu.

Ethics statement

The studies involving human participants were reviewed and approved by the Institutional Review Board at Children's Hospital Los Angeles in the United States. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

CG-Z, RL, and JW conceived and designed the study. SC, CV, BX, JS, and JW were involved in data acquisition and administrative duties. CG-Z, SC, CV, BX, JS, AJ, RL, and JW contributed to statistical analysis, manuscript writing, and designing figures. AJ, RL, and JW lead the first author's activities

and decisions during the research. JW is the last author of this manuscript. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by National Heart, Lung, and Blood Institute (Grants 1U01-HL-117718-01, 1R01-HL136484-01A1, and 1F31NS106828). The National Center for Research (5UL1-TR000130-05) through the Clinical Translational Science Institute at Children's Hospital Los Angeles. A Research Career Development Fellowship supports Chau Vu from Saban Research Institute at Children's Hospital Los Angeles. Additionally, Philips Healthcare provided support for protocol development and applications engineering on a support-in-kind basis.

Acknowledgments

We want to thank Dr. Matthew T. Borzage for the valuable discussions in the analysis of data and the paper's organization. In addition, we thank Noel Arugay, Dr. Adam Bush, and Dr. Xin Miao for their support with MRI data collection and Silvie Suriany and Honglei Liu for the bloodwork analysis. We also acknowledge Mr. Bertin Valdez and Obdulio Carreras for coordinating the patient study visits and Dr. Tom Hofstra, Dr. Jackie Bascom, Susan Carson, Trish Peterson, and Debbie Harris from the CHLA Hematology Division for their assistance with patient recruitment, and Benita Tamrazi, MD, for her help with radiological readings.

Conflict of interest

Author JW is a Consultant for BluebirdBio, Celgene, Apopharma, WorldcareClinical, and BiomeInformatics.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Chaparro CM, Suchdev PS. Anemia epidemiology, pathophysiology, and etiology in low- and middle-income countries. *Ann N Y Acad Sci.* (2019) 1450:15–31. doi: 10.1111/nyas.14092

2. Kassebaum NJ, GBD Anemia Collaborators. The global burden of anemia. *Hematol Oncol Clin North Am.* (2016) 30:247–308. doi: 10.1016/j.hoc.2015.11.002

3. Tsui AKY, Marsden PA, Mazer CD, Sled JG, Lee KM, Henkelman RM, et al. Differential HIF and NOS responses to acute anemia: defining organ-specific hemoglobin thresholds for tissue hypoxia. *Am J Physiol Regul Integr Comp Physiol.* (2014) 307:13–25. doi: 10.1152/ajpregu.00411.2013

4. Watts ME, Pocock R, Claudianos C. Brain energy and oxygen metabolism: emerging role in normal function and disease. *Front Mol Neurosci.* (2018) 11. doi: 10.3389/fnmol.2018.00216

5. Wolters FJ, Zonneveld HI, Licher S, Cremers LGM, Ikram MK, Koudstaal PJ, et al. Hemoglobin and anemia in relation to dementia risk and accompanying changes on brain MRI. *Neurology.* (2019) 93:917–26. doi: 10.1212/WNL.0000000000008003

6. Angastiniotis M, Eleftheriou A, Galanello R, Harteveld CL, Petrou M, Traeger-Synodinos J, et al. Chapter 2 - epidemiology of hemoglobinopathies. In: Old J, editor. *Prevention of Thalassaemias and Other Haemoglobin Disorders: Volume 1: Principles*. Nicosia: Thalassaemia International Federation (2013).

7. Wahed A, Dasgupta A. Chapter 4 - hemoglobinopathies and thalassemias. In: Wahed A, Dasgupta A, editors. *Hematology and Coagulation.* San Diego: Elsevier (2015). p. 55–80.

8. Metafratzi Z, Argyropoulou MI, Kiortsis DN, Tsampoulas C, Chaliassos N, Efremidis SC. T-2 relaxation rate of basal ganglia and cortex in patients with beta-thalassaemia major. *Br J Radiol.* (2001) 74:407–10. doi: 10.1259/bjr.74.881.740407

9. Kawadler JM, Clayden JD, Kirkham FJ, Cox TC, Saunders DE, Clark CA. Subcortical and cerebellar volumetric deficits in paediatric sickle cell anaemia. *Br J Haematol.* (2013) 163:373–6. doi: 10.1111/bjh.12496

10. Case M, Zhang HS, Mundahl J, Datta Y, Nelson S, Gupta K, et al. Characterization of functional brain activity and connectivity using EEG and fMRI in patients with sickle cell disease. *Neuroimage Clin.* (2017) 14:1–17. doi: 10.1016/j.nicl.2016.12.024

11. Cologner J, Kim Y, Bush A, Choi S, Balderrama MC, Coates TD, et al. Contrasting resting-state fMRI abnormalities from sickle and non-sickle anemia. *PLoS ONE.* (2017) 12:e0184860. doi: 10.1371/journal.pone.0184860

12. Sun B, Brown RC, Hayes L, Burns TG, Huamani J, Bearden DJ, et al. White matter damage in asymptomatic patients with sickle cell anemia: screening with diffusion tensor imaging. *Am J Neuroradiol.* (2012) 33:2043–9. doi: 10.3174/ajnr.A3135

13. Kawadler JM, Kirkham FJ, Clayden JD, Hollocks MJ, Seymour EL, Edey R, et al. White matter damage relates to oxygen saturation in children with sickle cell anemia without silent cerebral infarcts. *Stroke.* (2015) 46:1793–9. doi: 10.1161/STROKEAHA.115.008721

14. Stotesbury H, Kirkham FJ, Kolbel M, Balfour P, Clayden JD, Sahota S, et al. White matter integrity and processing speed in sickle cell anemia. *Neurology.* (2018) 90:E2042–50. doi: 10.1212/WNL.0000000000005644

15. Choi S, O'Neil SH, Joshi AA, Li J, Bush AM, Coates TD, et al. Anemia predicts lower white matter volume and cognitive performance in sickle and non-sickle cell anemia syndrome. *Am J Hematol.* (2019) 94:1055–65. doi: 10.1002/ajh.25570

16. Jacob M, Stotesbury H, Kawadler JM, Lapadaire W, Saunders DE, Sangeda RZ, et al. White matter integrity in tanzanian children with sickle cell anemia a diffusion tensor imaging study. *Stroke.* (2020) 51:1166–73. doi: 10.1161/STROKEAHA.119.027097

17. Fields ME, Mirro AE, Binkley MM, Guilliams KP, Lewis JB, Fellah S, et al. Cerebral oxygen metabolic stress is increased in children with sickle cell anemia compared to anemic controls. *Am J Hematol.* (2022) 97:682–90. doi: 10.1002/ajh.26485

18. Guilliams KP, Fields ME, Ragan DK, Chen Y, Eldeniz C, Hulbert ML, et al. Large-vessel vasculopathy in children with Sickle Cell Disease: a magnetic resonance imaging study of infarct topography and focal atrophy. *Pediatr Neurol.* (2016) 69:49–57. doi: 10.1016/j.pediatrneurol.2016.11.005

19. Usmani A, Machado RF. Vascular complications of sickle cell disease. *Clin Hemorheol Microcirc.* (2018) 68:205–21. doi: 10.3233/CH-189008

20. Russo AG, Ponticorvo S, Tartaglione I, Caiazza M, Roberti D, Elefante A, et al. No increased cerebrovascular involvement in adult beta-thalassemia by advanced MRI analyses. *Blood Cells Mol Dis.* (2019) 78:9–13. doi: 10.1016/j.bcmd.2019.05.001

21. Gevers S, Nederveen AJ, Fijnvandraat K, van den Berg SM, van Ooij P, Heijtel DF, et al. Arterial spin labeling measurement of cerebral perfusion in children with sickle cell disease. *J Magn Reson Imaging.* (2012) 35:779–87. doi: 10.1002/jmri.23505

22. Borzage MT, Bush AM, Choi S, Nederveen AJ, Vaclavu L, Coates TD, et al. Predictors of cerebral blood flow in patients with and without anemia. *J Appl Physiol.* (2016) 120:976–81. doi: 10.1152/japplphysiol.00994.2015

23. Kosinski PD, Croal PL, Leung J, Williams S, Odame I, Hare GMT, et al. The severity of anaemia depletes cerebrovascular dilatory reserve in children with sickle cell disease: a quantitative magnetic resonance imaging study. *Br J Haematol.* (2017) 176:280–7. doi: 10.1111/bjh.14424

24. Guilliams KP, Fields ME, Ragan DK, Eldeniz C, Binkley MM, Chen Y, et al. Red cell exchange transfusions lower cerebral blood flow and oxygen extraction fraction in pediatric sickle cell anemia. *Blood.* (2018) 131:1012–21. doi: 10.1182/blood-2017-06-789842

25. Afzali-Hashemi L, Baas KPA, Schrantee A, Coolen BF, van Osch MJP, Spann SM, et al. Impairment of cerebrovascular hemodynamics in patients with severe and milder forms of sickle cell disease. *Front Physiol.* (2021) 12:645205. doi: 10.3389/fphys.2021.645205

26. Juttukonda MR, Donahue MJ, Waddle SL, Davis LT, Lee CA, Patel NJ, et al. Reduced oxygen extraction efficiency in sickle cell anemia patients with evidence of cerebral capillary shunting. *J Cereb Blood Flow Metab.* (2021) 41:546–60. doi: 10.1177/0271678X20913123

27. Choi S, Leahy RM, Wood JC. Lower white matter volume in beta-thalassemia associated with anemia and cognitive performance. *Am J Hematol.* (2020) 95:E144–6. doi: 10.1002/ajh.25787

28. Mandonnet E, Sarubbo S, Petit L. The nomenclature of human white matter association pathways: proposal for a systematic taxonomic anatomical classification. *Front Neuroanat.* (2018) 12. doi: 10.3389/fnana.2018.00094

29. Chai Y, Ji CR, Coloigner J, Choi S, Balderrama M, Vu C, et al. Tract-specific analysis and neurocognitive functioning in sickle cell patients without history of overt stroke. *Brain Behav.* (2021) 11:e01978. doi: 10.1002/brb3.1978

30. Wang Y, Fellah S, Fields ME, Guilliams KP, Binkley MM, Eldeniz C, et al. Cerebral oxygen metabolic stress, microstructural injury, and infarction in adults with sickle cell disease. *Neurology.* (2021) 97:e902–12. doi: 10.1212/WNL.0000000000012404

31. Sandor S, Leahy R. Surface-based labeling of cortical anatomy using a deformable atlas. *IEEE Trans Med Imaging.* (1997) 16:41–54. doi: 10.1109/42.552054

32. Shattuck DW, Sandor-Leahy SR, Schaper KA, Rottenberg DA, Leahy RM. Magnetic resonance image tissue classification using a partial volume model. *Neuroimage.* (2001) 13:856–76. doi: 10.1006/nimg.2000.0730

33. Shattuck DW, Leahy RM. Automated graph-based analysis and correction of cortical volume topology. *IEEE Trans Med Imaging.* (2001) 20:1167–77. doi: 10.1109/42.963819

34. Joshi AA, Shattuck DW, Thompson PM, Leahy RM. Surface-constrained volumetric brain registration using harmonic mappings. *IEEE Trans Med Imaging.* (2007) 26:1657–69. doi: 10.1109/TMI.2007.901432

35. Joshi AA, Choi S, Liu Y, Chong M, Sonkar G, Gonzalez-Martinez J, et al. A hybrid high-resolution anatomical MRI atlas with sub-parcellation of cortical gyri using resting fMRI. *bioRxiv.* (2021) 2020.2009.2012.294322. doi: 10.1101/2020.09.12.294322

36. Andersson JLR, Graham MS, Zsoldos E, Sotiropoulos SN. Incorporating outlier detection and replacement into a non-parametric framework for movement and distortion correction of diffusion MR images. *Neuroimage.* (2016) 141:556–72. doi: 10.1016/j.neuroimage.2016.06.058

37. Andersson JLR, Sotiropoulos SN. An integrated approach to correction for off-resonance effects and subject movement in diffusion MR imaging. *Neuroimage.* (2016) 125:1063–78. doi: 10.1016/j.neuroimage.2015.10.019

38. Bhushan C, Haldar JP, Choi S, Joshi AA, Shattuck DW, Leahy RM. Co-registration and distortion correction of diffusion and anatomical images based on inverse contrast normalization. *Neuroimage.* (2015) 115:269–80. doi: 10.1016/j.neuroimage.2015.03.050

39. Varadarajan D, Haldar JP. A theoretical signal processing framework for linear diffusion MRI: Implications for parameter estimation and experiment design. *Neuroimage.* (2017) 161:206–18. doi: 10.1016/j.neuroimage.2017.08.048

40. Varadarajan D, Haldar JP. Towards optimal linear estimation of orientation distribution functions with arbitrarily sampled diffusion MRI data. *2018 IEEE 15th International Symposium on Biomedical Imaging (Isbi 2018)*, Washington, DC (2018). p. 743–6.

41. Yeh FC, Verstynen TD, Wang YB, Fernandez-Miranda JC, Tseng WYI. Deterministic diffusion fiber tracking improved by quantitative anisotropy. *PLoS ONE.* (2013) 8:e80713. doi: 10.1371/journal.pone.0080713

42. Jones DK, Knosche TR, Turner R. White matter integrity, fiber count, and other fallacies: The do's and don'ts of diffusion MRI. *Neuroimage* (2013) 73:239–54. doi: 10.1016/j.neuroimage.2012.06.081

43. Soares JM, Marques P, Alves V, Sousa N. A hitchhiker's guide to diffusion tensor imaging. *Front Neurosci.* (2013) 7:31. doi: 10.3389/fnins.2013.00031

44. Lebel C, Beaulieu C. Longitudinal development of human brain wiring continues from childhood into adulthood. *J Neurosci.* (2011) 31:10937–947. doi: 10.1523/jneurosci.5302-10.2011

45. Chang YS, Owen JP, Pojman NJ, Thieu T, Bukshpun P, Wakahiro MLJ, et al. White matter changes of neurite density and fiber orientation dispersion during human brain maturation. *PLoS ONE* (2015) 10:e0123656. doi: 10.1371/journal.pone.0123656

46. Yekutieli D, Benjamini Y. Resampling-based false discovery rate controlling multiple test procedures for correlated test statistics. *J Stat Plan Inference.* (1999) 82:171–96. doi: 10.1016/S0378-3758(99)00041-5

47. Manly BFJ. Randomization and regression methods for testing for associations with geographical, environmental and biological distances between populations. *Res Popul Ecol.* (1986) 28:201–18. doi: 10.1007/BF02515450

48. Manly BFJ. *Randomization, Bootstrap and Monte Carlo Methods in Biology*. New York, NY: Chapman and Hall (2007).

49. Winkler AM, Ridgway GR, Webster MA, Smith SM, Nichols TE. Permutation inference for the general linear model. *Neuroimage.* (2014) 92:381–97. doi: 10.1016/j.neuroimage.2014.01.060

50. R Core Team. *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing (2020).

51. Steinberg MH. Management of sickle cell disease. *N Eng J Med.* (1999) 340:1021–30. doi: 10.1056/NEJM199904013401307

52. Kavanagh PL, Sprinz PG, Vinci SR, Bauchner H, Wang CJ. Management of children with sickle cell disease: a comprehensive review of the literature. *Pediatrics.* (2011) 128:e1552–74. doi: 10.1542/peds.2010-3686

53. Yawn BP, Buchanan GR, Afenyi-Annan AN, Ballas SK, Hassell KL, James AH, et al. Management of sickle cell disease: summary of the 2014 evidence-based report by expert panel members. *JAMA.* (2014) 312:1033–48. doi: 10.1001/jama.2014.10517

54. Enninful-Eghan H, Moore RH, Ichord R, Smith-Whitley K, Kwiatkowski JL. Transcranial doppler ultrasonography and prophylactic transfusion program is effective in preventing overt stroke in children with Sickle cell disease. *J Pediatr.* (2010) 157:479–84. doi: 10.1016/j.jpeds.2010.03.007

55. Bush AM, Borzage MT, Choi S, Vaclavu L, Tamrazi B, Nederveen AJ, et al. Determinants of resting cerebral blood flow in sickle cell disease. *Am J Hematol.* (2016) 91:912–7. doi: 10.1002/ajh.24441

56. Fields ME, Guilliams KP, Ragan DK, Binkley MM, Eldeniz C, Chen Y, et al. Regional oxygen extraction predicts border zone vulnerability to stroke in sickle cell disease. *Neurology.* (2018) 90:e1134–44. doi: 10.1212/WNL.0000000000005194

57. Vu C, Bush A, Choi S, Borzage M, Miao X, Nederveen AJ, et al. Reduced global cerebral oxygen metabolic rate in sickle cell disease and chronic anemias. *Am J Hematol.* (2021) 96:901–13. doi: 10.1002/ajh.26203

58. Chai Y, Bush AM, Coloigner J, Nederveen AJ, Tamrazi B, Vu C, et al. White matter has impaired resting oxygen delivery in sickle cell patients. *Am J Hematol.* (2019) 94:467–74. doi: 10.1002/ajh.25423

59. Bush AM, Coates TD, Wood JC. Diminished cerebral oxygen extraction and metabolic rate in sickle cell disease using T2 relaxation under spin tagging MRI. *Magn Reson Med.* (2018) 80:294–303. doi: 10.1002/mrm.27015

60. Croal PL, Leung J, Phillips CL, Serafin MG, Kassner A. Quantification of pathophysiological alterations in venous oxygen saturation: a comparison of global MR susceptometry techniques. *Magn Reson Imaging.* (2019) 58:18–23. doi: 10.1016/j.mri.2019.01.008

61. Juttukonda MR, Donahue MJ, Davis LT, Gindville MC, Lee CA, Patel NJ, et al. Preliminary evidence for cerebral capillary shunting in adults with sickle cell anemia. *J Cereb Blood Flow Metab.* (2019) 39:1099–110. doi: 10.1177/0271678X17746808

62. Li W, Xu X, Liu P, Strouse JJ, Casella JF, Lu H, et al. Quantification of whole-brain oxygenation extraction fraction and cerebral metabolic rate of oxygen consumption in adults with sickle cell anemia using individual T2-based oxygenation calibrations. *Magn Reson Med.* (2020) 83:1066–80. doi: 10.1002/mrm.27972

63. Mackin RS, Insel P, Truran D, Vichinsky EP, Neumayr LD, Armstrong FD, et al. Neuroimaging abnormalities in adults with sickle cell anemia: associations with cognition. *Neurology.* (2014) 82:835–41. doi: 10.1212/WNL.0000000000000188

64. Kim JA, Leung J, Lerch JP, Kassner A. Reduced cerebrovascular reserve is regionally associated with cortical thickness reductions in children with sickle cell disease. *Brain Res.* (2016) 1642:263–9. doi: 10.1016/j.brainres.2016.03.041

65. Choi S, Bush AM, Borzage MT, Joshi AA, Mack WJ, Coates TD, et al. Hemoglobin and mean platelet volume predicts diffuse T1-MRI white matter volume decrease in sickle cell disease patients. *Neuroimage Clin.* (2017) 15:239–46. doi: 10.1016/j.nicl.2017.04.023

66. Ford AL, Ragan DK, Fellah S, Binkley MM, Fields ME, Guilliams KP, et al. Silent infarcts in sickle cell disease occur in the border zone region and are associated with low cerebral blood flow. *Blood.* (2018) 132:1714–23. doi: 10.1182/blood-2018-04-841247

67. Musallam KM, Taher AT, Karimi M, Rachmilewitz EA. Cerebral infarction in β-thalassemia intermedia: breaking the silence. *Thromb Res.* (2012) 130:695–702. doi: 10.1016/j.thromres.2012.07.013

68. Baldeweg T, Hogan AM, Saunders DE, Telfer P, Gadian DG, Vargha-Khadem F, et al. Detecting white matter injury in sickle cell disease using voxel-based morphometry. *Ann Neurol.* (2006) 59:662–72. doi: 10.1002/ana.20790

69. Ropper AH, Klein J, Samuels MA, Prasad S. *Adams and Victor's Principles of Neurology*. New York, N.Y: McGraw-Hill Education LLC (2019).

70. Caplan L. *Stroke*. Cary: Oxford University Press, Incorporated (2010).

71. Sam K, Conklin J, Holmes KR, Sobczyk O, Poublanc J, Crawley AP, et al. Impaired dynamic cerebrovascular response to hypercapnia predicts development of white matter hyperintensities. *Neuroimage Clin.* (2016) 11:796–801. doi: 10.1016/j.nicl.2016.05.008

72. Quinn CT. Minireview: clinical severity in sickle cell disease: the challenges of definition and prognostication. *Exp Biol Med.* (2016) 241:679–88. doi: 10.1177/1535370216640385

73. Novelli EMMD, Gladwin MTMD. Crises in sickle cell disease. *Chest.* (2016) 149:1082–93. doi: 10.1016/j.chest.2015.12.016

74. Darbari DS, Sheehan VA, Ballas SK. The vaso-occlusive pain crisis in sickle cell disease: definition, pathophysiology, and management. *Eur J Haematol.* (2020) 105:237–46. doi: 10.1111/ejh.13430

75. Field JJ. Five lessons learned about long-term pain management in adults with sickle cell disease. *Hematol Am Soc Hematol Edu Prog.* (2017) 2017:406–11. doi: 10.1182/asheducation-2017.1.406

76. Dang YH, Zhao Y, Xing B, Zhao XJ, Huo FQ, Tang JS, et al. The role of dopamine receptors in ventrolateral orbital cortex-evoked anti-nociception in a rat model of neuropathic pain. *Neuroscience.* (2010) 169:1872–80. doi: 10.1016/j.neuroscience.2010.06.050

77. Vachon-Presseau E, Tétreault P, Petre B, Huang L, Berger SE, Torbey S, et al. Corticolimbic anatomical characteristics predetermine risk for chronic pain. *Brain.* (2016) 139:1958–70. doi: 10.1093/brain/aww100

78. Ong W-Y, Stohler CS, Herr DR. Role of the prefrontal cortex in pain processing. *Mol Neurobiol.* (2019) 56:1137–66. doi: 10.1007/s12035-018-1130-9

79. Sheng H-Y, Lv S-S, Cai Y-Q, Shi W, Lin W, Liu T-T, et al. Activation of ventrolateral orbital cortex improves mouse neuropathic pain–induced anxiodepression. *JCI Insight.* (2020) 5:e133625. doi: 10.1172/jci.insight.133625

80. Smallwood RF, Laird AR, Ramage AE, Parkinson AL, Lewis J, Clauw DJ, et al. Structural brain anomalies and chronic pain: a quantitative meta-analysis of gray matter volume. *J Pain.* (2013) 14:663–75. doi: 10.1016/j.jpain.2013.03.001

81. Zhang B, Jung M, Tu Y, Gollub R, Lang C, Ortiz A, et al. Identifying brain regions associated with the neuropathology of chronic low back pain: a resting-state amplitude of low-frequency fluctuation study. *Br J Anaesth.* (2019) 123:e303–11. doi: 10.1016/j.bja.2019.02.021

82. Schweinhardt P, Bushnell MC. Pain imaging in health and disease - how far have we come? *J Clin Invest.* (2010) 120:3788–97. doi: 10.1172/JCI43498

83. Berkelhammer LD, Williamson AL, Sanford SD, Dirksen CL, Sharp WG, Margulies AS, et al. Neurocognitive sequelae of pediatric sickle cell disease: a review of the literature. *Child Neuropsychol.* (2007) 13:120–31. doi: 10.1080/09297040600800956

84. Elalfy MS, Aly RH, Azzam H, Aboelftouh K, Shatla RH, Tarif M, et al. Neurocognitive dysfunction in children with beta thalassemia major: psychometric, neurophysiologic and radiologic evaluation. *Hematology.* (2017) 22:617–22. doi: 10.1080/10245332.2017.1338212

85. Detterich JA. Simple chronic transfusion therapy, a crucial therapeutic option for sickle cell disease, improves but does not normalize blood rheology: What should be our goals for transfusion therapy? *Clin Hemorheol Microcirc.* (2018) 68:173–86. doi: 10.3233/CH-189006

frontiers | Frontiers in Neurology

TYPE Original Research
PUBLISHED 14 September 2022
DOI 10.3389/fneur.2022.846596

OPEN ACCESS

EDITED BY
Andria Ford,
Washington University in St. Louis,
United States

REVIEWED BY
Marialuisa Zedde,
IRCCS Local Health Authority of
Reggio Emilia, Italy
Fenella Jane Kirkham,
University College London,
United Kingdom

*CORRESPONDENCE
Françoise Bernaudin
francoise.bernaudin@chicreteil.fr

SPECIALTY SECTION
This article was submitted to
Neuroepidemiology,
a section of the journal
Frontiers in Neurology

RECEIVED 31 December 2021
ACCEPTED 08 August 2022
PUBLISHED 14 September 2022

CITATION
Bernaudin F, Arnaud C, Kamdem A, Hau I, Madhi F, Jung C, Epaud R and Verlhac S (2022) Incidence, kinetics, and risk factors for intra- and extracranial cerebral arteriopathies in a newborn sickle cell disease cohort early assessed by transcranial and cervical color Doppler ultrasound. *Front. Neurol.* 13:846596. doi: 10.3389/fneur.2022.846596

Incidence, kinetics, and risk factors for intra- and extracranial cerebral arteriopathies in a newborn sickle cell disease cohort early assessed by transcranial and cervical color Doppler ultrasound

Françoise Bernaudin[1,2]*, Cécile Arnaud[1], Annie Kamdem[1], Isabelle Hau[3], Fouad Madhi[3], Camille Jung[2], Ralph Epaud[3] and Suzanne Verlhac[4]

[1]Department of Pediatrics, Referral Center for Sickle Cell Disease, Intercommunal Créteil Hospital, University Paris Est, Créteil, France, [2]Clinical Research Center, Intercommunal Créteil Hospital, University Paris Est, Créteil, France, [3]Department of Pediatrics, Intercommunal Créteil Hospital, University Paris Est, Créteil, France, [4]Department of Medical Imaging, Referral Center for Sickle Cell Disease, Intercommunal Créteil Hospital, Créteil, France

The risk of stroke in children with sickle cell disease (SCD) is detected by abnormal intracranial arterial time-averaged mean of maximum velocities (TAMVs ≥200 cm/s). Recently, extracranial internal carotid artery (eICA) arteriopathy has been reported, and a cross-sectional study showed that eICA-TAMVs ≥160 cm/s are significantly associated with eICA kinkings and stenosis. The cumulative incidence of and predictive risk factors for intracranial arteriopathy are well described in sickle cell anemia (SCA=SS/Sβ0) but are lacking for SC/Sβ+ children, as is the cumulative incidence of eICA arteriopathy. We report a prospective longitudinal cohort study including 493 children with SCD (398 SCA, 95 SC/Sβ+), all assessed by transcranial and cervical color Doppler ultrasound. Cerebral MRI/MRA data were available in 375 children with SCD and neck MRA in 365 children. eICA kinkings were defined as eICA tortuosities on neck MRA, with an internal acute angle between the two adjacent segments <90°. The median follow-up was 10.6 years. The cumulative incidence of kinkings was significantly lower in SC/Sβ+ children than in children with SCA, and no SC/Sβ+ child developed intra- or extracranial stenotic arteriopathy. The 10-year KM estimate of cumulative incidence (95% CI) for eICA-TAMVs ≥160 cm/s revealed its development in the 2nd year of life in children with SCA, reaching a plateau of 17.4% (13.2–21.6%) by about 10 years of age, while the plateau for eICA stenosis was 12.3% (8.3–16.3%). eICA assessment identified 13.5% (9.3–17.7%) patients at risk of stroke who were not detected by transcranial color Doppler ultrasound. We also show, for the first time, that in addition to a congenital origin, eICA kinkings

sin patients with SCD can develop progressively with aging as a function of eICA-TAMVs, themselves related to anemia severity. Ongoing hydroxyurea treatment was significantly associated with a lower risk of abnormal intracranial arteriopathy and eICA kinkings. After adjustment with hydroxyurea, baseline low hemoglobin, high reticulocyte, and WBC counts remained independent risk factors for intracranial arteriopathy, while low hemoglobin and SEN β-haplotype number were independent risk factors for extracranial arteriopathy. The association between extracranial arteriopathy and SEN β-haplotype number suggested a genetic link between the ethnic origin and incidence of eICA kinkings. This prospective cohort study shows the importance of systematically assessing the eICA and of recording biological parameters during the 2nd year of life before any intensive therapy to predict the risk of cerebral arteriopathy and treat patients with severe baseline anemia.

KEYWORDS

sickle cell disease/anemia, transcranial and cervical color-Doppler, ultrasound, cerebral MRI/MRA, cerebral arterial stenosis, neck-MRA, hydroxyurea, silent cerebral infarct

Introduction

The cumulative incidence of and predictive risk factors for intracranial arteriopathy have been well described in children with sickle cell anemia (SCA; i.e., SS, Sβ0, and SDPunjab) but are lacking for SC/Sβ+ children. Strokes are most often associated with stenosis of the large arteries of the intracranial anterior circulation (1). Transcranial Doppler (TCD) screening assesses time-averaged mean of maximum velocities (TAMVs) in the middle cerebral artery (MCA), anterior cerebral artery (ACA), and internal carotid artery (ICA) (2, 3). Abnormal high TAMVs ($\geq$200 cm/s) identify patients with a 40% stroke risk within 36 months (4) and are associated with a risk of silent cerebral infarcts (SCI) (5–7). In 1998, prophylactic red cell chronic transfusion programs were shown to significantly reduce the incidence of strokes among patients at risk (STOP-I) (8).

While the involvement of the circle of Willis is certainly predominant in SCA cerebral vasculopathy, less attention has been given to the extracranial internal carotid artery (eICA), which can be the site of stenosis and/or occlusion and is also responsible for overt strokes and SCI (9–13). Contrary to intracranial arteriopathy detectable by TCD *via* a temporal window and by cerebral MRA, assessing the eICA requires using a color Doppler ultrasound through a submandibular approach (11) and neck MRA (9–14). In two large cohorts of stroke-free children with SCA, we reported in a cross-sectional study (14) that eICA-TAMVs $\geq$160 cm/s are highly associated with kinkings and eICA stenosis (14), which were independent risk factors for SCI, along with acute and chronic anemia (7).

Since this first description, there has been no report on the cumulative incidence of extracranial cerebral arteriopathy during childhood and the associated predictive risk factors. The aim of the present study was to evaluate the cumulative incidence of and predictive risk factors for extracranial cerebral arteriopathy and to compare them with cumulative incidence of and predictive risk factors for intracranial arteriopathy in a newborn SCD (SCA & SC/Sβ+) cohort, longitudinally assessed for cerebral intra- and extracranial arteriopathies.

Methods and patients

Since 1992, our center has systematically assessed patients as soon as 18 months of age by using a transcranial color Doppler ultrasound machine (14). The assessment of the eICA has been performed *via* submandibular windows using the same low-frequency probe as for the TCD since June 2011 (14). Starting in May 1993, MRI/MRA with a 1.5T magnet with FLAIR, T1, T2, SWI, diffusion-weighted sequences, circle of Willis, and 3D time-of-flight (TOF) angiography were performed without sedation every 2 years in children older than 5 years, but earlier in patients on chronic transfusion for abnormal TCD and systematically before hematopoietic stem cell transplantation. Neck 3D TOF angiography (neck MRA) was added in June 2011 (14). Neck MRA was systematically performed in children older than 5 years but earlier in those with abnormally high eICA velocities. All imaging data were reviewed by the same expert (SV). Arteries were assessed for shape deformations and stenosis, defined as at least a 25% decrease in the lumen based on diameter. Tortuosities can be classified into three types, namely, loop, coiling, and kinking (15, 16). Loops are defined as S- or C-shaped deformities coiling as a circular course, while kinking

is described as a sharp angulation. For the present study, only kinkings with an internal acute angle between the two adjacent segments <90° were retained for analysis. Stenosis was defined as a 25% decrease at least in the lumen of MCA, ICA, ACA, or eICA.

The present cohort included children born between Jan 1988 and Jan 2018 who were followed in the center at least until June 2012 in order to systematically assess annually by TCD and at least once by cervical color Doppler ultrasound. Parental written informed consent was obtained in accordance with the Declaration of Helsinki, and data were prospectively and systematically recorded in the clinical database of the referral center for SCD in Créteil (CNIL, N° 2069568). The use of the database was approved for this cohort study by the Créteil Institutional Review Board.

Indications for intensive therapy were as follows: Hydroxyurea has been prescribed since 1992, initially in patients older than 3 years and experiencing frequent vaso-occlusive crises (VOC) and/or acute chest syndrome (ACS) (17). Since 1998, a subset of patients with an abnormal TCD history but with normalized velocities on chronic transfusion and no stenosis on MRA have been prescribed hydroxyurea (18, 19). Moreover, because of the proven negative effect of anemia on cognitive performance (20), hydroxyurea has also been given to patients with normal TCD but hemoglobin <7 g/dl since 2000. Thereafter, hydroxyurea was also recommended to symptomatic children during the 2nd year of life after their first complete checkup including TCD. More recently, considering the safety and efficacy of the Baby HUG trial (21) and the NIH recommendations (22), hydroxyurea was occasionally prescribed as soon as 9 months of age. Chronic transfusion was recommended in children experiencing at least two acute splenic sequestrations until the recommended age for splenectomy, in those with intracranial or eICA-TAMVs ≥200 cm/s or stenosis on cerebral or neck MRA and in those still experiencing frequent VOC/ACS on hydroxyurea. Transplantation was recommended to patients with a matched sibling donor (MSD) who experienced frequent VOC/ACS despite hydroxyurea or to those with cerebral vasculopathy defined by the presence of intra- or extracranial stenotic arteriopathy, abnormal TAMV, or the presence of ischemic lesions.

The follow-up was from Jan 1988 to Sept 2019. Alpha-genes, beta-globin haplotypes (BEN, CAR, SEN, and others), and G6PD enzymatic activity were recorded. Average biologic parameters were obtained at baseline after the age of 12 months and before the age of 3 years, a minimum of 3 months away from a transfusion, 1 month from a painful episode, and before any intensive therapy.

Statistical analysis

Participant baseline characteristics were summarized through the use of percentages, and mean (standard deviation, SD) or median (range); 95% confidence intervals (95% CIs) around point estimates were computed. Fisher exact tests were used to compare proportions and Mann–Whitney tests to compare continuous distributions.

Birth date defined entry into the study. For Kaplan–Meier estimates of cumulative incidence (probability) of events, the participants were censored on the date of last visit or event, that is, TAMVs ≥200 cm/s for intracranial arteries and ≥160 cm/s for the eICA, or stenosis on cerebral and neck MRA. Failure time data curves were compared across baseline groups by the log-rank test. Because only six deaths occurred among 493 patients, they were not considered competing risks for studied events but treated as noninformative censoring observations at the time of death, similar to children who had not experienced any event of interest and were censored at the time of their last visit. The presence or not of ongoing treatment by hydroxyurea for at least 6 months was recorded for each event in each patient.

Association between outcomes and baseline variables was assessed using Cox regression with the estimated hazards ratio (HR) and 95% CI and adjusted with ongoing hydroxyurea treatment. The absence of violation of the proportional hazard assumption was checked in the analyses. Univariable models were fitted, and variables associated with the outcome at the 20% level were retained for introduction into a multivariable model, except for those with strong correlation such as hematocrit with hemoglobin and neutrophils with the WBC count. Multivariate analyses used a stepwise selection process that consists of a series of alternating forward selection and backward elimination steps. All statistical tests were two-sided, with p-values of 0.05 or less denoting statistical significance.

Statistical analyses were performed with SPSS version 24, R version 4.0.2, and MedCalc (Belgium) software packages.

Results

This study included 493 children (238 F, 255 M) with SCD [398 SCA (385 SS, 10 Sβ0, and three SDPunjab), 65 SC, and 30 Sβ+ patients], all assessed by TCD before 2 years of age and at least once with cervical Doppler. Those born after June 2009 ($N = 205$) were simultaneously assessed by intracranial and cervical Doppler before 2 years of age.

In the overall cohort, the median (range) follow-up was 10.6 (1.1–22.9) years, providing 5,335 patient-years of follow-up. A total of three children with SCA had an ischemic stroke: the first one had an abnormal TCD at 18 months of age (235 cm/s on left MCA) and had a stroke 1 month later, just before the confirmatory TCD; the second one had normal left-sided velocities but no available temporal window on the right side and had a stroke related to severe right MCA stenosis at 4.4 years of age; the third child had normal TCD but developed a febrile ACS at age 4.2 years and experienced a massive

bilateral MCA and ACA thrombosis and died. Overall, five deaths occurred in children with SCA: the first one at age 4.2 years was related to the overt stroke described before, two others were related to sepsis at ages 2 and 7 years, and two occurred after transition at age 19 years related to complicated haploidentical transplant and at age 20 years related to severe ACS; one death occurred in an Sβ+ patient at age 13 years during the course of ACS related to acute pulmonary hypertension with thrombosis of the inferior vena cava. Hydroxyurea was prescribed in 235/398 (59.0%) patients with SCA at the median age of 5.1 (0.7–17.8) years and in 3/95 (3.2%) Sβ+ patients at 3, 4, and 6 years. Indications for hydroxyurea were frequent VOC/ACS (N = 132), baseline hemoglobin lower than 7 g/dL (N = 28), a history of TAMV ≥ 200 cm/s after normalization on chronic transfusion, and no stenosis (N = 75). Chronic transfusion was started in 221 SCA children at a median age of 3.6 (0.5–17.1) years and in two Sβ+ children. Indications for chronic transfusion in children with SCA were abnormal TAMVs ≥ 200 cm/s (N = 116) in intracranial (N = 97) or in extracranial arteries (N = 19), intracranial stenosis or eICA stenosis not associated with abnormal TAMVs ≥ 200 cm/s (N = 25), and recurrent splenic sequestrations or frequent VOC/ACS despite hydroxyurea in the others. The patients with a history of abnormal TAMV placed on chronic transfusion who were switched to hydroxyurea (N = 75) received both until the maximal tolerated dose of hydroxyurea was reached. Stem cell transplantation was performed in 70 patients with SCA from a matched sibling donor (N = 66) or haploidentical donor (N = 4) at a median age of 6.7 (3.2–19.7) years. The main indication for transplantation was the presence of cerebral vasculopathy (N = 40): history of overt stroke (N = 1), intra- or extracranial arteriopathy on MRA (N = 24), abnormal TAMV without stenosis (N = 11), presence of silent cerebral infarcts without stenotic arteriopathy but associated with other complications (N = 6), or hydroxyurea failure to decrease the rate of VOC/ACS or to avoid other complications (N = 30).

Among the 493 patients with SCD, G6PD activity was available in 439 patients, and deficit was present in 63 of them (13.9%). Alpha genes were available in 454 patients, and 167 (36.8%) had a deletion of at least one α-gene. Beta-haplotypes were available in 417 patients, and 268 of them were homozygous: CAR/CAR (N = 131), BEN/BEN (N = 91), and SEN/SEN (N = 46), while the others were heterozygous. Baseline mean (SD) biological parameters of patients with SCA and SC/Sβ+ patients and in patients with CAR/CAR, BEN/BEN, and SEN/SEN are shown (Table 1). Proportions of patients with intra- or extracranial arteriopathy according to genetic markers and mean (SD) biological parameters in each type of arteriopathy are shown in Table 2.

The flowchart of the study is presented in Figure 1.

Comparative cumulative incidence (95% CI) of intra- and extracranial arteriopathies in children with SCD

All data were calculated at 10 years of age (median follow-up).

Intracranial arteriopathy

Intracranial TAMVs ≥200 cm/s

TAMVs ≥200 cm/s were observed in 97 children with SCA at a median age of 3.8 (1.3–8.7) years. The cumulative incidence of intracranial TAMVs ≥200 cm/s was 27.6% (22.8–32.4%) in children with SCA, reaching a plateau by 9–10 years of age, while no SC/Sβ+ child developed abnormal intracranial TAMV (log rank; $p < 0.001$) (Figure 2A).

Intracranial stenosis

Intracranial MRA was available in 375 patients with SCD (321 SCA, 54 SC/Sβ+). Intracranial stenosis by MRA was observed only in 35 children with SCA at the median age of 4.8 (2.2–12.0) years. The cumulative incidence of intracranial stenosis was 11.1% (7.5–14.7%) in children with SCA, while no SC/Sβ+ child developed intracranial stenosis (log rank: p = 0.001) (Figure 2B). It was significantly higher in those with a history of intracranial TAMVs ≥200 cm/s (N = 97): 24.1% (15.1–33.1%) than in the others (N = 278): 4.2% (1.7–6.6%) (log rank; $p < 0.001$) (Figure 2C).

Extracranial arteriopathy

eICA-TAMVs ≥160 cm/s

eICA-TAMVs ≥160 cm/s were observed in 61 of 398 children with SCA and temporarily in one of 95 SC/Sβ+ child. eICA-TAMVs ≥160 cm/s were isolated (without intracranial TAMVs ≥200 cm/s) in 50 of 62 children with SCD. Among the 205 patients with SCD simultaneously assessed with transcranial and cervical color Doppler ultrasound since the 2nd year of life, eICA-TAMVs ≥160 cm/s were observed at the median age of 3.6 (1.3–9.5) years. The cumulative incidence of eICA-TAMVs ≥160 cm/s was 17.4% (13.2–21.6%) in children with SCA and 1.1% (0–3.4%) in SC/Sβ+ children (log rank; $p < 0.001$) (Figure 3A).

In children with SCA, the cumulative incidence of isolated (without intracranial TAMVs ≥200 cm/s) eICA-TAMVs ≥160 cm/s was 14.4% (12.6–16.2%), while that of isolated eICA-TAMVs ≥200 cm/s was 6.2% (3.2–9.2%).

TABLE 1 Baseline biological parameters in SCD patients, according to genotype (SCA vs. SC/Sb+) and beta-haplotype categories.

	Genotype			Beta-Haplotypes			
Biological parameters	**SS/Sb0**	**SC/Sb+**	***P*-value**	**CAR/CAR**	**BEN/BEN**	**SEN/SEN**	**Other**
Hemoglobin level (g/dL)	8.4 ± 1.3	10.4 ± 0.9	*<0.001*	8.0 ± 1.1	8.6 ± 1.3	9.0 ± 1.6	9.2 ± 1.4
Hematocrit (%)	24.8 ± 4.0	29.9 ± 3.1	<0.001	23.8 ± 3.5	25.4 ± 3.9	25.9 ± 5.9	26.7 ± 3.7
Reticulocyte count (10^9/L)	292 ± 116	129 ± 63	*<0.001*	297 ± 104	276± 113	275 ± 120	234 ± 138
WBC count (10^9/L)	13.9 ± 5.0	8.8 ± 2.9	*<0.001*	14.0 ± 5.2	13.7 ± 4.8	13.1 ± 5.1	12.0 ± 5.0
Neutrophil count (10^9/L)	5.3 ± 2.9	3.4 ± 1.5	*<0.001*	5.5 ± 3.2	5.3 ± 3.0	4.6 ± 2.0	4.6 ± 2.5
Platelet count (10^9/L)	341 ± 116	321 ± 99	*NS*	340 ± 120	356 ± 122	329 ± 83	328 ± 108
MCV (fL)	76.0 ± 9.3	66.0 ± 6.4	*<0.001*	74.8 ± 11.0	77.0 ± 7.8	78.3 ± 6.7	71.8 ± 9.4
Bilirubin (micromol/L)	29.7 ± 16.2	13.2 ± 5.1	*<0.001*	31.9 ± 15.3	28.2 ± 20.0	29.6 ± 16.5	21.4 ± 12.9
LDH (IU/L)	710 ± 342	394 ± 151	*<0.001*	828 ± 406	663 ± 282	558 ± 233	549 ± 292
HbF (%)	17.1 ± 7.9	9.0 ± 7.8	*<0.001*	14.1 ± 6.7	18.7 ± 8.2	21.7 ± 7.8	12.1 ± 8.3

Average biologic parameters were obtained at baseline after the age of 12 months and before the age of 3 years, a minimum of 3 months away from a transfusion, 1 month from a painful episode, and before any intensivetherapy.

Except platelet count, which is similar in SCA and SC/Sb+ children, all other parameters are highly significantly ($p < 0.001$) different in both populations, that is, in SCA compared to SC/Sb+ children, hemoglobin and hematocrit were lower, while WBC, neutrophils, reticulocyte counts, MCV, bilirubin, LDH, and HbF% werehigher.

Hemoglobin and hematocrit were significantly lower in patients with CAR/CAR than in those with BEN/BEN ($p = 0.001$) and ($p = 0.005$), respectively, and in patients with SEN/SEN ($p = 0.001$) and ($p = 0.010$), respectively, but were not different between BEN/BEN and SEN/SENpatients.

Hemoglobin F% was significantly lower in patients with CAR/CAR than in patients with BEN/BEN ($p < 0.001$) and SEN/SEN ($p < 0.001$) and was lower in patients with BEN/BEN than in those with SEN/SEN patients but not significantly (p =0.054).

LDH was significantly higher in patients with CAR/CAR than in those with BEN/BEN ($p = 0.004$) and in those with SEN/SEN ($p < 0.001$) and was higher in patients with BEN/BEN than in those with SEN/SEN but not significantly (p =0.051).

Thus, patients with CAR/CAR were the most anemic with the most hemolysis and with the lowest HbF%, while patients with SEN/SEN were the less anemic with the least hemolysis and had the highestHbF%.

10.3389/fneur.2022.846596

TABLE 2 Proportions of patients with SCD with intra- and extracranial arteriopathies according to genetic markers, baseline biologic parameters, and ongoing hydroxyurea treatment for at least 6 months at each event.

		Overall SCD population: SCA and SC/Sb+ ($N = 493$)					
		Proportions					
		Intracranial TAMV≥200 97/493 (19.7%)	**eICA Stenosis** 35/375 (9.3%)	**eICA TAMV≥160** 62/493 (12.6%)	**eICA Tortuosities** 100/365 (30.6%)	**No intra 200& Stenosis** 38/365 (10.4%)	**No eICA≥160** 348/493 (70.6%)
Genetic markers	***N***						
Genotype ($N = 493$)							
SCA	398	97/398 (24.4%)	10.9%	15.3%	33.5%	11.8%	63.8%
SC/Sb+	95	0/95 (0%)	0/39 (0%)	1/95 (1.1%)	11.6%	0%	98.9%
Gender ($N = 493$)							
M	255	18.4%	8.6%	12.5%	33.3%	12.2%	72.9%
F	238	21%	10.1%	12.6%	27.9%	8.7%	68.1%
G6PD activity ($N = 439$)							
normal	376	18.9%	7.0%	12.5%	30.2%	8.6%	71.3%
deficient	63	28.6%	15.6%	9.5%	26.3%	14.0%	65.1%
Alpha-Thalassemia ($N = 454$)							
absent	287	21.6%	10.8%	13.6%	28.6%	11.1%	68.6%
present	167	18.0%	5.0%	12.6%	33.1%	9.6%	71.3%
Beta Haplotype ($N = 417$)							
CAR/CAR	131	28.2%	11.4%	16.8%	30.4%	12.3%	58.0%
BEN/BEN	91	26.4%	10.4%	13.2%	32.3%	10.4%	64.8%
SEN/SEN	46	13%	2.6%	23.9%	45.9%	17.9%	69.6%
Other	149	16.7%	9.4%	9.3%	26.2%	6.3%	76.0%
Biological parameters				**mean ± SD**			
Hemoglobin level (g/dL)	428	7.8 ± 1.2	7.6 ± 1.0	8.2 ± 1.4	8.3 ± 1.3	8.1 ± 1.2	9.2 ± 1.4
Hematocrit (%)	427	23.4 ± 3.5	21.7 ± 5.5	24.2 ± 4.0	24.6 ± 3.7	24.4 ± 3.7	26.9 ± 4.3
Reticulocyte count (10^9/L)	419	345 ± 134	369 ± 131	277 ± 101	284 ± 105	293 ± 99	230 ± 119
WBC count (10^9/L)	428	16.4 ± 5.4	17.7 ± 6.6	13.6 ± 3.9	13.7 ± 4.4	13.7 ± 5.1	11.7 ± 4.7
Neutrophil count (10^9/L)	419	6.3 ± 3.3	6.5 ± 4.0	5.4 ± 2.6	5.0 ± 2.4	4.9 ± 2.8	4.5 ± 2.5
Platelet count (10^9/L)	426	336 ± 130	342 ± 102	314 ± 105	337 ± 112	318 ± 104	337 ± 110
MCV (fL)	424	79.0 ± 7.4	81.9 ± 6.2	76.4 ± 7.7	75.6 ± 7.9	76.7 ± 8.3	71.9 ± 10.0
Bilirubin (mmol/L)	334	34.8 ± 17.7	30.3 ± 14.8	29.9 ± 13.5	29.5 ± 14.4	27.6 ± 10.9	23.0 ± 15.2
LDH (IU/L)	372	858 ± 385	1026 ± 412	662 ± 312	657 ± 313	675 ± 327	586 ± 311
HbF (%)	416	14.2 ± 6.4	14.5 ± 5.5	16.4 ± 8.4	16.5 ± 8.0	16.7 ± 7.2	15.5 ± 9.0
				Proportions			
Hemoglobin < 7g/dL	40	21/40: 52.5%	7/38: 18.4%	11/40: 27.5%	12/35: 34.3%	5/35: 14.3%	11/40: 27.5%
WBC count > 20 x 109/L	38	20/38: 52.6%	8/34: 23.5%	5/38: 13.2%	9/32: 28.1%	5/33: 15.1%	15/38: 39.5%
Reticulocyte count > 400 x 10^9/L	54	21/54: 38.9%	8/45: 17.8%	6/54: 11.1%	13/41: 31.7%	4/43: 9.3%	28/54: 51.9%
				Ongoing hydroxyurea treatment at each event			
	N	143/493	102/375	216/493	169/365	206/365	NA
Event on ongoing HU treatment		12/143: 8.4%	11/102: 5.4%	22/216: 10.2%	36/169: 21.3%	13/206: 6.3%	NA

10.3389/fneur.2022.846596

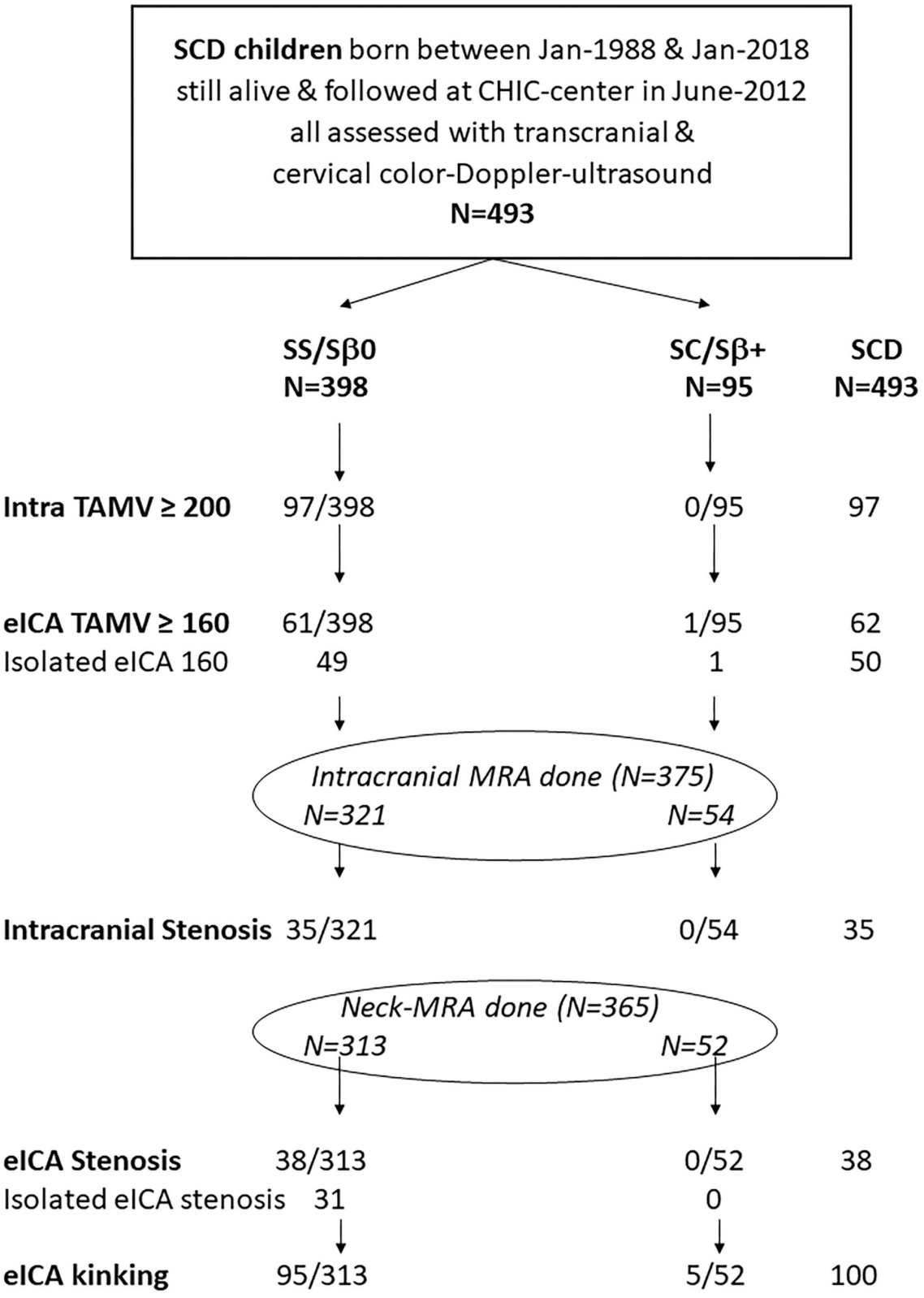


FIGURE 1
Flowchart of the newborn SCD cohort study systematically assessed by transcranial and cervical color Doppler ultrasound.

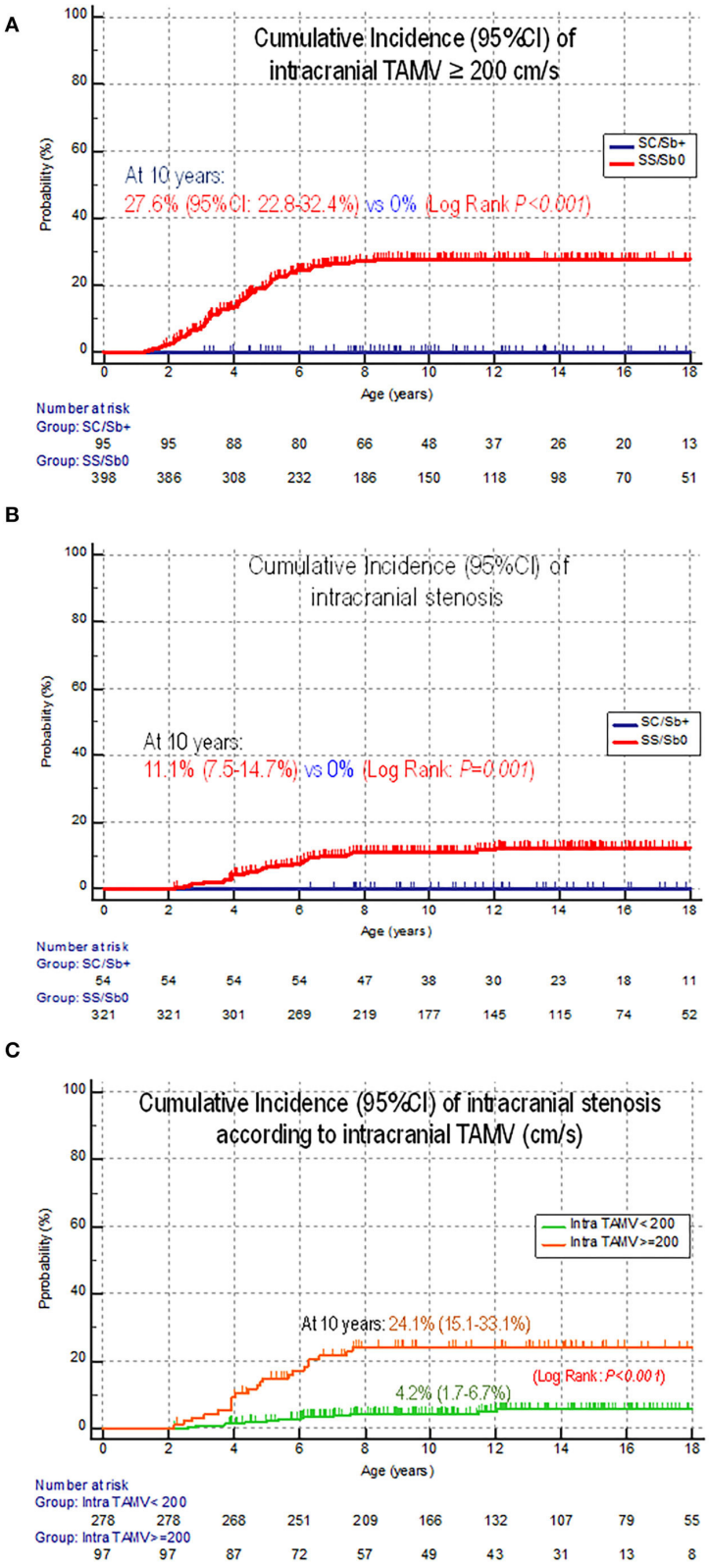


FIGURE 2
Comparative cumulative incidence of intra- and extracranial arteriopathies in SCA vs SC/Sb+ children and according to TAMV. **(A)** Intracranial TAMV ≥ 200 cm/s. **(B)** Intracranial stenosis. **(C)** Intracranial stenosis according to a history of TAMV (< or ≥ 200 cm/s). Of note, among the 13 patients with stenosis but no history of TAMVs ≥200 cm/s, 10 had a history of conditional TAMV (170–199 cm/s), one had no temporal window with underlying severe intracranial arteriopathy, one had a stroke related to extracranial arteriopathy, and no obvious reason was found in one patient.

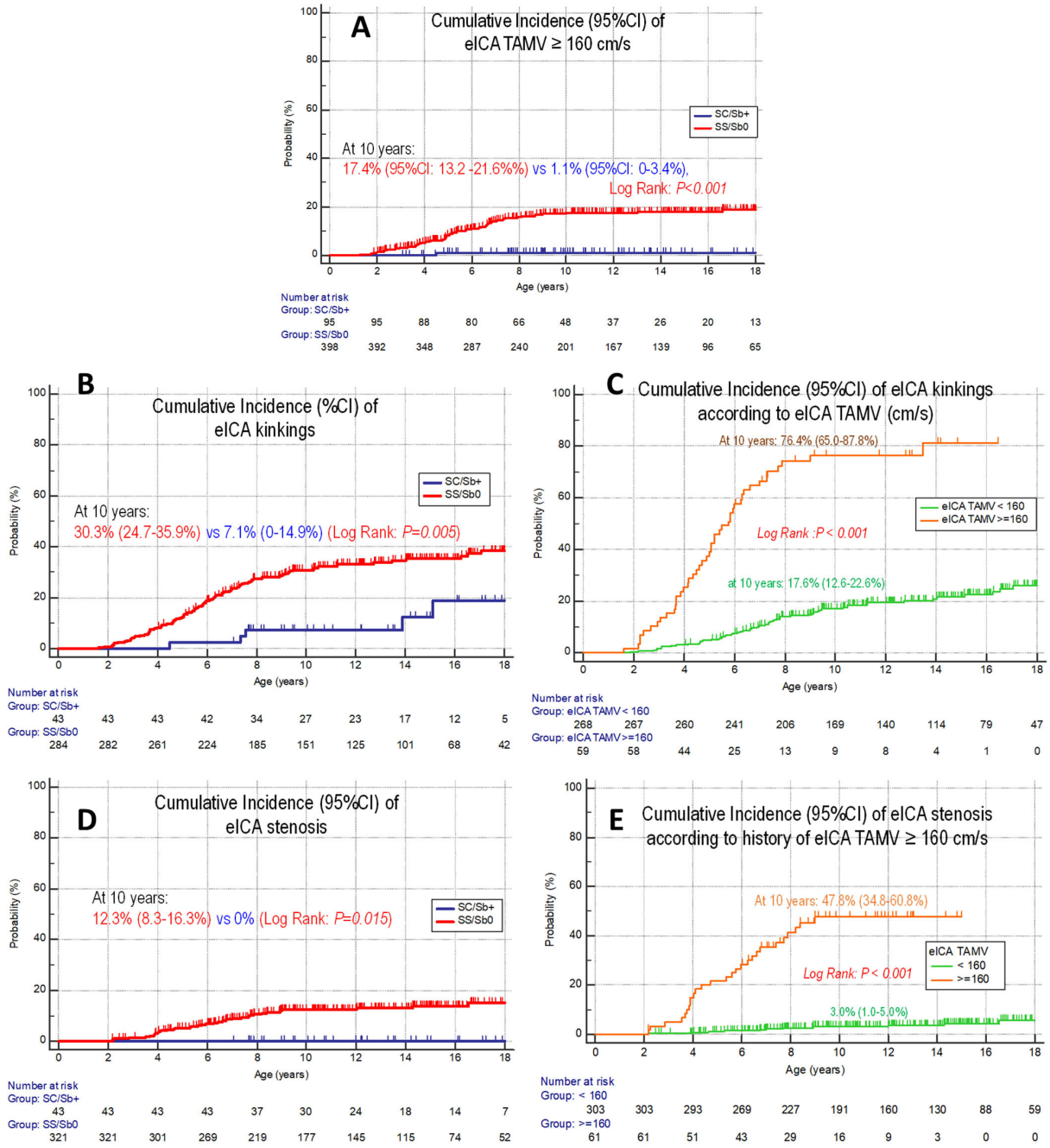


FIGURE 3
Comparative cumulative incidence of eICA arteriopathy in SCA vs SC/Sb+ children and according to TAMV. **(A)** eICA ≥ 160 cm/s. **(B)** eICA kinkings. **(C)** eICA kinkings according to eICA TAMV (< or ≥ 160 cm/s). **(D)** eICA stenosis. **(E)** eICA stenosis according to eICA TAMV (< or ≥ 160 cm/s).

eICA kinkings

eICA kinkings were identified by neck MRA in 95 children with SCA and only in five SC/Sβ+ children. Kinkings were present at the first neck MRA in the majority of children but developed secondarily in 12 SCA- and 1 SC/Sβ+ children. The cumulative incidence of eICA kinkings was 30.3% (24.7–35.9%) in children with SCA and 7.1% (0–14.9%) in SC/Sβ+ patients (log rank; $p = 0.005$) (Figure 3B). In children with a history of eICA-TAMVs ≥160 cm/s, the probability of kinkings was 76.4% (64.9–87.9%) vs 17.1% (12.4-21.8%) in those with

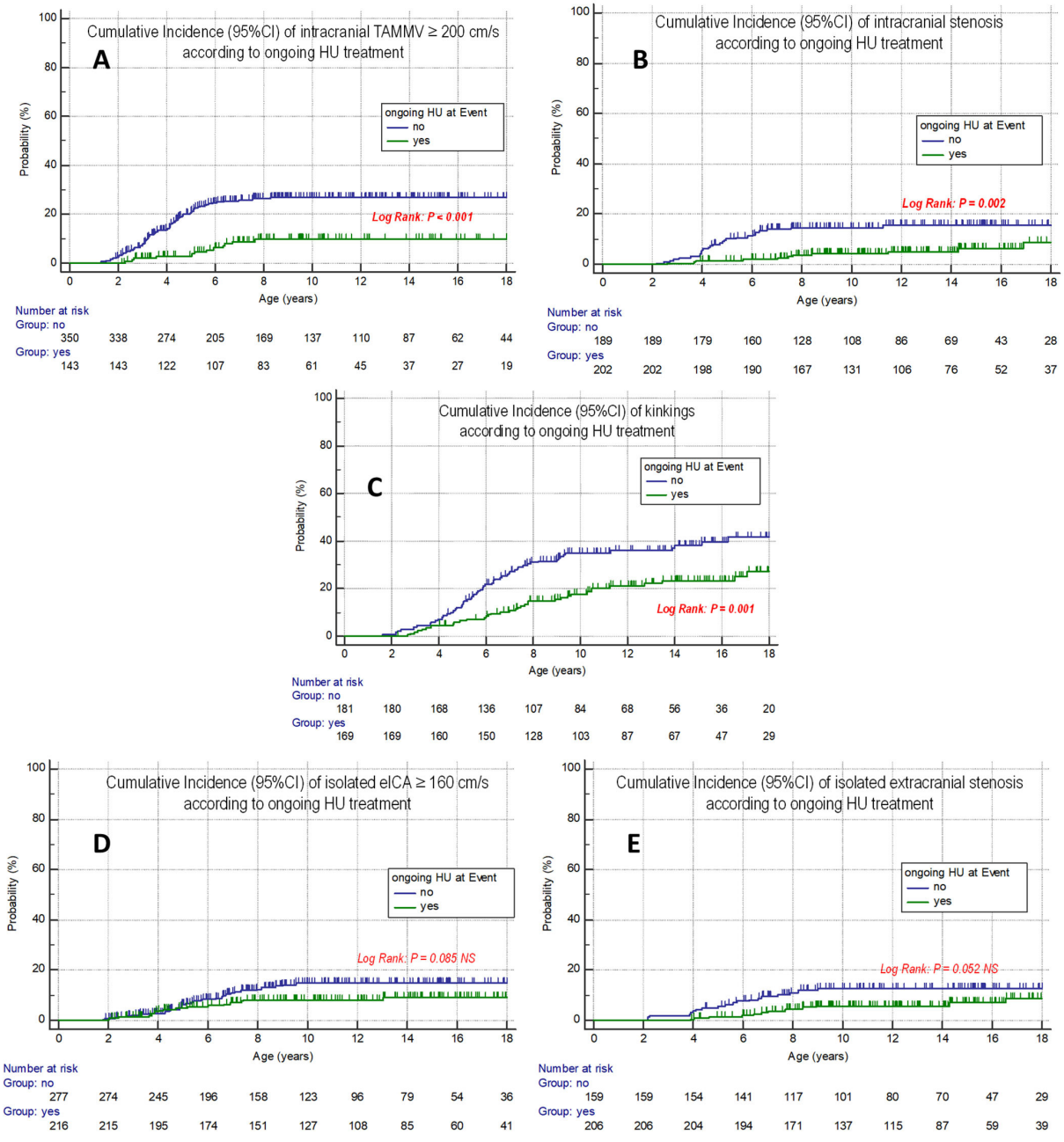


FIGURE 4
Cumulative incidence of intra- and extracranial arteriopathies in SCD children according to ongoing hydroxyurea treatment at each event. **(A)** Intracranial TAMV ≥ 200 cm/s. **(B)** Intracranial stenosis. **(C)** eICA kinking. **(D)** eICA-TAMV ≥ 160 cm/s. **(E)** eICA stenosis.

no history of eICA-TAMVs ≥160 cm/s (log rank; $p < 0.001$) (Figure 3C).

eICA stenosis

Neck MRA was available in 365 children with SCD. The first neck MRA was performed at the median age of 5.0 (1.6–8.6) years in children born after June 2009. eICA stenosis was present in 38 and isolated (without intracranial stenosis) in 31 children with SCD. All patients with eICA stenosis were children with SCA and not SC/Sβ+ children. The cumulative incidence of eICA stenosis was 13.0% (8.6–17.4%) in children with SCA and 0% in SC/ Sβ+ children (log rank; $p = 0.015$) (Figure 3D) and was 47.8% (34.8–60.8%) in children with a history of eICA-TAMVs ≥ 160 cm/s, but only 3% (0.9–5.1%) in those with eICA-TAMVs <160 cm/s (log rank; $p < 0.001$)

(Figure 3E). The cumulative incidence of isolated eICA stenosis was 11.1% (7.3–14.9%).

Of note, chronic transfusion was also required in 13.5% (9.3–17.7%) additional children who had no intracranial arteriopathy but had eICA-TAMVs ≥200 cm/s or eICA stenosis.

Impact of ongoing hydroxyurea treatment at event

Ongoing hydroxyurea treatment at event (Figure 4) was significantly associated with a lower risk of intracranial TAMV ≥ 200 cm/s (log rank; $p < 0.001$), intracranial stenosis (log rank; $p = 0.002$), and eICA kinkings (log rank; $p = 0.001$) and a trend to a lower risk of eICA-TAMV 160 cm/s and eICA stenosis.

Predictive risk factors for intra- and extracranial arteriopathies

Considering the impact of hydroxyurea, univariate COX regression analyses with baseline biological parameters were carried out after adjustment for ongoing hydroxyurea treatment at event and are presented in Table 3. Moreover, all multivariate analyses were also adjusted with ongoing hydroxyurea treatment.

Intracranial arteriopathy

Abnormal high intracranial TAMVs ≥200 cm/s

Multivariate Cox regression analysis introducing all risk factors <0.2 presented in Table 3 (except hematocrit and neutrophil counts strongly correlated with hemoglobin and WBC counts, respectively) retained as significant and independent predictive risk factors: baseline hemoglobin level, which, per unit increase, decreased the risk [HR = 0.495 (0.401–0.613), $p < 0.001$], and the WBC count, which increased the risk [HR = 1.098 (1.055–1.143), $p < 0.001$], independently of ongoing hydroxyurea treatment. For the model predicting abnormal high intracranial TAMV including hemoglobin and WBC, the P-value for the proportional hazard (PH) assumption test was $P = 0.84$, indicating no violation of the PH assumption.

Using thresholds, the analysis retained baseline hemoglobin <7g/dL [HR = 4.630 (2.703–7.937), $p < 0.001$], WBC >20x10 (9)/L [HR = 3.774 (1.876-6.494), $p < 0.001$], and reticulocytes >400 x 10^9/L [HR = 2.247 (1.321–3.817), $p = 0.003$] as significant and independent predictive risk factors.

Intracranial stenosis

Multivariate analysis retained only as predictive risk factors: baseline hemoglobin [HR = 0.537 (0.382–0.755), $p < 0.001$], which decreased the risk, and WBC count [HR = 1.098 (1.033–1.168), $p = 0.003$], which increased the risk of intracranial stenosis occurrence independently of ongoing hydroxyurea treatment.

Extracranial arteriopathy

Isolated eICA-TAMVs ≥160 cm/s

Multivariate Cox regression analysis retained as independent predictive risk factors: baseline hemoglobin [HR = 0.739 (95% CI: 0.583–0.937), $p = 0.013$] and SEN beta-haplotype number [HR = 1.653 (95% CI: 1.064–2.566), $p = 0.025$], which per unit increase decreased and increased the risk of eICA-TAMVs ≥160 cm/s, respectively. For the model predicting abnormal high eICA-TAMVs including hemoglobin and SEN beta-haplotype number, the P-value for the PH assumption test was $P = 0.96$, indicating no violation of the PH assumption.

eICA kinkings

Multivariate Cox regression analysis retained as significant and independent predictive risk factors for eICA kinkings: baseline hemoglobin [HR = 0.757 (95% CI: 0.627–0.915), $p = 0.004$], which, per unit increase, decreased the risk and SEN beta-haplotype number [HR = 1.453 (95% CI: 1.108–1.905), $p = 0.007$], which, per unit increase, increased the risk, independently of ongoing hydroxyurea treatment.

Of note, the prevalence of kinkings in SCD patients without SEN beta-haplotype was 29.2% (66/226) vs. 35.1% (13/37) in those with one and 45.9% (17/37) in those with two SEN beta-haplotypes.

eICA stenosis

Multivariate Cox regression analysis retained as significant and independent predictive risk factors for eICA stenosis: baseline hemoglobin [HR = 0.681 (95% CI: 0.506–0.917), $p = 0.011$] and SEN beta-haplotype number [HR = 1.697 (95% CI: 1.096–2.626), $p = 0.018$], respectively, independently of ongoing hydroxyurea treatment.

Impact of the SEN beta-haplotype

While the presence of SEN beta-haplotypes was protective for the development of intracranial TAMVs ≥200 cm/s, it was a risk factor for eICA-TAMVs ≥160 cm/s and eICA kinkings, with a maximum impact in the presence of two SEN beta-haplotypes (Figures 5A,B). As children with SEN/SEN have the highest baseline hemoglobin compared to other beta-haplotypes, the lowest probability of intracranial

TABLE 3 Predictive risk factors for intra- and extracranial arteriopathies in patients with SCD.

	SCD patients ($N = 493$)									
	Intracranial TAMV≥200 $N = 97/493$		**Intracranial stenosis $N = 35/375$**		**Isolated eICA TAMV≥160 $N = 50/493$**		**eICA kinkings $N = 100/365$**		**Isolated eICA Stenosis $N = 31/365$**	
	COX regression analysis after Adjustment with ongoing HU treatment at event Hazard Ratio (HR) and 95% Confidence Interval									
	HR (95% CI)	***P*-value**	**HR (95% CI)**	***P*-value**	**HR (95% CI)**	***P*-value**	**HR (95% CI)**	***P*-value**	**HR (95% CI)**	***P*-value**
	Per presence									
Genetic markers										
Gender F vs. M		>0.2		>0.2		>0.2		>0.2		>0.2
G6PD deficiency	1.610 (0.960–2.703)	0.071	2.347 (1.048–5.236)	**0.038**		>0.2		>0.2		>0.2
Alpha-Thalassemia		>0.2	0.436 (0.190–1.002)	0.051		>0.2		>0.2		>0.2
	Per beta-haplotype number									
Beta Haplotype										
Car	1.415 (1.137–1.760)	**0.002**	1.351 (0.946–1.930)	0.098	1.280 (0.939–1.744)	0.118		>0.2		>0.2
Ben		>0.2		>0.2		>0.2		>0.2		>0.2
Sen	0.624 (0.423–0.921)	**0.017**		>0.2	1.311 (0.904–1.901)	0.154	1.330 (1.027–1.723)	**0.031**	1.579 (1.027–2.427)	**0.037**
	Per unit increase									
Biological parameters										
Hemoglobin level (g/dL)	0.196 (0.101–0.382)	**<0.001**	0.461 (0.336–0.633)	**<0.001**	0.683 (0.554–0.843)	**<0.001**	0.774 (0.671–0.894)	**<0.001**	0.721(0.551–0.945)	**0.018**
Hematocrit (%)	0.888 (0.863–0.914)	**<0.001**	0.871 (0.832–0.911)	**<0.001**	0.901 (0.851–0.953)	**<0.001**	0.925 (0.887–0.965)	**<0.001**	0.929 (0.862–1.002)	0.055
Reticulocyte count (10^9/L)	1.006 (1.004–1.007)	**<0.001**	1.007 (1.004–1.009)	**<0.001**	1.002 (1.000–1.004)	0.077	1.002 (1.001–1.004)	**0.003**	1.002 (0.999–1.005)	0.126
WBC count (10^9/L)	1.149 (1.111–1.187)	**<0.001**	1.145 (1.086—1.206)	**<0.001**	1.038 (0.984–1.095)	0.167		>0.2		>0.2

(Continued)

10.3389/fneur.2022.846596

TABLE 3 (Continued)

	SCD patients ($N = 493$)									
	Intracranial TAMV≥200 $N = 97/493$		**Intracranial stenosis $N = 35/375$**		**Isolated eICA TAMV≥160 $N = 50/493$**		**eICA kinkings $N = 100/365$**		**Isolated eICA Stenosis $N = 31/365$**	
	COX regression analysis after Adjustment with ongoing HU treatment at event Hazard Ratio (HR) and 95% Confidence Interval									
	HR (95% CI)	***P*-value**	**HR (95% CI)**	***P*-value**	**HR (95% CI)**	***P*-value**	**HR (95% CI)**	***P*-value**	**HR (95% CI)**	***P*-value**
Neutrophil count (10^9/L)	1.152 (1.090–1.217)	**<0.001**	1.119 (1.014-1.235)	**0.025**	1.077 (0.978–1.185)	0.131		>0.2		>0.2
Platelet count (10^9/L)		>0.2		>0.2		0.198		>0.2		>0.2
MCV (fL)	1.069 (1.043–1.095)	**<0.001**	1.089 (1.043–1.137)	**<0.001**	1.030 (0.997–1.063)	0.072		>0.2		>0.2
Bilirubin (mmol/L)	1.030 (1.018–1.042)	**<0.001**		>0.2	1.015 (0.998-1.032)	0.079	1.011 (0.998–1.025)	0.085		>0.2
LDH (IU/L)	1.001 (1.001–1.002)	**<0.001**	1.002 (1.001–1.003)	**<0.001**		>0.2		>0.2		>0.2
HbF (%)		>0.2		>0.2		0.2	1.019 (0.995-1.043)	0.115		>0.2
Thresholds										
Hemoglobin < 7g/dL	5.525 (3.247–9.346)	**<0.001**	2.278 (0.853-6.060)	0.100	2.247 (1.033–4.878)	**0.041**		>0.2		>0.2
WBC count > 20 x 10^9/L	5.236 (3.115–8.772)	**<0.001**	3.363 (1.558-8.621)	**0.003**		>0.2		>0.2		>0.2
Reticulocyte count > 400 x 10^9/L	3.817 (2.299–6.329)	**<0.001**	3.559 (1.541–8.264)	**0.003**		>0.2		>0.2		>0.2

Univariate Cox regression analysis after adjustment with ongoing hydroxyurea treatment at each event. Levels of thresholds used were the 10th percentile for hemoglobin (7.0 g/dL) and near the 90th percentile for WBC (19.7 x 10^9/L) and reticulocyte counts (422 x 10^9/L) but rounded for easier clinicaluse.
The bold values indicates the significant *P*-values (<0.05).

10.3389/fneur.2022.846596

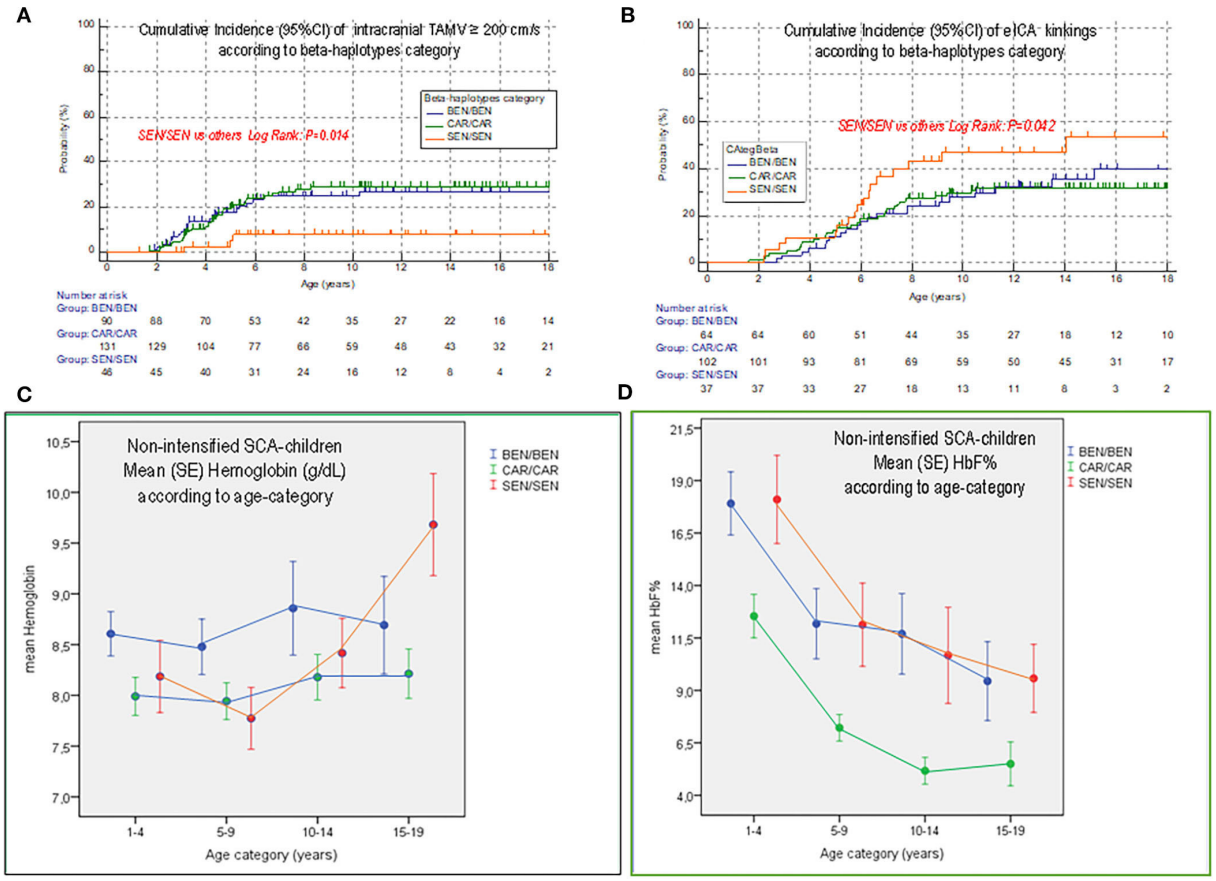


FIGURE 5
Comparison of cumulative incidence of intra- and extracranial TAMV and kinkings according to homozygous beta-haplotype category and outcome of hemoglobin level and HbF% in non-intensified children according to the three beta-haplotypes categories. **(A)** Cumulative incidence of intracranial TAMV ≥ 200 cm/s according to homozygous beta-haplotypes category. **(B)** Cumulative incidence of eICA kinkings according to homozygous beta-haplotypes category. **(C)** Outcome of hemoglobin level during aging in non-intensified patients (not on hydroxyurea, or on chronic transfusion, or transplanted) according to homozygous beta-haplotypes category. **(D)** Outcome of HbF% level during aging in non-intensified patients according to homozygous beta-haplotypes category. These values were recorded at annual checkup in non-intensified children with SCA. While mean hemoglobin remained relatively stable during aging in children with BEN/BEN and CAR/CAR, hemoglobin decreased in children with SEN/SEN between 5 and 10 years of age before significantly increasing thereafter. Thus, children with SEN/SEN were the most anemic between 5 and 10 years of age and the less anemic after 15 years of age. This age period corresponds with the occurrence of abnormal TAMV and eICA kinkings in patients with SEN/SEN.

arteriopathy was not surprising, but the highest risk of extracranial arteriopathy was unexpected. As the mean (SD) age at hydroxyurea initiation in SEN/SEN [6.2 yr, (3.2)] was slightly higher than that in BEN/BEN [5.2 yr, (2.8)] and CAR/CAR [5.4 yr, (2.9)] children, although not significantly, we determined the changes in hemoglobin and HbF% with aging in the non-intensified patients (no hydroxyurea, chronic transfusion, or transplantation). We observed that in children with SEN/SEN, but not in children with other haplotypes, hemoglobin strongly decreased between 5 and 10 years of age, a period during which they developed intracranial TAMVs ≥200 cm/s and eICA kinkings (Figures 5C,D).

Discussion

Here, we present the first longitudinal cohort study including 493 children with SCD simultaneously assessed by transcranial and cervical color Doppler ultrasound. In the present SCD cohort study, only children with SCA and not SC/Sβ+children developed both intra- and extracranial macro-arteriopathies during infancy. Lower intracranial TAMVs in patients with SC were previously reported (23), but the cumulative incidence of cerebral arteriopathy in longitudinal cohort studies has been missing in the literature, and the benefit of TCD screening in children with compound heterozygous SCD is not well defined (24). The present study showing the absence

of cerebral macro-arteriopathy in SC/Sβ+ children raises the issue of the usefulness of early TCD screening in this population. However, as kinkings were observed in several SC/Sβ+ children in this cohort, and SCI has been reported in this population, (25, 26) the data suggest that the presence of eICA stenosis should be investigated in adult patients.

In SCA, most ischemic strokes are linked to intracranial arteriopathy, but the circle of Willis is normal in approximately 25% of cases (9), indicating an additional underlying mechanism. Interestingly, several cases of strokes have been reported to be associated with stenosis/occlusion of eICA (1, 10–13). Moreover, the incidence of SCI was significantly associated with the presence of eICA stenosis (7). Until now, cerebral MRA and TCD have been mainly used to explore the major intracranial vessels. Compared with those of the intracranial vasculature, morphologic changes of the extracranial vasculature in patients with SCD are less well described and understood. Cervical color Doppler ultrasound scanning has only recently been used in SCD. Using a submandibular approach, Gorman et al. (11) detected abnormal eICA-TAMVs in four of 131 children, three of whom had a stroke, while 13 of 236 children had eICA stenosis or occlusion in Deane et al.'s study (12). Telfer et al. used neck MRA in 67 SCA patients with a history of stroke or abnormal TCD and found that 10 of 67 (15%) had eICA occlusion (13). We reported a cross-sectional study between June 2011 and April 2012 in two stroke-free cohorts including 435 SCA cases assessed by transcranial and cervical Doppler, with intracranial and neck MRA available in 104 of 435 subjects (14). We showed that TAMVs were about 25% lower in the eICA than in the MCA and that eICA-TAMVs $\geq$160 cm/s were highly associated with eICA stenosis and kinkings.

The present longitudinal cohort study is the first to show that the kinetics of extracranial arteriopathy in patients with SCA are similar to those of intracranial arteriopathy as abnormal eICA-TAMV and stenosis were observed as soon as the 2nd year of life, in line with Telfer's study reporting the occurrence of four stroke episodes associated with eICA occlusion in young children at 2 to 4 years of age (13). Importantly, we show that the cumulative incidence of abnormal eICA-TAMV reaches a plateau by about 10 years of age, as previously shown for intracranial abnormal TAMV in children with SCA (17). The importance of systematically detecting extracranial arteriopathy is supported by the ability of eICA assessment to identify 13.5% additional patients at risk of stroke because of eICA-TAMVs $\geq$200 cm/s or eICA stenosis in the absence of intracranial arteriopathy. A cross-sectional study in 167 children and adults with SCA assessed by cerebral MRI/MRA and neck MRA reported the presence of intra- or extracranial stenosis in 20 and nine patients, respectively, but extracranial stenosis was always associated with the presence of intracranial stenoses and could not be evaluated as an independent risk factor for stroke (27). Thus, the authors suggested that systematic detection of extracranial stenosis may have limited clinical utility in patients with SCD (27). However, Verlhac et al. (28) recently reported in the Debré, Paris, cohort the presence of eICA stenosis in 48 children with SCA, of whom eight had a history of stroke; importantly, five of eight had isolated eICA stenosis, confirming the clinical utility to systematically assess the extracranial part of the internal carotid artery.

We also show for the first time that in addition to a congenital origin, eICA kinkings in patients with SCD can develop progressively with aging as a function of eICA-TAMVs, themselves related to anemia severity. Kinkings are known to be increased in a variety of connective tissue genetic disorders, such as Loeys–Dietz syndrome (29). They are associated with a risk of eICA dissection (30) and transient cerebral arteriopathy and may represent a clinically relevant imaging biomarker of vascular biology for pediatric strokes (31). The congenital or acquired origin of kinkings is still debated (32). Their non-association with age has been reported, suggesting that they are the result of alterations during embryological development, rather than vascular remodeling secondary to aging (33). In SCA children, they may promote the formation of stenosis by disturbing blood flow, for example, in the intracranial carotid siphon and bifurcation. However, other studies have described predisposing factors such as older age, female gender, and hypertension, suggesting vascular remodeling. A study compared the presence of eICA kinkings assessed by neck MRA in 56 patients with SCD with 56 controls and showed significantly greater tortuosity in the eICA and vertebral arteries in patients with SCD than in controls, suggesting that they could be due to aberrations in hemodynamics from nonlaminar flow in these vessels (34). Our findings showing progressive development of eICA kinkings during infancy argues in favor of vascular remodeling as a consequence of high blood flow associated with severe chronic anemia. This is also supported by studies using an arteriovenous fistula model in rabbits, showing that high blood flow and high shear stress [defined as the tangential force per unit area exerted by the wall on the fluid (35)] induced endothelial cell activation, proliferation, (36) dilatation, elongation, and tortuosity, and smooth muscle cell proliferation (37). When arteries are enlarged and remodeled in response to high flow conditions, wall shear stress is subsequently reduced, and long-term exposure to low wall shear stress ($<$ 5 dynes/cm^2) induces severe intimal thickening (37). Worsening of eICA tortuosity can induce a blood pressure drop (38) responsible for cerebral ischemia occurrence when self-regulatory mechanisms cannot compensate for the blood flow drop. This could explain the association between eICA stenosis and the presence of SCI (7). Nevertheless, additional genetic factors promoting eICA kinkings cannot be excluded, as suggested by the increased incidence of kinkings and extracranial arteriopathy observed in this series in patients with SEN beta-haplotype.

While the SCA genotype and low baseline hemoglobin level were predictive risk factors for intra- and extracranial

arteriopathies, high baseline reticulocyte count and LDH, the surrogate markers for hemolysis, and WBC count were only predictive of intracranial arteriopathy risk. SEN-β-haplotypes which were found associated with a lower risk for intracranial-arteriopathy, were unexpectedly associated with higher risk for extracranial arteriopathy, but showed in non-intensified patients that SEN/SEN children had decreased hemoglobin between 5 and 10 years of age, the period when they start developing eICA-kinkings with higher frequency compared to other beta-haplotypes. Another explanation could be a genetic link between the ethnic origin from Senegal and the presence of kinkings. Thus, it would be important to compare the prevalence of kinkings in non-SCD patients from Senegal with other African countries. This finding could explain the higher incidence of SCI observed in patients with the SEN β-haplotype (39), as we previously showed an association between eICA kinkings and SCI (7).

Could our findings impact the choice of therapy for cerebral arteriopathy prevention? The present prospective cohort study was not designed to test the impact of hydroxyurea treatment, which was only given in patients with normal cerebral velocities or in those with a history of abnormal cerebral velocities who had normalized them on chronic transfusion and had no arterial stenosis. However, we show that ongoing hydroxyurea treatment for at least 6 months at event was associated with a lower risk of intracranial arteriopathy, and we show, for the first time, an association with a lower risk of eICA kinking development. As severe baseline anemia is the major predictive risk factor for intra-/extracranial arteriopathy and SCI (7, 40), it is clear that any treatment improving anemia such as hydroxyurea or voxelotor (41) could be useful to decrease the risk of intra-/extracranial arteriopathy. Hydroxyurea induces the increase in HbF, MCV, and hemoglobin; decreases WBC, neutrophil, and platelet counts; improves hemolytic markers [decreased reticulocyte count and LDH (42)]; and significantly decreases the rate of VOC, ACS, and transfusion needs (43). The safety and efficacy of hydroxyurea in young children have been established in the United States (22) and in malaria-endemic sub-Saharan Africa (44, 45), justifying early hydroxyurea initiation, not only to prevent crises but also to secondary strokes in low-income countries where safe blood products are rare and costly. As hemolytic anemia and high WBC count are the major risk factors for intracranial arteriopathy, hydroxyurea is a good candidate for primary stroke prevention. Open-label trials in the United States and Nigeria (46–51), and several randomized trials comparing hydroxyurea with the placebo such as BABY HUG (21, 52) (the United States), SCATE (53) (the United States, Jamaica, and Brazil), and NOHARM (54) (Uganda) have shown that hydroxyurea lowers velocities in patients with elevated intracranial velocities and reduces the risk of conversion to abnormal velocities (46–51) and of first clinical strokes. Thus, to date, there are robust data showing that hydroxyurea decreases the risk of abnormal intracranial TAMVs. In France, providers were initially reluctant to systematically treat asymptomatic young children with hydroxyurea due to the cytotoxic effect of hydroxyurea on dividing cells, including spermatogonia (55–57). However, a recent report in patients treated with hydroxyurea prior to puberty, whose sperm parameters were analyzed after treatment suspension and several months on chronic transfusion, showed no specific effect of hydroxyurea on sperm parameters above those SCD-related (58), supporting its early use. Nevertheless, in the absence of systematic cerebral MRI/MRA and neck MRA in those studies comparing hydroxyurea with placebo, the impact of hydroxyurea on decreasing the risk of stenosis, SCI, and extracranial arteriopathy remains unknown. The HUSTLE study, evaluating the prevalence of SCI in a cohort treated with hydroxyurea at maximum tolerated dose, found only a small increase in the prevalence of SCI on hydroxyurea (38% at baseline and 41% after 6 years) (59). By contrast, an increase in detection and progression of SCI despite hydroxyurea was reported in another study (60). Our data showing a higher risk of extracranial arteriopathy in patients with SEN β-haplotype who have a higher baseline HbF level do not argue for a favorable impact of hydroxyurea on extracranial arteriopathy. It would be important to evaluate in independent cohorts with a high prevalence of patients with SEN β-haplotype if early initiation of hydroxyurea before age 2 years could prevent the strong decrease in hemoglobin observed in our cohort among SEN/SEN patients between 5 and 10 years of age and the extracranial arteriopathy. However, we have reported that compared to children with SEN/SEN, children with BEN/BEN and CAR/CAR had the highest hemoglobin and HbF levels at baseline, and they had significantly lower hemoglobin and HbF levels on hydroxyurea than children with BEN/BEN who had the best response to hydroxyurea (61). Other factors may explain the variable impact of hydroxyurea on cerebral vasculopathy. The increased affinity of HbF for oxygen can decrease tissue oxygen delivery and explain the variable efficiency of hydroxyurea in preventing SCI, priapism, and pulmonary hypertension (62). Moreover, the increased MCV observed on hydroxyurea could be deleterious in the absence of sufficient improvement of deformability. Thus, in the presence of severe cerebral arteriopathy, chronic transfusion appears more efficient than hydroxyurea to reduce stroke recurrence, as demonstrated in the SWiTCH trial (63), and transplantation, which can cure 98% of children with SCA (64), and reduces TAMVs (65) and stenosis scores (66) more efficiently than chronic transfusion, as demonstrated in the DREPAGREFFE trial (65). Finally, gene therapy using lentiviral transfer of a marked β-globin (β^{A-T87Q}) gene (67), which results in near pancellular β^{A-T87Q} expression with reduced sickling and hemolysis and increased total hemoglobin (68), seems quite promising for cerebral vasculopathy prevention.

This study has some limitations. The relatively low number of SC/Sβ+ children in this series does not allow to conclude that

this population never develops extracranial arteriopathy during infancy. For several outcomes, including intracranial TAMVs $\geq$ 200 cm/s, intracranial stenosis, eICA TAMVs $\geq$ 160 cm/s, and eICA stenosis, the number of events in the patients with SC/Sβ+ was either 0 or very small, and it was therefore not possible to accurately calculate hazard ratios. It was not designed to evaluate the impact of hydroxyurea on cerebral vasculopathy prevention as hydroxyurea was only given to children with SCA with normal TAMVs and no stenosis. We had a high proportion of patients receiving chronic transfusion, and our results may not be generalizable to cohorts with lower rates of transfusion. Moreover, the associations between biological parameters and cerebral arteriopathy described here are only descriptive and do not demonstrate any causal relationship. We also acknowledge that our results will need to be further validated in independent cohorts with a sufficient proportion of patients with SEN β haplotypes and homogenously treated early with hydroxyurea. However, this is the first prospective longitudinal cohort study reporting the kinetics and predictive risk factors for extracranial arteriopathy in children with SCD followed in the same referral center since birth, and highlighting the predictive and critical values of baseline parameters recorded during the 2nd year of life before any intensive therapy. These blood parameters are representative of the influence of all genetic biomarkers on the different blood components. Thus, children with baseline hemoglobin <7g/L, WBC count >20 x 10^9/L, or reticulocyte >400 x 10^9/L should be considered as candidates for early intensified therapy, transplantation in the presence of matched sibling donor, or gene therapy.

In conclusion, only children with SCA appear to be at risk of intra- and extracranial arteriopathies during infancy. Extracranial arteriopathy is most often isolated, and eICA assessment detects 13.5% more patients at risk of stroke not detected by intracranial assessment, showing the importance of systematically assessing the eICA in children with SCA. In addition to a congenital origin, eICA kinkings in patients with SCD can develop progressively with aging as a function of eICA-TAMVs, themselves related to anemia severity, and are risk factors for stenotic extracranial arteriopathy. This prospective cohort study shows the importance of recording biological parameters during the 2nd year of life before any intensive therapy to predict the risk of cerebral arteriopathy and treat patients with severe baseline anemia.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

Ethics statement

The studies involving human participants were reviewed and approved by Créteil-Institutional-Review-Board. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

FB designed and performed the research, collected the data, performed the statistical analyses, interpreted the data, and wrote the manuscript. SV designed and performed the research, performed Doppler ultrasound scans and MRI/MRA, collected, analyzed, and interpreted data, and co-wrote the manuscript. CA, AK, and CJ designed the study, collected and interpreted data, and co-wrote the manuscript. FB, CA, AK, IH, FM, and RE participated in the management of patient care. All authors critically reviewed and approved the manuscript.

Acknowledgments

The authors would like to thank all the patients and their families for their participation in this observational longitudinal cohort study and the statistician Sylvie Chevret (Statistic department, St-Louis hospital, Paris) who checked with R software the proportional hazard assumption based on weighted residuals (Grambsch–Therneau test).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Rothman SM, Fulling KH, Nelson JS. Sickle cell anemia and central nervous system infarction: a neuropathological study. *Ann. Neurol.* (1986) 20:684–90. doi: 10.1002/ana.410200606

2. Adams RJ, McKie V, Nichols F, Carl E, Zhang DL, McKie K, et al. The use of transcranial ultrasonography to predict stroke in sickle cell disease. *N Engl J Med.* (1992) 326:605–10. doi: 10.1056/NEJM199202273260905

3. Verlhac S, Bernaudin F, Tortrat D, Brugieres P, Mage K, Gaston A, et al. Detection of cerebrovascular disease in sickle cell disease children by transcranial Doppler sonography. Correlation with MRI and MRA and conventional angiography. *Pediatr Radiol.* (1995) 25:S14–9. doi: 10.1007/BF03545573

4. Adams RJ, McKie VC, Carl EM, Nichols FT, Perry R, Brock K, et al. Long-term stroke risk in children with sickle cell disease screened with transcranial Doppler. *Ann Neurol.* (1997) 42:699–704. doi: 10.1002/ana.410420505

5. Thangarajh M, Yang G, Fuchs D, Ponisio MR, McKinstry RC, Jaju A, et al. Magnetic resonance angiography-defined intracranial vasculopathy is associated with silent cerebral infarcts and glucose-6-phosphate dehydrogenase mutation in children with sickle cell anaemia. *Br J Haematol.* (2012) 159:352–9. doi: 10.1111/bjh.12034

6. Arkuszewski M, Krejza J, Chen R, Ichord R, Kwiatkowski JL, Bilello M, et al. Sickle cell anemia: intracranial stenosis and silent cerebral infarcts in children with low risk of stroke. *Adv Med Sci.* (2014) 59:108–13. doi: 10.1016/j.advms.2013.09.001

7. Bernaudin F, Verlhac S, Arnaud C, Kamdem A, Vasile M, Kasbi F, et al. Chronic and acute anemia and extracranial internal carotid stenosis are risk factors for silent cerebral infarcts in sickle cell anemia. *Blood.* (2015) 125:1653–61 doi: 10.1182/blood-2014-09-599852

8. Adams RJ, McKie VC, Hsu L, Files B, Vichinsky E, Pegelow C, et al. Prevention of a first stroke by transfusions in children with sickle cell anemia and abnormal results on transcranial Doppler ultrasonography. *N Engl J Med.* (1998) 339:5–11. doi: 10.1056/NEJM199807023390102

9. Gerald B, Sebes JI, Langston JW. Cerebral infarction secondary to sickle cell disease: arteriographic findings. *AJR Am J Roentgenol.* (1980) 134:1209–12. doi: 10.2214/ajr.134.6.1209

10. Calviere L, Viguier A, Guidolin B, Tall P, Larrue V. Cervical artery stenoses in sickle cell disease. *Eur Neurol.* (2007) 58:120–1. doi: 10.1159/000103650

11. Gorman MJ, Nystrom K, Carbonella J, Pearson H. Submandibular TCD approach detects post-bulb ICA stenosis in children with sickle cell anemia. *Neurology.* (2009) 73:362–5. doi: 10.1212/WNL.0b013e3181ae2361

12. Deane CR, Goss D, Bartram J, Pohl KR, Height SE, Sibtain N, et al. Extracranial internal carotid arterial disease in children with sickle cell anemia. *Haematologica.* (2010) 95:1287–92. doi: 10.3324/haematol.2010.022624

13. Telfer PT, Evanson J, Butler P, Hemmaway C, Abdulla C, Gadong N, et al. Cervical carotid artery disease in sickle cell anemia: clinical and radiological features. *Blood.* (2011) 118:6192–9. doi: 10.1182/blood-2011-03-337915

14. Verlhac S, Balandra S, Cussenot I, Kasbi F, Vasile M, Kheniche A, et al. Extracranial carotid arteriopathy in stroke-free children with sickle cell anemia: detection by submandibular Doppler sonography. *Pediatr Radiol.* (2014) 44:587–96. doi: 10.1007/s00247-014-2880-9

15. Weibel J, Fields WS. Tortuosity, coiling, and kinking of the internal carotid artery. II Relationship of morphologica variation to cerebrovascular insufficiency. *Neurology.* (1965) 15:462–8 doi: 10.1212/WNL.15.5.462

16. Ciuricǎ S, Lopez-Sublet M, Loeys BL, Radhouani I, Natarajan N, Vikkula M, et al. Arterial Tortuosity Novel Implications for an Old Phenotype. *Hypertension.* (2019) 73:951–60. doi: 10.1161/HYPERTENSIONAHA.118.11647

17. Bernaudin F, Verlhac S, Arnaud C, Kamdem A, Chevret S, Hau I, et al. Impact of early transcranial Doppler screening and intensive therapy on cerebral vasculopathy outcome in a newborn sickle cell anemia cohort. *Blood.* (2011) 117:1130–40. doi: 10.1182/blood-2010-06-293514

18. Bernaudin F, Verlhac S, Coïc L, Lesprit E, Brugières P, Reinert P. Long term follow-up of pediatric sickle cell disease patients with abnormal high velocities on transcranial Doppler. *Pediatric Radiol.* (2005) 3:242–8. doi: 10.1007/s00247-005-1419-5

19. Bernaudin F, Verlhac S, Arnaud C, Kamdem A, Hau I, Leveillé E, et al. Long-term treatment follow-up of children with sickle cell disease monitored with abnormal transcranial Doppler velocities. *Blood.* (2016) 127:1814–22 doi: 10.1182/blood-2015-10-675231

20. Bernaudin F, Verlhac S, Freard F, Roudot-Thoraval F, Benkerrou M, Thuret I, et al. Multicenter prospective study of children with sickle cell disease: radiographic and psychometric correlation. *J Child Neurol.* (2000) 15:333–43. doi: 10.1177/088307380001500510

21. Wang WC, Ware RE, Miller ST, Iyer RV, Casella JF, Minniti CP, et al. BABY HUG investigators. Hydroxycarbamide in very young children with sickle-cell anaemia: a multicentre, randomised, controlled trial (BABY HUG). *Lancet.* (2011) 377:1663–72. doi: 10.1016/S0140-6736(11)60355-3

22. Yawn BP, Buchanan GR, Afenyi-Annan AN, Ballas SK, Hassell KL, James AH, et al. Management of sickle cell disease: summary of the 2014 evidence-based report by expert panel members. *JAMA.* (2014) 312:1033–48 doi: 10.1001/jama.2014.10517

23. Vieira C, de Oliveira CN, de Figueiredo LA, Santiago RP, Adanho CS, Santana SS, et al. Transcranial Doppler in hemoglobin SC disease. *Pediatr Blood Cancer.* (2017) 64:e26342. doi: 10.1002/pbc.26342

24. DeBaun MR, Jordan LC, King AA, Schatz J, Vichinsky E, Fox CK, et al. American Society of Hematology 2020 guidelines for sickle cell disease: prevention, diagnosis, and treatment of cerebrovascular disease in children and adults. *Blood Adv.* (2020) 4:1554–88. doi: 10.1182/bloodadvances.2019001142

25. Armstrong FD, Thompson RJ Jr, Wang W, Zimmerman R, Pegelow CH, Miller S. et al. Cognitive functioning and brain magnetic resonance imaging in children with sickle cell disease. Neuropsychology committee of the cooperative study of sickle cell disease. *Pediatrics.* (1996) 97:864–70. doi: 10.1542/peds.97.6.864

26. Pegelow CH, Macklin EA, Moser FG, Wang WC, Bello JA, Miller ST, et al. Longitudinal changes in brain magnetic resonance imaging findings in children with sickle cell disease. *Blood.* (2002) 99:3014–8. doi: 10.1182/blood.V99.8.3014

27. Schlotman AA, Donahue MJ, Kassim AA, Lee CA, Waddle SL, Pruthi S, et al. Intracranial and extracranial vascular stenosis as risk factors for stroke in sickle cell disease. *Pediatr Neurol.* (2021) 114:29–34. doi: 10.1016/j.pediatrneurol.2020.10.006

28. Verlhac S, Ithier G, Bernaudin F, Oloukoi C, Cussenot I, Balandra S et al. Evolution of extracranial internal carotid artery disease in children with sickle cell anemia. *Stroke.* (2022) 53:2637–46. doi: 10.1161/STROKEAHA.121.037980

29. Kirton A, Crone M, Benseler S, Mineyko A, Armstrong D, Wade A, et al. Fibromuscular dysplasia and childhood stroke. *Brain.* (2013) 136:1846–56. doi: 10.1093/brain/awt111

30. Saba L, Argiolas GM, Sumer S, Siotto P, Raz E, Sanfilippo R, et al. Association between internal carotid artery dissection and arterial tortuosity. *Neuroradiology.* (2015) 57:149–53. doi: 10.1007/s00234-014-1436-x

31. Wei F, Diedrich KT, Fullerton HJ. deVeber G, Wintermark M, Hodge J, et al. Arterial Tortuosity: An imaging biomarker of childhood stroke pathogenesis? *Stroke.* (2016) 47:1265–70. doi: 10.1161/STROKEAHA.115.011331

32. Leipzig TJ, Dohrmann GJ. The tortuous or kinked carotid artery: pathogenesis and clinical considerations. A historical review. *Surg Neurol.* (1986) 25:478–86. doi: 10.1016/0090-3019(86)90087-X

33. Beigelman R, Izaguirre AM, Robles M, Grana DR, Ambrosio G, Milei J. Are kinking and coiling of carotid artery congenital or acquired? *Angiology.* (2010) 61:107–12. doi: 10.1177/0003319709336417

34. Buch K, Arya R, Shah B, Nadgir RN, Saito N, Qureshi MM, et al. Quantitative analysis of extracranial arterial tortuosity in patients with sickle cell disease. *J Neuroimaging.* (2017). 27:421–7 doi: 10.1111/jon.12418

35. Katritsis D, Kaiktsis L, Chaniotis A, Pantos J, Efstathopoulos EP, Marmarelis V. Wall shear stress: theoretical considerations and methods of measurement. *Prog Cardiovasc Dis.* (2007) 49:307–29. doi: 10.1016/j.pcad.2006.11.001

36. Sho E, Sho M, Singh TM, Nanjo H, Komatsu M, Xu C, et al. Arterial enlargement in response to high flow requires early expression of matrix metalloproteinases to degrade extracellular matrix. *Exp Mol Pathol.* (2002) 73:142–53. doi: 10.1006/exmp.2002.2457

37. Sho E, Nanjo H, Sho M, Kobayashi M, Komatsu M, Kawamura K, et al. Arterial enlargement, tortuosity, and intimal thickening in response to sequential exposure to high and low wall shear stress. *J Vasc Surg.* (2004) 39:601–12. doi: 10.1016/j.jvs.2003.10.058

38. Wang L, Zhao F, Wang D, Hu S, Liu J, Zhou Z, et al. Pressure drop in tortuosity/kinking of the internal carotid artery: simulation and clinical investigation. *Biomed Res Int.* (2016) 2916:2428970. doi: 10.1155/2016/2428970

39. Kinney TR, Sleeper LA, Wang WC Zimmerman RA, Pegelow CH, Ohene-Frempong K, et al. Silent cerebral infarcts in sickle cell anemia: a risk factor analysis. The cooperative study of sickle cell disease. *Pediatrics.* (1999) 103:640–5. doi: 10.1542/peds.103.3.640

40. Debaun MR, Sarnaik SA, Rodeghier MJ, Minniti CP, Howard TH, Iyer RV, et al. Associated risk factors for silent cerebral infarcts in sickle cell anemia:

low baseline hemoglobin, gender and relative high systolic blood pressure. *Blood.* (2012) 119:3684–90. doi: 10.1182/blood-2011-05-349621

41. Vichinsky E, Hoppe CC, Ataga KI, Ware RE, Nduba V, El-Beshlawy A, et al. HOPE trial investigators. a phase 3 randomized trial of Voxelotor in sickle cell disease. *N Engl J Med.* (2019) 381:509–19. doi: 10.1056/NEJMoa1903212

42. Charache S, Dover GJ, Moore RD, Eckert S, Ballas SK, Koshy M, et al. Hydroxyurea: effects on hemoglobin F production in patients with sickle cell anemia. *Blood.* (1992) 79:2555–65 doi: 10.1182/blood.V79.10.2555.bloodjournal79102555

43. Charache S, Terrin ML, Moore RD, Dover GJ, Barton FB, Eckert SV, et al. Effect of hydroxyurea on the frequency of painful crisis in sickle cell anemia. *N Engl J Med.* (1995) 332:1317–22 doi: 10.1056/NEJM199505183322001

44. Opoka RO, Ndugwa CM, Latham TS, Lane A, Hume HA, Kasirye P, et al. Novel use of hydroxyurea in an african region with malaria (NOHARM): a trial for children with sickle cell anemia. *Blood.* (2017) 130:2585–93. doi: 10.1182/blood-2017-06-788935

45. Tshilolo L, Tomlinson G, Williams TN, Santos B, Olupot-Olupot P, Lane A et al. REACH investigators. Hydroxyurea for children with sickle cell Anemia in Sub-Saharan Africa. *N Engl J Med.* (2019) 380:121–31. doi: 10.1056/NEJMoa1813598

46. Zimmerman SA, Schultz WH, Burgett S, Mortier NA, Ware RE. Hydroxyurea therapy lowers transcranial Doppler flow velocities in children with sickle cell anemia. *Blood.* (2007) 110:1043–7 doi: 10.1182/blood-2006-11-057893

47. Lagunju I, Brown BJ, Sodeinde O. Hydroxyurea lowers transcranial Doppler flow velocities in children with sickle cell anaemia in a Nigerian cohort. *Pediatr Blood Cancer.* (2015) 62:1587–91. doi: 10.1002/pbc.25529

48. Adegoke SA, Macedo-Campos RS, Braga JAP, Figueiredo MS, Silva GS. Changes in transcranial Doppler flow velocities in children with sickle cell disease: the impact of hydroxyurea therapy. *J Stroke Cerebrovasc Dis.* (2018) 27:425–31. doi: 10.1016/j.jstrokecerebrovasdis.2017.09.020

49. Lagunju I, Brown BJ, Oyinlade AO, Asinobi A, Ibeh J, Esione A, et al. Annual stroke incidence in Nigerian children with sickle cell disease and elevated TCD velocities treated with hydroxyurea. *Pediatr Blood Cancer.* (2019) 66:e27252. doi: 10.1002/pbc.27252

50. Galadanci NA, Umar Abdullahi S, Vance LD, Musa Tabari A, Ali S, Belonwu R, et al. Feasibility trial for primary stroke prevention in children with sickle cell anemia in Nigeria (SPIN trial). *Am J Hematol.* (2017) 92:780–8 doi: 10.1002/ajh.24770

51. Galadanci NA, Abdullahi SU, Shehi Ali Abubakar SA, Wudil Jibir B, Aminu H, Tijjani A, et al. Moderate fixed-dose hydroxyurea for primary prevention of strokes in Nigerian children with sickle cell disease: Final results of the SPIN trial. *Am J Hematol.* (2020) 95:E247–E250. doi: 10.1002/ajh.25900

52. Thornburg CD, Files BA, Luo Z, Miller ST, Kalpatthi R, Iyer R, et al. BABY HUG Investigators. Impact of hydroxyurea on clinical events in the BABY HUG trial. *Blood.* (2012) 120:4304–10; quiz 4448 doi: 10.1182/blood-2012-03-419879

53. Hankins JS, McCarville MB, Rankine-Mullings A, Reid ME, Lobo CL, Moura PG, et al. Prevention of conversion to abnormal transcranial Doppler with hydroxyurea in sickle cell anemia: A Phase III international randomized clinical trial. *Am J Hematol.* (2015) 90:1099–105. doi: 10.1002/ajh.24198

54. Opoka RO, Hume HA, Latham TS, Lane A, Williams O, Tymon J, et al. Hydroxyurea to lower transcranial Doppler velocities and prevent primary stroke: the Uganda NOHARM sickle cell anemia cohort. *Haematologica.* (2020) 105:e272–5. doi: 10.3324/haematol.2019.231407

55. Berthaut I, Guignedoux G, Kirsch-Noir F, de Larouziere V, Ravel C, Bachir D, et al. Influence of sickle cell disease and treatment with hydroxyurea on sperm parameters and fertility of human males. *Haematologica.* (2008) 93:988–93. doi: 10.3324/haematol.11515

56. Berthaut I, Bachir D, Kotti S, Chalas C, Stankovic K, Eustache F, et al. Adverse effect of hydroxyurea on spermatogenesis in patients with sickle cell anemia after 6 months of treatment. *Blood.* (2017) 130:2354–6. doi: 10.1182/blood-2017-03-771857

57. DeBaun MR. Hydroxyurea therapy contributes to infertility in adult men with sickle cell disease: a review. *Expert Rev Hematol.* (2014) 7:767–73. doi: 10.1586/17474086.2014.959922

58. Joseph L, Jean C, Manceau S, Chalas C, Arnaud C, Kamdem A, et al. Effect of hydroxyurea exposure before puberty on sperm parameters in males with sickle cell disease. *Blood.* (2021) 137:826–9. doi: 10.1182/blood.2020006270

59. Nottage KA, Ware RE, Aygun B, Smeltzer M, Kang G, Moen J, et al. Hydroxycarbamide treatment and brain MRI/MRA findings in children with sickle cell anaemia. *Br J Haematol.* (2016) 175:331–858. doi: 10.1111/bjh.14235

60. Rigano P, De Franceschi L, Sainati L, Piga A, Piel FB, Cappellini MD, et al. Italian multicenter study of hydroxyurea in sickle cell anemia investigators. Real-life experience with hydroxyurea in sickle cell disease: a multicenter study in a cohort of patients with heterogeneous descent. *Blood Cells Mol Dis.* (2018) 69:82–9. doi: 10.1016/j.bcmd.2017.08.017

61. Bernaudin F, Arnaud C, Kamdem A, Hau I, Lelong F, Epaud R, et al. Biological impact of α genes, β haplotypes, and G6PD activity in sickle cell anemia at baseline and with hydroxyurea. *Blood Adv.* (2018) 2:626–37. doi: 10.1182/bloodadvances.2017014555

62. Gordeuk, V.R., Campbell, A., Rana, S., Nouraie M, Niu X, Minniti CP, et al. Relationship of erythropoietin, fetal hemoglobin, and hydroxyurea treatment to tricuspid regurgitation velocity in children with sickle cell disease. *Blood.* (2009) 114:4639–44. doi: 10.1182/blood-2009-04-218040

63. Ware RE, Helms RW. SWiTCH Investigators. Stroke with transfusions changing to hydroxyurea (SWiTCH). *Blood.* (2012) 119:3925–32. doi: 10.1182/blood-2011-11-392340

64. Bernaudin F, Dalle JH, Bories D, Peffault de, Latour RP, Robin M, et al. Long-term event-free survival, chimerism and fertility outcomes in 234 patients with sickle-cell anemia younger than 30 years after myeloablative conditioning and matched-sibling transplantation in France. *Haematologica.* (2020) 105:91–101. doi: 10.3324/haematol.2018.213207

65. Bernaudin F, Verlhac S, Peffault de Latour R, Dalle JH, Brousse V, Petras E, et al. Association of matched sibling donor hematopoietic stem cell transplantation with transcranial doppler velocities in children with sickle cell anemia. *JAMA.* (2019) 321:266–76. doi: 10.1001/jama.2018.20059

66. Verlhac S, Gabor F, Paillard C, Chateil JF, Jubert C, Petras M, et al. Improved stenosis outcome in stroke-free sickle cell anemia children after transplantation compared to chronic transfusion. *Br J Haematol.* (2021) 193:188-193. doi: 10.1111/bjh.17178

67. Ribeil JA, Hacein-Bey-Abina S, Payen E, Magnani A, Semeraro M, Magrin E, et al. Gene therapy in a patient with sickle cell disease. *N Engl J Med.* (2017) 376:848–55. doi: 10.1056/NEJMoa1609677

68. Thompson AA, Walters MC, Mapara MY, Hongeng S, Yannaki E, Andreas E, et al. Resolution of serious vaso-occlusive pain crises and reduction in patient-reported pain intensity: results from the ongoing phase 1/2 HGB-206 group c study of lentiglobin for sickle cell disease (bb1111) Gene Therapy. ASH 2020. *Blood.* (2020) 677a. doi: 10.1182/blood-2020-134940

frontiers | Frontiers in Neurology

TYPE Original Research
PUBLISHED 20 October 2022
DOI 10.3389/fneur.2022.1000889

OPEN ACCESS

EDITED BY
Deqiang Qiu,
Emory University, United States

REVIEWED BY
Adam Bush,
Stanford University, United States
Clio González-Zacarías,
University of Southern California,
United States

*CORRESPONDENCE
Karin Shmueli
k.shmueli@ucl.ac.uk

SPECIALTY SECTION
This article was submitted to Stroke,
a section of the journal Frontiers in Neurology

RECEIVED 22 July 2022
ACCEPTED 27 September 2022
PUBLISHED 20 October 2022

CITATION
Murdoch R, Stotesbury H, Kawadler JM, Saunders DE, Kirkham FJ and Shmueli K (2022) Quantitative susceptibility mapping (QSM) and R2* of silent cerebral infarcts in sickle cell anemia. *Front. Neurol.* 13:1000889. doi: 10.3389/fneur.2022.1000889

Quantitative susceptibility mapping (QSM) and R2* of silent cerebral infarcts in sickle cell anemia

Russell Murdoch[1], Hanne Stotesbury[2], Jamie M. Kawadler[2], Dawn E. Saunders[2], Fenella J. Kirkham[2,3] and Karin Shmueli[1]*

[1]Department of Medical Physics and Biomedical Engineering, University College London, London, United Kingdom, [2]Developmental Neurosciences, UCL Great Ormond Street Institute of Child Health, London, United Kingdom, [3]University Hospital Southampton NHS Foundation Trust, and Clinical and Experimental Sciences, University of Southampton, Southampton, United Kingdom

Silent cerebral infarction (SCI) is the most commonly reported radiological abnormality in patients with sickle cell anemia (SCA) and is associated with future clinical stroke risk. To date, there have been few histological and quantitative MRI studies of SCI and multiple radiological definitions exist. As a result, the tissue characteristics and composition of SCI remain elusive. The objective of this work was therefore to investigate the composition of segmented SCI lesions using quantitative MRI for R_2^* and quantitative magnetic susceptibility mapping (QSM). 211 SCI lesions were segmented from 32 participants with SCA and 6 controls. SCI were segmented according to two definitions (FLAIR+/−T1w-based threshold) using a semi-automated pipeline. Magnetic susceptibility (χ) and R_2^* maps were calculated from a multi-echo gradient echo sequence and mean SCI values were compared to an equivalent region of interest in normal appearing white matter (NAWM). SCI χ and R_2^* were investigated as a function of SCI definition, patient demographics, anatomical location, and cognition. Compared to NAWM, SCI were significantly less diamagnetic ($\chi = -0.0067$ ppm vs. -0.0153 ppm, $p < 0.001$) and had significantly lower R_2^* (16.7 s^{-1} vs. 19.2 s^{-1}, $p < 0.001$). SCI definition had a significant effect on the mean SCI χ and R_2^*, with lesions becoming significantly less diamagnetic and having significantly lower R_2^* after the application of a more stringent T1w-based threshold. SCI-NAWM R_2^* decrease was significantly greater in patients with SCA compared with controls (-2.84 s^{-1} vs. -0.64 s^{-1}, $p < 0.0001$). No significant association was observed between mean SCI–NAWM χ or R2* differences and subject age, lesion anatomical location, or cognition. The increased χ and decreased R_2^* in SCI relative to NAWM observed in both patients and controls is indicative of lower myelin or increased water content within the segmented lesions. The significant SCI–NAWM R_2^* differences observed between SCI in patients with SCA and controls suggests there may be differences in tissue composition relative to NAWM in SCI in the two populations. Quantitative MRI techniques such as QSM and R_2^* mapping can be used to enhance our understanding of the pathophysiology and composition of SCI in patients with SCA as well as controls.

KEYWORDS

quantitative susceptibility mapping (QSM), R2*, sickle cell anemia (SCA), composition, silent cerebral infarction (SCI), infarction, magnetic susceptibility

Introduction

Covert or silent cerebral infarction (SCI) on magnetic resonance imaging (MRI) is the most commonly reported radiological abnormality in patients with sickle cell anemia (SCA) (1). SCI are lesions which appear hyperintense on T2-weighted Fluid Attenuated Inversion Recovery (FLAIR) MRI found in patients with normal neurological assessments (2). In SCA, the most common radiological definition of SCI was introduced in the Silent Infarct Transfusion (SIT) trial, where SCI were defined as a hyperintense lesion visible on at least two planes of a FLAIR image, measuring at least 3 mm in one dimension, and occurring in a subject without focal neurological symptoms (3). A more stringent SCI definition, applied in a study of adults with SCA, additionally required the lesion to appear hypointense on T1-weighted images (4).

Despite the absence of focal symptoms, the presence of SCI is associated with increased risk of future overt stroke (5) and, in some studies, with reduced full scale IQ (6–8). SCI may be secondary to cerebral small vessel disease (9), the mechanism considered likely in adults in the general population as they age (10). However, SCI mechanisms and pathophysiology remain poorly understood (11). The appearance of SCI on MRI is consistent with hypoxia-ischemia (insufficient oxygen and/or blood flow). Disease of the arterioles, microembolism, demyelination (12), and residual damage from venous sinus thrombosis (13) or posterior reversible encephalopathy syndrome have all been proposed as potential mechanisms. Risk factors include previous seizures, male sex, vascular stenosis, relatively low hemoglobin and high blood pressure (14). To date, few pathological studies of SCI have been reported and the tissue characteristics and composition of SCI remain elusive.

In most studies in patients with SCA, SCI has been studied as a binary variable. Few studies have considered measures such as lesion count or volume. Where these have been considered, lesion characteristics have been assumed to be homogenous across the brain, with all lesions having equivalent effects upon cognition and stroke risk. Our recent study attempted to further characterize lesions, beyond count and volume, by investigating the potential effects of additional factors such as lesion depth in white matter and lesion brain lobe on the relationship between SCI and cognition (15). We found no significant relationship between any global measures of SCI burden and cognitive impairment in patients with SCA, irrespective of SCI definition. This study agrees with other recent studies, which suggest that additional factors such as MRI field strength and FLAIR image resolution may affect the relationship between SCI and cognition, along with patient characteristics and treatment regimens (16, 17).

The objective of this study was to build on that initial research, using transverse relaxation rate (R_2^*) and quantitative susceptibility mapping (QSM) to investigate SCI composition and lesion heterogeneity. We investigated the magnetic susceptibility (χ) and R_2^* of SCI relative to normal appearing white matter (NAWM) in the contralateral hemisphere. QSM calculates the spatial distribution of χ from the phase images acquired using gradient echo MRI (18). χ is an intrinsic property of tissue, determined by tissue composition and microstructure (19). In white matter, where most SCI occur (11), myelin is the key susceptibility source and is relatively diamagnetic. However, iron (paramagnetic, primarily in ferritin), calcifications (diamagnetic) and deoxygenated hemoglobin (paramagnetic) may also contribute to the measured χ in white matter regions (19). We chose to incorporate R_2^* into this study as it can be calculated from the gradient echo magnitude data acquired for QSM and provides complementary information to QSM. That is, R_2^* increases as the total number of microscopic susceptibility sources in a voxel increases, irrespective of whether the sources are paramagnetic or diamagnetic. In other words, tissue R2* would decrease if there was a loss of diamagnetic molecules whilst χ would increase. This means that R_2^* can be used together with QSM to disambiguate changes in positive and negative χ sources (20, 21).

More specifically, the two main aims of this study were to: 1) examine the χ and R_2^* of SCI lesions relative to NAWM; and 2) investigate lesion χ and R_2^* as a function of other lesion characteristics, participant demographics and cognition.

Materials and methods

Patients

Patients with SCA (hemoglobin-SS) and age and race-matched healthy control (HC) participants were recruited to two studies with overlapping MRI protocols between 2016 and 2019: the Sleep Asthma Cohort follow-up (SAC) (22) and the Prevention of Morbidity in Sickle Cell Anemia baseline investigation (POMS) (23). Exclusion criteria have been reported previously (24).

Ethical approval was granted by West London NHS (05/Q0408/42, 11/EM/0084, 15/LO/0347), Yorkshire NHS (15/YH/0213) and University College London (14475/001) ethics committees. Fully informed consent and assent according to the Declaration of Helsinki were obtained from participants and, for children, from their parent/guardian.

MRI acquisition

All patients were imaged on a 3T Siemens (Erlangen, Germany) Magnetom Prisma MRI system using a 64-channel head coil. The MRI protocol included: a T2-weighted fluid attenuated inversion recovery (FLAIR) sequence for SCI identification and definition [voxel dimensions = 0.65 ×

0.65 × 1 mm^3, matrix size = 306 × 384 × 240, repetition time (TR) = 5,000 ms, echo time (TE) = 395 ms, inversion time (TI) = 1,800 ms], a T1-weighted magnetization-prepared rapid gradient-echo (MP-RAGE) sequence for white matter segmentation and SCI definition [voxel dimensions = 1 × 1 × 1 mm^3, 256 × 240 × 256, TE_1 = 2.74 ms, TR = 2,300 ms, flip angle = 8°], and a multi-echo gradient echo (ME-GRE) sequence used to calculate R_2^* and χ maps [voxel dimensions=1.15 × 1.15 × 1.15 mm^3, matrix size = 180 × 220 × 166, echoes = 7, TE_1 = 3 ms, ΔTE = 4 ms, flip angle = 15°].

SCI segmentation

The full details of the SCI segmentation pipeline have been published previously (15) and are described briefly below. Each FLAIR image was assessed by an experienced neuroradiologist (D.S.) who identified SCI and recorded the lobe in which SCI were located. The SIT trial definition was used; an area of hyperintense signal, measuring at least 3 mm in greatest dimension, and occurring in a patient with no focal neurological symptoms. A generous region of interest (ROI) was manually drawn over the region of increased signal. As in prior studies, a minimum intensity threshold, derived from the mean cortical FLAIR intensity ($1.02 \cdot Mean_{FLAIR-cortex}$) (25), was applied to the ROI to remove voxels which did not appear hyperintense on the FLAIR image.

To meet the more stringent SCI definition (4, 26), a second maximum intensity threshold, derived from the mean cortical T1-weighted MP-RAGE intensity ($1.02 \cdot Mean_{T1-cortex}$) was applied to the ROI to remove voxels which did not appear hypointense on the T1 image. Example SCI segmentations based upon the two definitions are shown in Figure 1.

NAWM ROIs in the contralateral hemisphere were segmented by mirroring each segmented SCI across the brain midline and shifting the segmentation to a manually selected position in NAWM in the same transverse slice, avoiding any SCI in the contralateral hemisphere.

Exclusion criteria

Lesions which consisted of 2 voxels or fewer after interpolation into the native QSM space were excluded from this study as they were no longer considered to meet the requirement of measuring 3 mm in at least one dimension.

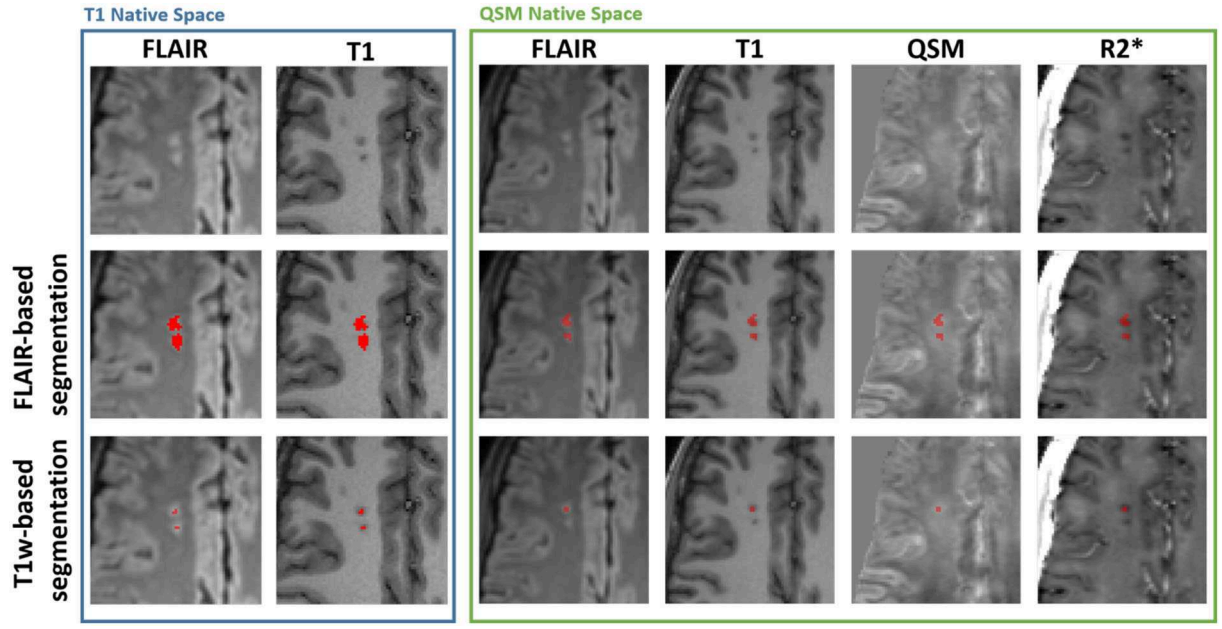


FIGURE 1
Example silent cerebral infarct segmentations. Blue Box: Axial view of two silent cerebral infarcts (SCI) segmented in a representative patient with sickle cell anemia (SCA) overlaid on FLAIR and T1-weighted images in the native T1w space. The example SCI appear hyperintense on FLAIR images and hypointense on T1-weighted images. The application of a more stringent T1w-based maximum intensity threshold (bottom) reduces the volume of the segmented SCI lesions relative to the standard FLAIR-based segmentation (middle). Green Box: Axial view of the same SCI after interpolation into the native quantitative susceptibility mapping (QSM) space, overlaid over FLAIR, T1w, QSM and R2* images. The SCI appear bright on QSM and dark on R2* maps relative to normal appearing white matter. Note that the coregistration of the lesions into the QSM space results in the lower SCI segmentation appearing in the adjacent slice.

MRI image processing

QSM was calculated from the ME-GRE images using the following pipeline: Coil combination was performed using a phase difference approach (27). Total field maps were estimated from a non-linear fit of the reconstructed complex images (28), with residual wraps in the fitted field removed using SEGUE phase unwrapping (29). Images were realigned with the main magnetic field direction (30) prior to background field removal carried out using projection onto dipole fields (31). The χ maps were calculated using the iterative Tikhonov method (32), with regularization parameter ($\alpha = 0.013$) selected using L-curve methods in six representative subjects (33). R_2^* maps were calculated from a linear fit of the log-transformed ME-GRE magnitude images as a function of echo time. QSM and R_2^* image processing was performed using MATLAB (MATLAB 2021b, MathWorks Inc., Natick, MA, USA).

Cortex, ventricle and white matter segmentations were obtained using Freesurfer, applied to the T1-weighted MP-RAGE images (34, 35). The white matter segmentation was further subdivided into juxtacortical, periventricular, or deep voxels by calculating the respective distance between the voxels and the cortex and ventricle Freesurfer segmentations. Juxtacortical voxels were defined as the 75th percentile in the distance to the cortex map and periventricular voxels were defined as the 5th percentile in the distance to the ventricle map (15). The separate white matter segmentations were used to classify the depth of SCI within white matter.

FLAIR images were affinely registered to their corresponding T1-weighted MP-RAGE using FSL FLIRT (36, 37). The T1-weighted MP-RAGE images were affinely registered to the tilt-corrected first echo ME-GRE magnitude image and the resultant affine transformation was applied to each of the SCI/contralateral NAWM segmentations (before and after the application of the additional T1w threshold), and the juxtacortical/periventricular/deep white matter maps with nearest neighbor interpolation.

Cognitive assessment

Participants enrolled in the SAC study who were over 16 years old underwent the Wechsler Adult Intelligence Scale (WAIS) test to assess their cognitive performance. The WAIS test is designed to measure full scale IQ (FSIQ) using a series of tests across several domains (38). WAIS also provides measures of working memory index (WMI), reflecting the ability to retain and manipulate information over a short period, and processing speed index (PSI), reflecting mental speed. Participants younger than 16 years completed the equivalent Wechsler Intelligence scale for Children (WISC) (39). Participants enrolled in the POMS study completed the Wechsler Abbreviated Scale of Intelligence (WASI) (40), a shorter form of the WAIS/WISC which provides comparable results (41), as well as the WAIS/WISC subtests necessary to measure PSI and WMI.

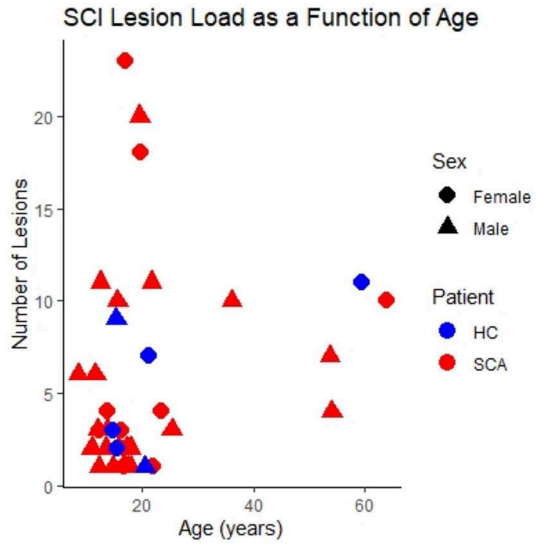


FIGURE 2
SCI lesion count. The number of silent cerebral infarcts (SCI) segmented in each participant as a function of age, sex, and sickle cell status in the study cohort. Lesions were defined according to the FLAIR-based SIT trial definition. This is the case for all subsequent figures unless indicated otherwise.

Statistical analysis

All statistical analyses were performed in R Statistical Software. (v4.1.2; R Core Team 2021). Group-wise differences in Age/FSIQ/WMI/PSI between patients with SCA and controls were examined using Wilcoxon Rank Sum Tests, and sex differences were considered using Chi-Squared tests.

ROI mean χ and R_2^*, and voxel count were calculated within each lesion before and after the application of the T1w maximum intensity threshold in the native QSM space. For each SCI/NAWM ROI the mean χ and R_2^* and voxel count were calculated within each of the three white matter segmentations, i.e., for SCI with voxels in multiple WM regions (e.g., deep and juxta-cortical) separate mean values were calculated for the two WM regions.

Mean SCI χ and R_2^* were investigated relative to the mean values measured within NAWM in the contralateral hemisphere. Mean values in the SCI and NAWM were compared using correlation, Bland-Altman analysis, and paired *t*-tests. Paired *t*-tests were applied to consider differences in the mean SCI χ and R_2^* before and after the application of the T1w-based intensity threshold.

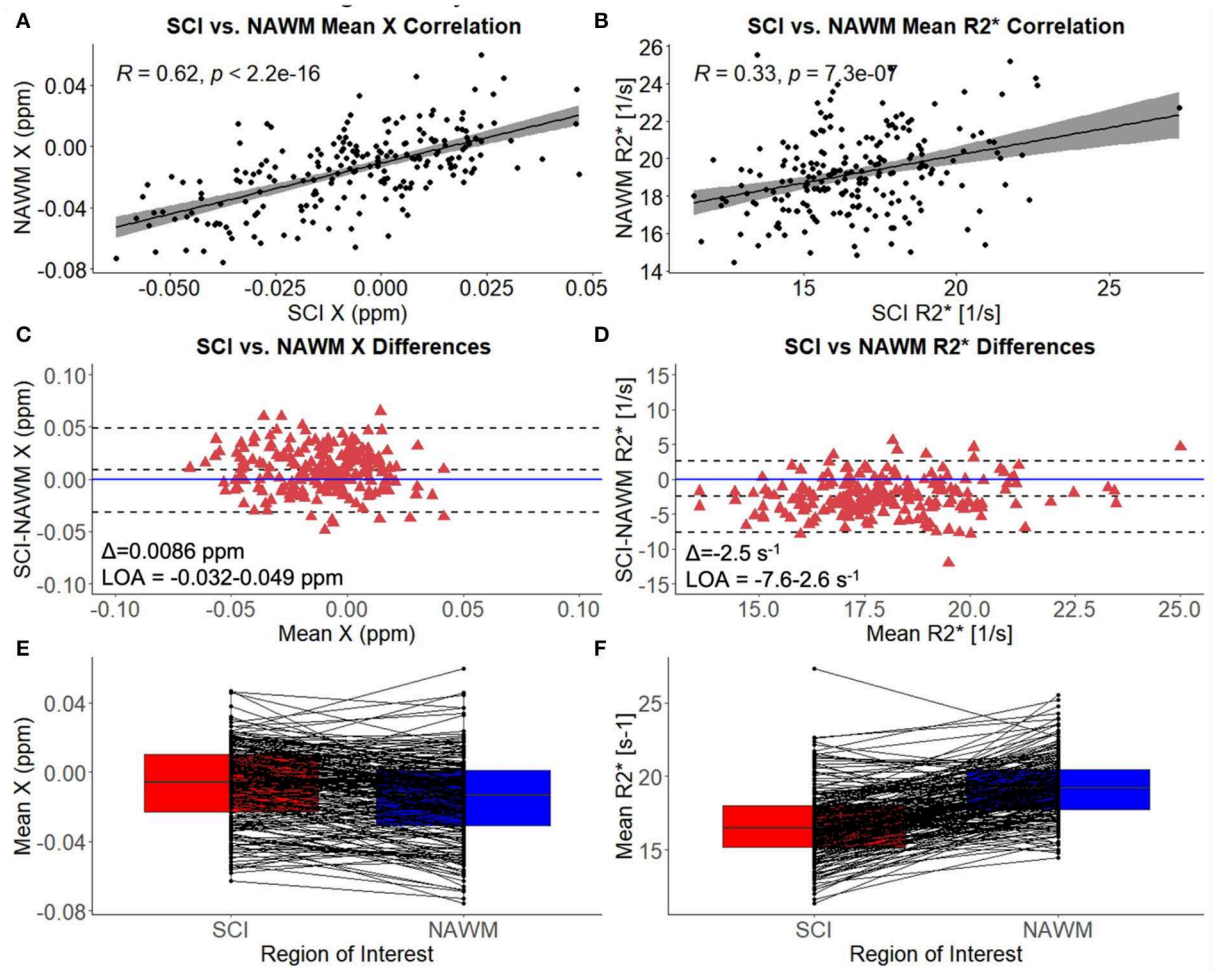


FIGURE 3
SCI vs. NAWM χ and R_2^*. **(A)** Correlation between the mean susceptibility (χ) in segmented silent cerebral infarcts (SCI) and contralateral normal appearing white matter (NAWM). **(B)** Correlation between mean SCI and NAWM R_2^*. **(C)** Bland-Altman analysis of SCI-NAWM χ differences. The mean bias (Δ) is positive suggesting that on average the SCI are less diamagnetic than NAWM. **(D)** Bland-Altman analysis of SCI-NAWM R_2^* differences. The mean bias is negative indicating that on average the SCI have lower R_2^* than NAWM. Bland-Altman plots also show the limits of agreement (LOA) and the horizontal blue line is located at SCI-NAWM = 0. **(E)** Boxplot comparing mean SCI and NAWM χ, showing that mean χ is significantly elevated in SCI. **(F)** Boxplot showing that mean R2* is significantly reduced in SCI relative to NAWM.

Analysis of variance (ANOVA) was used to investigate whether subject age (log-transformed), sickle cell status, brain lobe and lesion volume (log-transformed) had a significant effect on the measured SCI χ and R_2^*.

Groupwise differences between age-corrected SCI χ and R_2^* in patients with SCA and controls were examined using student's *t*-test. *Post-hoc* differences in age-corrected SCI χ and R_2^* as a function of brain lobe were investigated using ANOVA and Tukey's honest significance test. Correlation analysis was used to investigate the association between lesion volume and age-corrected SCI χ and R_2^*, The effect of white matter depth on age-corrected SCI χ and R_2^* was considered in a separate ANOVA, as some lesions were split across multiple white matter regions. In the SCA cohort only, for whom blood samples were available, correlations were examined between age-corrected SCI χ and R_2^* and blood hemoglobin levels, a measure of anemia.

To consider the potential association between cognitive performance and mean SCI χ/R_2^*, mean χ/R_2^* were calculated across SCI in participants with multiple SCI. Correlations were then investigated between mean SCI χ/R_2^* per subject and measures of cognitive performance.

Results

A total of 93 participants with SCA and 33 controls were eligible for inclusion in this investigation after being imaged with the three necessary MRI sequences (T1w MP-RAGE, T2w FLAIR, and ME-GRE) as part of the two clinical studies (Table 1). The presence of SCI was identified in 38/93 SCA subjects (40.8%) and 10/33 controls (3 Hb-AA, 7 Hb-AS) (30.3%). 2 patients with SCA and 1 control were excluded due to

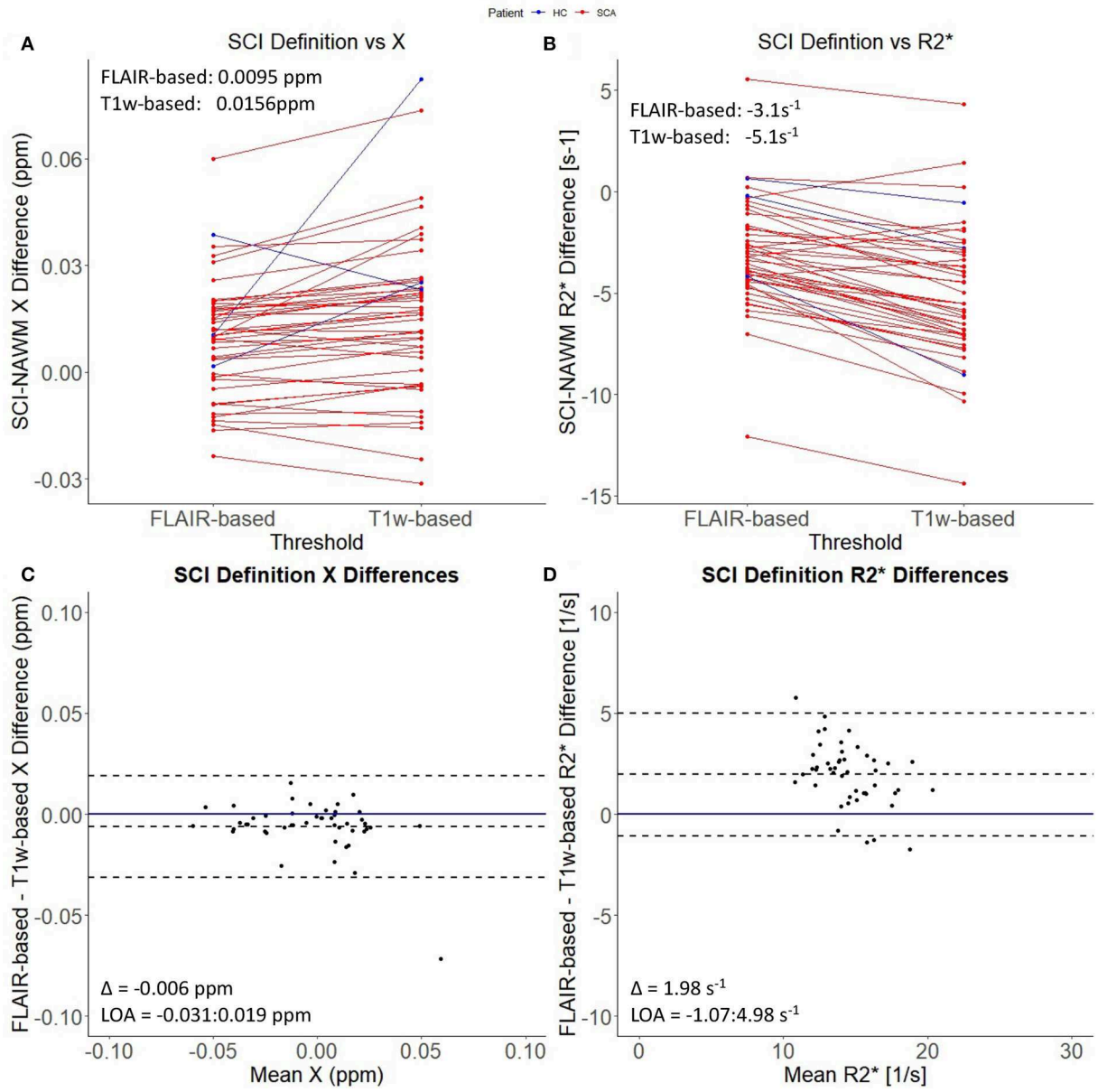


FIGURE 4
Effect of SCI definition on SCI-NAWM χ and R_2^*. **(A)** Comparison of mean silent cerebral infarct (SCI) and normal appearing white matter (NAWM) susceptibility (χ) differences before and after the application of the more stringent T1w-based threshold. **(B)** Comparison of mean SCI-NAWM R2* differences before and after application of the T1w-based threshold. Mean SCI-NAWM χ and R_2^* differences for the two definitions are shows in **(A,B)** respectively. **(C)** Bland-Altman analysis of mean SCI-NAWM χ differences before and after application of the T1w-based threshold. The mean bias (Δ) and limits of agreement (LOA) are annotated within the plot. **(D)** Bland-Altman analysis of the mean SCI-NAWM R_2^* differences.

poor image quality in at least one of the three image contrasts. SCI in 4 patients with SCA and 3 controls did not meet the inclusion criteria after interpolation into the native QSM space. As a result, SCI segmented in 32 patients with SCA, and 6 controls were considered in this study.

211 SCI Segmentations Persisted after Interpolation into the native QSM space, 178 (∼84%) in patients with SCA and 33 (∼16%) in controls. Most lesions were in either the frontal (125/211, 59.2%) or parietal lobe (66/211, 31.3%), with fewer SCI located in the occipital (8/211, 3.8%) or temporal lobe (7/211, 3.3%). Heatmaps showing the distribution and frequency of SCI segmented in the SCA and control subjects in this cohort has been published previously (15). Lesion distributions were found to be similar in the patient and control groups.

Of the 211 lesion segmentations which persisted after interpolation into the native QSM space, 47 (∼22%) persisted

TABLE 1 Overview of participant and lesion characteristics in patients with sickle cell anemia (SCA) and healthy controls (HC).

Participants	SCA	HC	P
N	32	6	–
Male (Yes/No)	22/10	2/4	0.1
Age (mean ± sd) [years]	20.6 ± 13.1	24.5 ± 17.4	0.54
Age (min-max) [years]	8.5-63.9	14.8-59.5	–
Hematocrit (mean ± sd) (g/dL)	8.82 ± 1.47	NA	–
Chronic transfusion (yes|no)	2|30	NA	–
Transfusion last 6 Months	4|28	NA	–
Abnormal MRA (yes|no)	7|25	0|6	–
Severe vasculopathy (yes|no)	1|31	0|6	–
FSIQ (mean ± sd)	91.9 ± 13.5	89.5 ± 7.4	0.52
WMI (mean ± sd)	89.6 ± 13.0	94.7 ± 7.8	0.48
PSI (mean ± sd)	87.6 ± 11.1	86 ± 19.0	0.55
Lesion count (mean ± sd)	5.6 ± 5.8	5.5 ± 4.1	0.75
Lesions (FLAIR|T1w)			
N	178|44	33|3	–
Frontal	113|35 (63.5|79.5%)	12|1 (36.4|33.3%)	–
Occipital	3|0 (1.7|0.0%)	5|1 (15.2|33.3%)	–
Parietal	53|6 (29.8|13.6%)	13|1 (39.4|33.3%)	–
Temporal	4|0 (2.2|0.0%)	3|0 (9.1|0.0%)	–
Other	4|3 (2.2|4.5%)	0|0 (0.0|0.0%)	–
Right Hemisphere	92|25 (51.7|56.8%)	9|1 (27.3|33.3%)	–
Left Hemisphere	86|19 (48.3| 43.2%)	24|2 (72.7|66.7%)	–

Lesions characteristics are shown for both the FLAIR-based and T1-weighted-based SCI segmentations. *P*-values are reported from the Wilcoxon Rank Sum Tests and Chi-Squared tests used to compare differences in the SCA and HC cohorts.

after application of the additional, more stringent T1w-based threshold. Of these, 44/47 (∼94%) were present in sickle cell subjects and 3/47 (∼6%) were present in healthy controls. Following application of the T1w-based threshold, a much lower proportion of lesions persisted in the parietal lobe (7/66, 10.6%) compared with the frontal lobe (36/125, 28.8%).

The distribution of SCI as a function of age, sex and SCA status within the study cohort is shown in Figure 2.

Comparison of χ and R_2^* in SCI vs. NAWM

Mean χ (and R_2^*) values were strongly correlated between SCI and corresponding NAWM ROIs (χ: $r = 0.62$, $p < 0.001$, R_2^*: $r = 0.33$, $p = < 0.001$) (Figures 3A,B). Paired t-tests indicated that mean χ was significantly higher (less diamagnetic) in SCI compared to NAWM in the contralateral hemisphere (SCI: – 0.0067 ppm vs. NAWM: – 0.0153 ppm, $\Delta = 0.0086 \pm 0.0014$ ppm, $p < 0.001$), while mean R_2^* was significantly reduced (SCI: 16.7 s^{-1} vs. 19.2 s^{-1}, $\Delta = – 2.5 \pm 0.2$ s^{-1}, $p < 0.001$). The mean and distribution of differences between SCI and NAWM are shown in the Bland-Altman plots in Figures 3C,D and the boxplots in Figures 3E,F.

TABLE 2 Results of an ANOVA investigating the effect of subject age, sickle cell status, brain lobe and lesion volume on SCI and NAWM χ and R_2^*.

ANOVA	LN (Age)	SCA	Lobe	Ln (Volume)
SCI χ	**0.0129**	0.0756	**<0.0001**	0.0815
NAWM χ	**0.0045**	0.15	**0.0002**	0.87
SCI-NAWM χ	0.48	0.85	**0.037**	0.11
SCI R_2*	**0.009**	**<0.0001**	0.16	**0.01**
NAWM R_2*	**0.007**	0.26	0.07	0.08
SCI-NAWM R_2*	0.997	**<0.001**	0.09	0.45

Presented in the table are *p*-values indicating which variables were significantly associated with differences in SCI and NAWM χ and R_2^* values.
$P < 0.05$ were considered significant and are highlighted in bold.

Investigating SCI χ and R_2^* as a function of lesion definition

Figure 1 demonstrates that the signal abnormalities in the FLAIR images extend beyond regions of hypointense signal in the T1–weighted images. Applying the T1w-based threshold reduced the mean SCI volume size by ∼86% (FLAIR threshold: 118.7 ± 217.5 mm^3 vs. FLAIR + T1-w threshold: 16.7 ± 43.2 mm^3).

Prior to T1w-based thresholding, no significant difference in SCI–NAWM χ difference was found between lesions which did and did not persist using the stricter definition. ($\Delta\chi_{\text{Persistent}}$: 0.0095 ppm vs. $\Delta\chi_{\text{Non-persistent}}$: 0.0084 ppm, $p = 0.7$). A trend toward lower SCI–NAWM R_2^* differences was observed in lesions which did persist using the more stringent definition relative to those that did not ($\Delta R_{2\text{ Persistent}}^*$: −3.14 s^{-1} vs. $\Delta R_{2\text{ Non-persistent}}^*$: −2.31 s^{-1}, $p = 0.06$). Bland Altman analysis comparing SCI and NAWM χ and R_2^* in the lesions which did and did not persist after the application of the T1w-based threshold is shown in Supplementary Figure 1.

In the lesions which did persist, paired *t*-tests showed that, after thresholding, lesion χ was significantly less diamagnetic in the T1w thresholded ROIs relative to the FLAIR segmentations (SCI χ_{T1w}: 0.001 ppm vs. SCI χ_{FLAIR}: – 0.005 ppm, $p = 0.002$) which manifests as larger χ differences relative to NAWM ($\Delta\chi_{\text{T1w}}$: 0.0156 ppm vs $\Delta\chi_{\text{FLAIR}}$: 0.0095 ppm, $\Delta = 0.0061 \pm 0.0019$ ppm, $p = 0.002$) (Figure 4). Similarly, SCIs defined using the T1w-based threshold had significantly lower mean R_2^* (SCI $R_{2\text{T1w}}^*$: 13.6 s^{-1} vs. SCI $R_{2\text{FLAIR}}^*$: 15.6 s^{-1}, $\Delta = 2.0 \pm 0.2$ s^{-1}, $p < 0.001$), corresponding to larger SCI–NAWM R_2^* differences ($\Delta R_{2\text{T1w}}^*$: −5.1 s^{-1} vs $\Delta R_{2\text{FLAIR}}^*$: −3.1 s^{-1}).

Investigating SCI χ and R_2^* as a function of subject demographic, anatomical location and volume

The results of the ANOVA investigating the effect of subject age, sickle cell status, brain lobe and lesion volume upon SCI χ and R_2^* are shown in Table 2.

Figure 5 shows SCI and NAWM χ and R_2^* plotted as a function of log-transformed age. SCI and NAWM χ were both significantly negatively correlated with log-transformed subject age, consistent with increasing myelin content in this relatively young cohort (42). However, no significant association with age was observed for the SCI–NAWM χ difference (Figure 5). In contrast, SCI and NAWM mean R_2^* were positively associated with age, in agreement with increased myelin content, and no significant correlation was observed in the SCI–NAWM R_2^* difference.

When considering the relationship between SCI–NAWM χ and R_2^* in SCI which persisted on application of the T1w threshold, no significant correlation between χ / R_2^* and age was observed (Supplementary Figure 2). In subsequent results the SCI and NAWM χ and R_2^* are age-corrected to remove any confounding effects of age on the effect of SCA, brain lobe and lesion volume on χ and R_2^* values.

Comparing age-corrected SCI and NAWM χ in patients with SCA relative to controls, no significant difference was observed in the SCI mean χ (SCI χ_{SCA}: − 0.006 ppm vs. SCI χ_{HC}: − 0.012 ppm, $p = 0.11$), or the SCI–NAWM differences ($\Delta\chi_{SCA}$: 0.0087 ppm vs. $\Delta\chi_{HC}$: 0.0085 ppm, $p = 0.96$). Age-corrected SCI R_2^* was significantly lower in patients with SCA relative to controls (SCI R_{2SCA}^*: 16.3 s^{-1} vs. SCI R_{2HC}^*: 18.9 s^{-1}, $p < 0.0001$), and this was also reflected in the SCI–NAWM difference (ΔR_{2SCA}^*: −2.84 s^{-1} vs ΔR_{2HC}^*: −0.64s^{-1}, $p < 0.0001$) (Figure 6). Participant numbers precluded further comparisons of the T1w-thresholded lesions between patients with SCA and controls, with such lesions only persisting in 2 controls.

Lesion χ and R_2^* were investigated as a function of brain lobe. Increased χ within SCI in the frontal lobe, relative to the parietal lobe, mirrored increased χ observed in NAWM, and no significant SCI–NAWM χ differences were observed between brain lobes (Figure 7). Inter-lobe mean R_2^*differences were not observed, but SCI–NAWM R_2^* differences were slightly elevated in the frontal vs parietal lobe ($\Delta R_{2frontal}^*$: − 2.86 s^{-1} vs. $\Delta R_{2parietal}^*$: − 2.13 s^{-1}, $p = 0.05$).

Similar χ trends as for the FLAIR defined lesions were observed between T1w-thresholded lesions in the frontal and parietal lobes (Supplementary Figure 3). However, the between-lobe SCI–NAWM ΔR_2^* difference between the frontal and parietal lobes observed for FLAIR lesion segmentations was no longer statistically significant for the more stringently defined T1w-thresholded SCI.

Despite significant correlations between age-corrected SCI χ and R_2^* and log (lesion volume) in the FLAIR-defined SCI, no significant associations between SCI–NAWM differences in χ (and R_2^*) and lesion size were found (Figure 8). Furthermore, no significant relationship between the age-corrected SCI–NAWM χ and R_2^* difference and white matter depth were observed for the juxta-cortical, deep, or periventricular SCI (Supplementary Figure 5).

No significant associations were found between age-corrected SCI mean χ and R_2^* (or SCI-NAWM χ and R_2^* differences) and blood hemoglobin, a measure of anemia severity only available in patients with SCA.

Investigating effect on cognitive impairment as a function of SCI χ and R_2^*

No significant associations were found between any of the cognitive measures (FSIQ/WMI/PSI) and mean SCI–NAWM χ or R_2^* differences (or mean age-corrected SCI χ and R_2^*) when considering the mean lesion values in participants with multiple lesions (Figure 9). No associations were found for either the combined SCA and HC cohort, or in the SCA cohort only.

Discussion

Using QSM and R_2^*, the objectives of this study were to investigate the composition of SCI relative to NAWM in patients with SCA and controls and investigate SCI χ and R_2^* as a function of other lesion characteristics, participant demographics and cognition. Mean χ was significantly higher (less diamagnetic) and mean R_2^* was significantly reduced in SCI compared to NAWM in the contralateral hemisphere. Lesion χ was significantly less diamagnetic with significantly lower mean R_2^*in the persisting T1w- thresholded ROIs relative to the FLAIR-based segmentations. SCI-NAWM R_2^* differences were larger in patients with SCA relative to controls. There was no effect of lesion size or anatomical location and no association with cognitive performance.

The rate of SCI within patients with SCA eligible for inclusion in this study (40.8%) was in line with rates reported previously in SCA literature (43). SCI were reported in 30.3% of healthy control subjects, which may seem higher than expected given the age range of controls in this cohort. However, previous high resolution MRI studies of SCI, with similar age ranges of controls, have reported high prevalence of SCI within the control cohorts, as high as 70% (44).

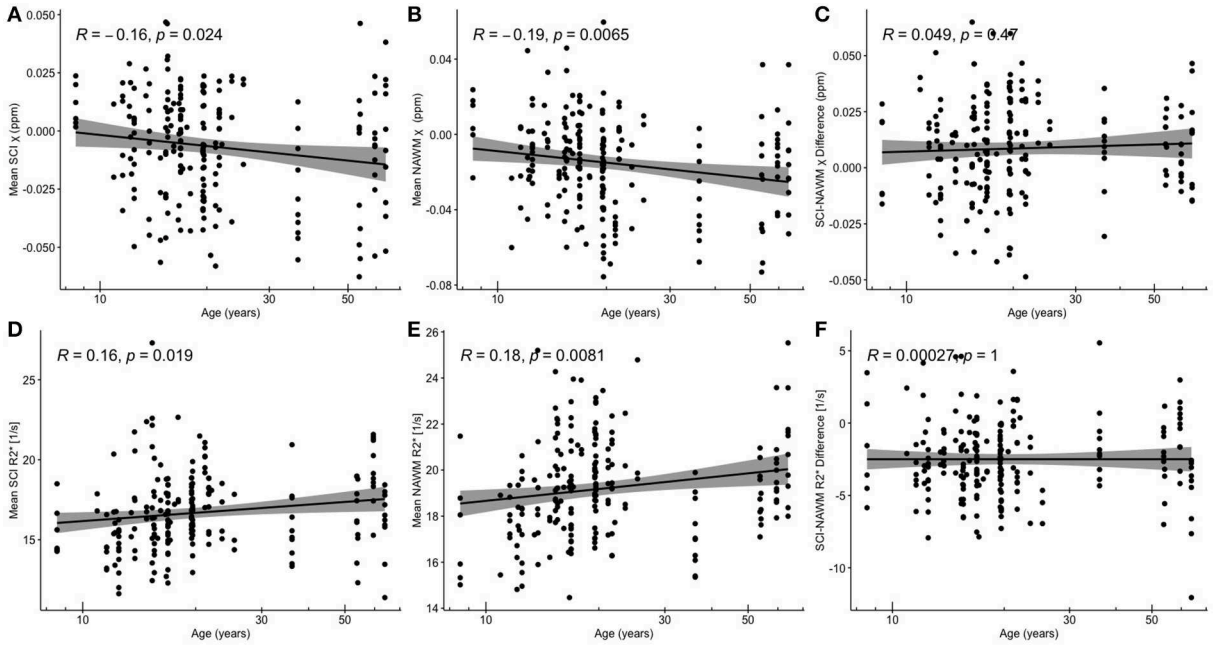


FIGURE 5
Correlations between SCI and NAWM χ and R_2^* and age. **(A)** Mean silent cerebral infarct (SCI) susceptibility (χ) **(B)** mean normal appearing white matter (NAWM) χ **(C)** SCI-NAWM χ difference **(D)** mean SCI R_2^* **(E)** mean NAWM R2* **(F)** mean SCI-NAWM R2* difference as a function of age plotted on a logarithmic scale. SCI were segmented using the FLAIR-based SIT trial definition.

Comparison of χ and R_2^* in SCI vs NAWM

QSM and R_2^* mapping showed increased mean χ and reduced mean R_2^* within SCI segmented using the SIT-trial (FLAIR-based) definition relative to NAWM in the contralateral hemisphere. This can be seen visually in Figure 1, with the segmented SCI appearing brighter on χ maps and darker on R_2^* maps relative to the surrounding NAWM.

An increased mean χ relative to NAWM could be caused by either an increase in the concentration of paramagnetic substances, such as iron in the form of ferritin or deoxygenated hemoglobin, or a reduction in diamagnetic χ sources such as myelin. The reduction of R_2^* in SCI suggests a loss of susceptibility sources (less myelin) as opposed to additional χ sources, since increased iron accumulation would be expected to increase R_2^* within the lesions.

Cerebral infarction is the process of tissue death caused by ischemia and hypoxia which results in a region of necrotic tissue. These necrotic regions are believed to be what is segmented based upon the accepted definitions in the SCA literature. As necrosis results in cell swelling, membrane rupture and the release of cell contents into the extracellular space triggering an inflammatory response, it is reasonable that susceptibility sources are reduced within the SCI, with loss of myelin, either primary or secondary to infarction, a potential explanation for our observations.

Ischemia and hypoxia have been linked to brain iron accumulation. Ischemia may be a key inflammatory trigger in SCA, and inflammatory processes have been linked to iron accumulation in numerous neurodegenerative conditions including Parkinson's (45–47) and Alzheimer's (48, 49). However, it appears that iron is preferentially taken up in the deep gray matter regions, where iron is predominately stored within ferritin macromolecules, as opposed to regions surrounding the sites of infarction (50).

Changes in myelin content may not be the sole contributor to the χ and R_2^* changes observed in SCI. Increased water content causes similar χ and R_2^* changes relative to normal appearing white matter, as reduced myelin content. Increased tissue water content causes the bulk tissue χ to become closer to zero in the calculated χ maps (which are intrinsically referenced to the χ of water). Therefore, in white matter, increased tissue water content causes the bulk χ of the regions to increase (become less diamagnetic). The lower concentration of susceptibility sources, caused by excess water, reduces R_2^* relative to NAWM, comparable to loss of myelin content. Regions of increased water content, in conditions such as edema, appear bright on T2-weighted MRI sequences and dark on T1-weighted MRI sequences relative to NAWM, analogous to regions of infarction. Therefore, it is challenging to separate the two mechanisms. Future imaging studies should aim to differentiate between increased water content and regions of infarction using

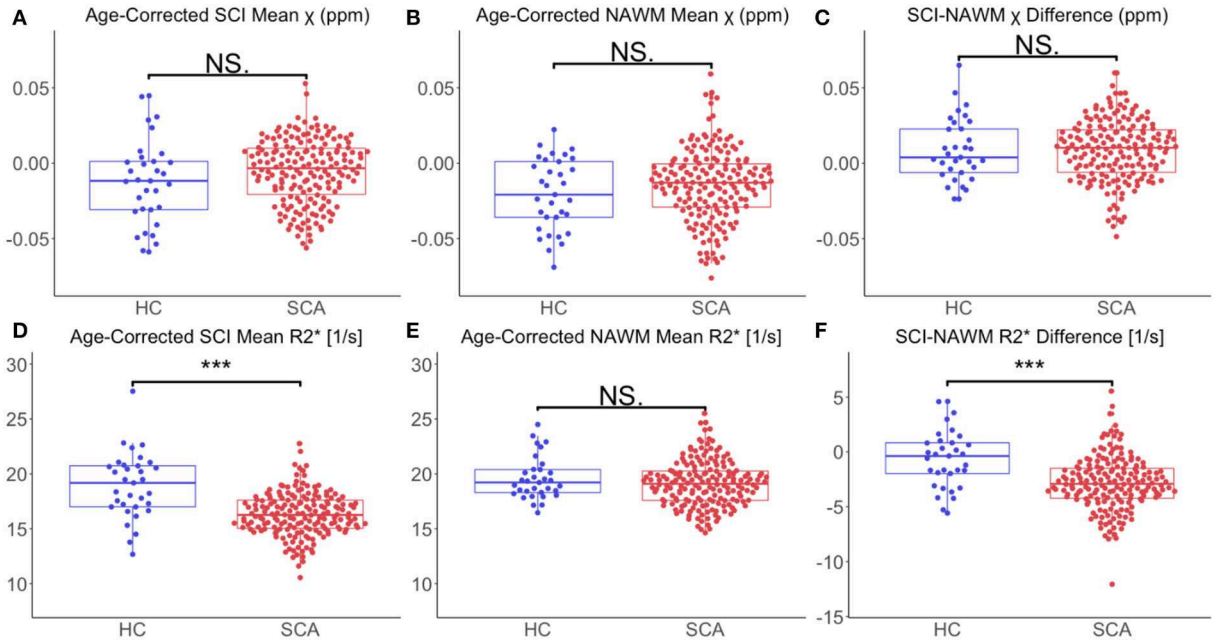


FIGURE 6
Effect of SCA on SCI and NAWM χ and R_2^*. **(A)** Comparison of mean age-corrected silent cerebral infarct (SCI) susceptibility (χ) in patients with sickle cell anemia (SCA) and healthy controls (HC). **(B)** Comparison of mean age-corrected normal appearing white matter (NAWM) χ in SCA and HC. **(C)** No significant differences in mean SCI-NAWM χ difference were observed between the SCA and HC groups (0.009 vs. 0.006 ppm, $p = 0.5$). **(D)** Comparison of mean age-corrected SCI R_2^* in SCA and HC. **(E)** Comparison of mean age-corrected NAWM R_2^* in SCA and HC. **(F)** The SCI–NAWM R_2^* difference was significantly lower in patients with SCA relative to HC (– 2.84 vs. – 0.64 s^{-1}, $p < 0.0001$). SCI were segmented using the FLAIR-based SIT trial definition. ***$p < 0.001$.

diffusion weighted imaging, where edema appears bright, or CT imaging where edema results in reduced attenuation relative to normal appearing white matter.

Investigating SCI χ and R_2^* as a function of lesion definition

The most common (FLAIR-based) definition of SCI, developed for the SIT trial, required a signal abnormality measuring at least 3 mm in greatest dimension and visible in at least two planes (3). This definition is dependent upon acquisition parameters such as voxel dimensions, which will determine whether lesions are visible in two planes or not, depending on the size of the lesion relative to the voxel dimensions. Therefore, advances in MRI hardware and acquisition have resulted in increased sensitivity to signal abnormalities compared with images obtained when the definitions were published.

In this study, all hyperintense signal abnormalities in control subjects were considered SCI in agreement with most current SCA literature. However, these lesions could also be reported as white matter hyper intensities (WMH), which have been the subject of numerous studies in healthy adults (51, 52). While the term has also been used in some SCA literature (25, 53), the vast majority of studies use the term 'SCI'.

In this study, we found that SCI definition had a significant effect on the mean χ and R_2^* of the segmented SCI. Applying the T1w-threshold increased the magnitude of the χ and R_2^* differences relative to normal appearing white matter. These differences suggest that the T1w-based segmentations may be more sensitive to regions of abnormal χ and R_2^* relative to NAWM, which may reflect necrosis, compared to the FLAIR based definitions. If the presence of SCI were linked to cognitive impairment in patients with SCA, it may be expected that SCI which persist after applying the more stringent T1w-based threshold would have a greater impact on cognitive performance, given the larger differences relative to NAWM. However, no significant association between the presence of SCI, which met the stringent T1w-based SCI definition, and cognitive performance was found in the previous study in this cohort (15).

MRI signal abnormalities were identified and labeled as SCI based upon two definitions applied in previous studies of sickle cell anemia, considering T2-weighted FLAIR and T1-weighted MP-RAGE contrasts. In the broader ischemic stroke literature, diffusion weighted imaging (DWI) has been used to identify the presence of acute ischemic lesions (54, 55). Acute lesions appear hyperintense on DWI images, and hypointense on maps

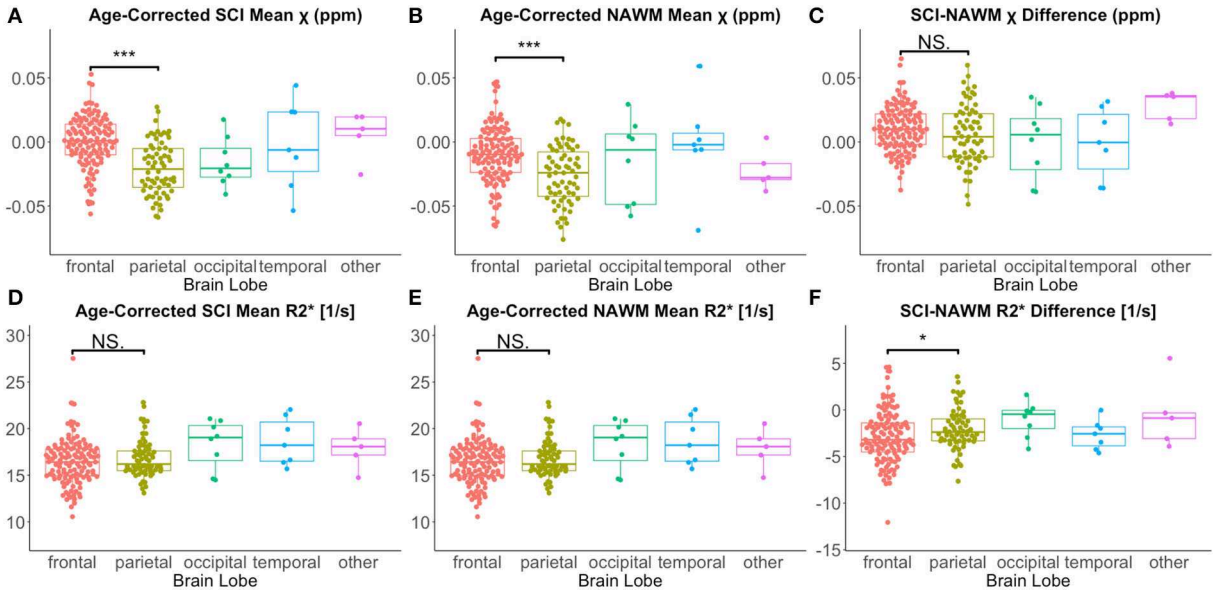


FIGURE 7
Effect of anatomical location on SCI and NAWM χ and R_2^*. Comparison of mean susceptibility (χ) and R_2^* measured in silent cerebral infarcts (SCI) in each of the brain lobes. **(A)** SCI in the frontal lobe were significantly more paramagnetic relative to lesions in the parietal lobe. **(B)** This lobar χ difference was also observed in normal appearing white matter (NAWM). **(C)** This resulted in no significant differences in the SCI–NAWM susceptibility difference between the lobes. **(D,E)** No significant differences were observed between lobes in the SCI and NAWM mean R2* comparisons. **(F)** A small, significant difference was observed between the SCI–NAWM R_2^* difference between lesions in the frontal and parietal lobes. SCI were segmented using the FLAIR-based SIT trial definition. $^*p < 0.05$. $^{***}p < 0.001$.

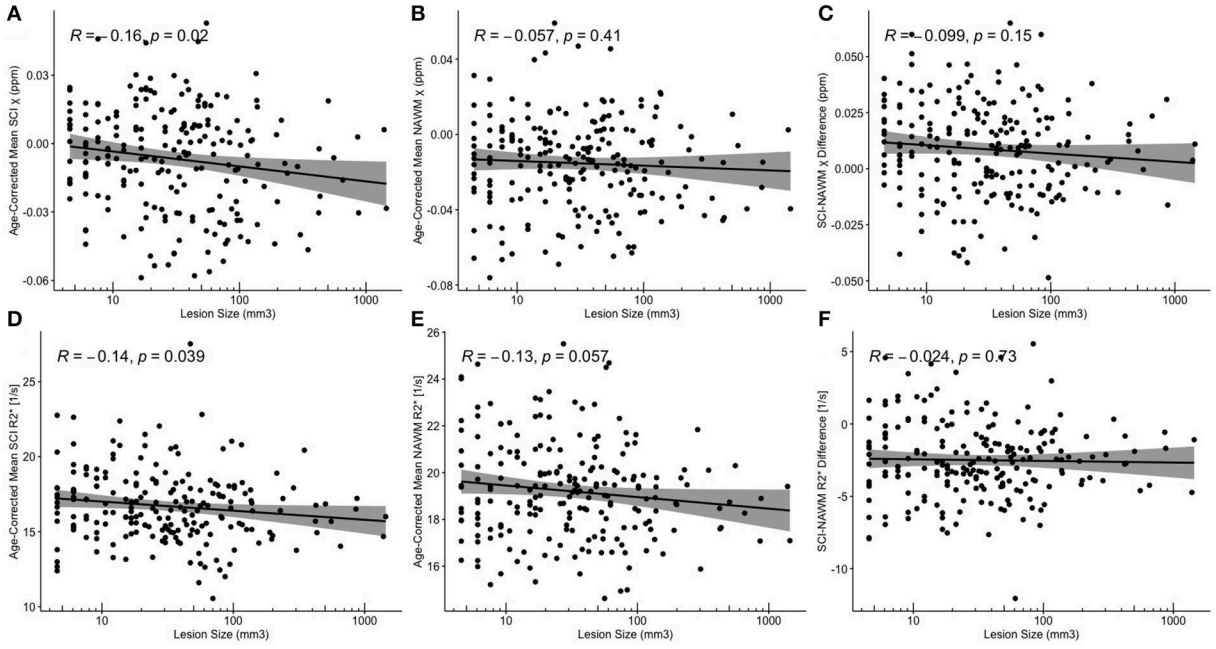


FIGURE 8
Correlations between SCI and NAWM χ and R_2^* and lesion volume. **(A,D)** Mean silent cerebral infarct (SCI) **(B,E)** normal appearing white matter (NAWM) **(C,F)** SCI-NAWM differences in susceptibility (χ) and R2* as a function of lesion volume plotted on a logarithmic scale. Mean SCI χ and R2* in regions segmented based upon the FLAIR-based definition.

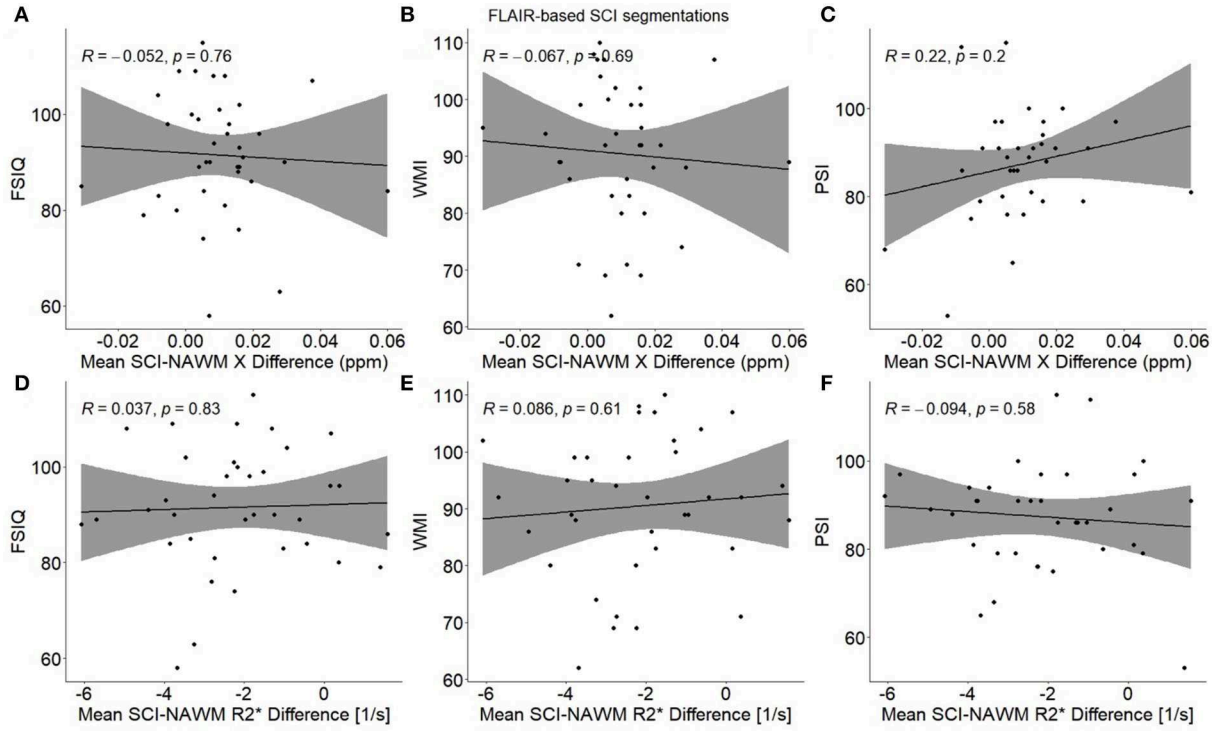


FIGURE 9
Association between participant mean SCI–NAWM χ and R_2^* differences and measures of cognitive performance. Within each participant, mean silent cerebral infarcts (SCI) χ and R_2^* differences relative to normal appearing white matter (NAWM) were calculated across all lesions, and associations were examined with full scale IQ (FSIQ) **(A,D)**, working memory index (WMI) **(B,E)** and processing speed index (PSI) **(C,F)**. No significant associations were observed between SCI–NAWM χ and R_2^* and any measures of cognition. SCI were segmented using the FLAIR-based SIT trial definition.

of apparent diffusion coefficient (ADC). Acute silent cerebral ischaemic events (ASCIE) have been investigated in patients with SCA, and it has been shown that not all ASCIE develop into chronic infarcts (56, 57). The use of FLAIR and T1-weighted image contrasts for SCI identification and segmentation ensured that this study focussed on chronic SCI lesions.

Investigating SCI χ and R_2^* as a function of subject demographics, anatomical location and volume

The greater SCI-NAWM R_2^* difference observed in patients with SCA relative to controls (Figure 6) indicates that the myelin content reduction in SCI may be more severe in these patients. This may be secondary to the hemodynamic stress observed in patients with SCA (24, 58). The increased SCI-NAWM R_2^* differences in SCI in patients with SCA relative to controls may also reflect the larger of proportion of SCI which persist using the T1w-based definition in patients with SCA. The reduced R_2^* of the SCI segmented in patients with SCA relative to controls show that there are larger differences in SCA SCI relative to NAWM. This suggests that more white matter damage/necrosis occurs in SCI in patients with SCA relative to controls, and that lesions in patients with SCA and controls may arise from different mechanisms.

No significant association between SCI–NAWM χ or R_2^* difference was observed with age in this cohort. If the mechanisms for SCI were dynamic and lesion composition evolved with time, then we might expect to see such associations. However, their absence suggests that negligible changes in lesion composition occur within the lesion once it has been sustained. It may be more valid to investigate lesion composition as a function of lesion age as opposed to subject age, to determine if changes in lesion composition occur with time. Note that the age of the infarcts in this study is not known and, therefore, the χ of the SCI could not be investigated as a function of lesion age. Future, longitudinal studies would allow examination of the formation of new lesions and evaluation of their evolution with time.

Both SCI and NAWM χ did have a significant negative association with the log-transformed patient age, and R_2^* values showed a significant positive association for the FLAIR-based segmentations (Figure 5). In the NAWM, decreasing

χ and increasing R_2^* with age is consistent with increasing myelin content, as expected in these young subjects with active myelination. The results of the SCI–NAWM comparison showed reduced susceptibility sources in the SCI relative to NAWM. However, the associations of SCI χ and R_2^* with age suggest that some myelin content remains in these regions and that the amount of myelin increases as a function of age. If there were no myelin present within the segmented SCI, we would expect no relationship with age. The SCI, and corresponding NAWM, segmented using the T1w-based definition demonstrated no significant associations with age (Supplementary Figure 2), probably due to the low number of lesions persisting after the more stringent definition.

No significant effect of lesion position in the brain (neither brain lobe nor lesion depth in white matter) was observed on mean SCI–NAWM χ or R_2^* difference (Figure 7, Supplementary Figures 4, 5).

While significant differences were observed between mean χ in NAWM in the frontal and parietal lobes, no such differences were observed between R_2^* values. Similar differences between lobes may be expected for both for the χ and R_2^* values, as they are both dependent upon the concentration of susceptibility sources. The discrepancy between R_2^* and χ values may have been caused by differences in the χ anisotropy of the regions or residual background fields present only in the χ maps. Emerging QSM techniques such as weak-harmonic QSM can be used to remove residual background fields when calculating χ maps and could help isolate the reason for this discrepancy between the χ and R_2^* results (59).

Differences in the mean SCI χ/R_2^* as a function of white matter depth may have been expected if differences in lesion composition were present. In this predominantly SCA cohort, a large proportion of the lesions were present in deep and juxtacortical white matter, which differs from WMH in elderly healthy populations where the majority of lesions are found in periventricular regions (60).

In QSM, χ is assumed to be an isotropic property which is independent of the direction of the applied magnetic field. This assumption is known to break down in white matter, where the highly structured axons result in susceptibility anisotropy (61–63). As a result, when comparing lesions in different regions of the brain, changes in χ could be caused by differences in orientation relative to the main magnetic field, in addition to changes in tissue composition.

It may be more informative to examine the white matter tract in which the lesion resides, as opposed to considering the lesion depth within white matter. This could be achieved by incorporating information from diffusion tensor imaging (DTI). Furthermore, spatial variation could be considered by examining the position of the SCI relative to the arterial territories. SCI are most commonly reported in the border zone region between arterial territories (11); however this finding comes mostly from low-resolution studies, and our recent high-resolution study suggests that smaller SCI may be just as common in juxta-cortical regions (64).

Investigating effect on cognitive impairment as a function of SCI χ and R_2^*

It has previously been widely reported that the presence of SCI is linked to increased cognitive deficits in patients with SCA (8). However, our previously published data in this cohort found no significant associations between measures of cognitive performance (FSIQ, WMI, and PSI) and conventional SCI lesion metrics (binary lesion presence/absence, number of lesions, and lesion volume) for any of the lesion definitions considered (15). Here, we investigated whether SCI tissue characteristics i.e., χ and R_2^* are associated with cognitive impairment and found no associations.

The cognitive difficulties present in patients with SCA have been reported to worsen with age. In the general population, white matter hyperintensities appear to be associated with longitudinal intellectual decline, particularly in those with mild cognitive impairment and stroke (65). Autopsy data are consistent with an effect on myelin secondary to arteriosclerosis and ischemia (66), associated with decline in perceptual speed, with some evidence of amyloid-β accumulation eventually (67). There are however few MRI data on WMH composition with which to compare our SCI data.

Limitations

A limitation of this study is the differences in resolution between the three MRI images used i.e., FLAIR: 0.65 × 0.65 × 1 mm^3, T1w MP-RAGE:1 × 1 × 1 mm^3, ME-GRE:1.15 × 1.15 × 1.15 mm^3. The SCI segmentations underwent a series of transformations and resampling with nearest neighbor interpolation before mean χ and R_2^* values were calculated in the native space of the tilt-corrected multi-echo gradient echo images. These interpolations often resulted in the loss of voxels from the SCI segmentations (e.g., see Figure 1) and introduced registration errors, labeling non-SCI voxels as lesions. Future studies should aim to utilize more consistent fields of view and voxel dimensions across the multiple MRI sequences acquired.

Partial volume effects will be present in this study due to the affine transformations applied to the lesion segmentations and the different resolutions of the three image contrasts. Partial volume effects may have led to the inclusion of normal appearing white matter tissue within the SCI segmentations. This would be expected to reduce SCI-NAWM differences in both χ and R_2^*.

Partial volume is likely to have a greater effect on larger SCI segmentations. A significant negative correlation was observed

between log-transformed lesion volume and mean SCI χ. This correlation may be affected by the inclusion, in larger segmentations, of normal appearing white matter, which is more diamagnetic than regions of infarction according to the SCI-NAWM comparison.

The effect of applying the T1w-threshold significantly reduced the volume of the SCI segmentations. Applying the T1w-based threshold increased the mean χ and reduced the mean R_2^*, increasing the SCI χ and R_2^* differences relative to NAWM. We might expect the lesions that persist after T1w-based thresholding to have more severe composition changes relative to NAWM, which would explain why these lesions also appear hypointense on T1-weighted images. However, the reduced volume is also less likely to contain partial volume effects so these may also contribute to the significant difference observed between lesion χ and R_2^* before and after the application of the more stringent lesion definition.

From the SAC and POMS studies 275 SCI lesions were present in subjects with good quality images from the three necessary MRI contrasts. Following interpolation into the native QSM space, 64/275 lesions had ≤ 2 voxels and were, therefore, excluded. Therefore, the results of this study will contain a smaller contribution from lesions consisting of just of few voxels in the original segmentations, as larger lesions were more likely to persist and be included in the final results

Although the ME-GRE sequence acquired in this study had a final echo time of 27 ms, which is shorter than the mean T2* of the SCI measured in this study (61 ms), the sequence had regular sampling of the magnitude signal decay and, therefore, provided accurate SCI R_2^* measurements (68).

After quality control and interpolation into the native QSM space, SCI were examined in 6 healthy controls, compared to 32 patients with SCA. The greater number of patients with SCA recruited in the SAC and POMS studies, relative to the number of controls, and the greater incidence rate of SCI within SCA relative to controls in the participants, means that the results of this study are dominated by SCI in SCA. This study found significant R_2^* differences between SCI in patients with SCA and controls. To increase confidence in this result, future work should look to compare SCI within a more evenly distributed cohort of SCA and control participants.

Participants imaged in this study were aged 8.5–63.9 years, with the majority of participants aged between 15 and 25 years. There were few data in participants aged between 25 and 50 years old. To fully understand changes in SCI lesion composition as function of age, future studies should increase the number of SCI investigated in participants within the range 25–50 years.

Regions of normal appearing white matter were positioned in the contralateral side, making sure to avoid the locations of identified infarcts. However, diffusion tensor imaging studies have shown that in some white matter regions, white matter integrity is decreased in patients with SCA relative to healthy controls, even in the absence of any focal regions of white matter damage (69). Therefore, regions which appeared as visually normal on the T1-weighted images and were, therefore, classified as normal appearing white matter (NAWM), may have shown changes associated with SCA.

Conclusion

R_2^* and quantitative susceptibility mapping were used to non-invasively investigate the composition of SCI relative to normal appearing white matter in patients with sickle cell anemia and controls. We demonstrated that SCI were significantly less diamagnetic and had lower R_2^* relative to NAWM, suggesting lower myelin concentrations and/or increased water content in both patients and controls. The SCI-NAWM R_2^* decrease observed within SCI was significantly larger in patients with SCA compared with controls. SCI definition had a significant effect on mean lesion χ and R_2^*; in SCI which persisted after the more stringent T1w-based SCI definition was applied, mean R_2^* was significantly reduced and mean χ significantly increased. Thus, the T1w-based segmentation appears to be more sensitive to changes in SCI χ and R_2^* relative to NAWM. We have shown that future SCI studies can use quantitative MRI methods such as R_2^* and QSM to enhance our understanding of the pathophysiology and composition of SCI in patients with SCA as well as controls.

Data availability statement

Full anonymized data will be shared on request from the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by West London NHS (05/Q0408/42, 11/EM/0084, 15/LO/0347), Yorkshire NHS (15/YH/0213) and University College London (14475/001) Ethics Committees. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

RM: manuscript preparation, QSM/R2* processing, and contralateral NAWM ROI segmentation. HS: data acquisition, SCI segmentation, and manuscript review. JK: data acquisition. DS: manuscript review and SCI detection. FK: manuscript review and data acquisition. KS: manuscript review and QSM/R2* processing. All

authors contributed to the article and approved the submitted version.

Funding

HS was funded by Action Medical Research (GN2509) and JK was funded by Great Ormond Street Children's Charity (V4615). This work was supported by the National Institute for Health Research and Biomedical Research Center at Great Ormond Street Hospital for Children NHS Foundation Trust and the Institute of Child Health (IS-BRC-1215-20012). KS was supported by European Research Council Consolidator Grant DiSCo MRI SFN 770939. The National Institute for Health Research (UK; PB-PG-1112-29099) and NHLBI (R01HL079937) provided funding for patient recruitment. RM was supported by the EPSRC-funded UCL Center for Doctoral Training in Medical Imaging (EP/L016478/1).

Acknowledgments

The authors would like to thank the patients and controls who participated in this research and their families. They would also like to thank the Great Ormond Street Hospital research radiographers for their efforts; Tina Banks, Jessica Cooper, Nichola Sellers, Paul Xavier, Bella Said, Jorvaar Gill, and Michelle Quigley.

Conflict of interest

FK was grant holder for GN2509, V4615, PB-PG-1112-29099, and R01HL079937 and has received honoraria from Global Blood Therapeutics, Bluebird Bio, Novartis, BIAL, Shire and Johnson and Johnson outside this work and not relevant to it.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2022.1000889/full#supplementary-material

References

1. Debaun MR, Kirkham FJ. Central nervous system complications and management in sickle cell disease. *Blood.* (2016) 127:829–38. doi: 10.1182/blood-2015-09-618579

2. Houwing ME, Grohssteiner RL, Dremmen MHG, Atiq F, Bramer WM, de Pagter APJ, et al. Silent cerebral infarcts in patients with sickle cell disease: a systematic review and meta-analysis. *BMC Med.* (2020) 18:1–17. doi: 10.1186/s12916-020-01864-8

3. Casella JF, King AA, Barton B, White DA, Noetzel MJ, Ichord RN, et al. Design of the silent cerebral infarct transfusion (SIT) trial. *Pediatr Hematol Oncol.* (2010) 27:69–89. doi: 10.3109/08880010903360367

4. Vichinsky E, Neumayr LD, Gold JI, Weiner MW, Rule RR, Truran D, et al. Neuropsychological dysfunction and neuroimaging abnormalities in neurologically intact adults with sickle cell anemia. *JAMA.* (2014) 303:1823–31. doi: 10.1001/jama.2010.562

5. Pegelow CH, Macklin EA, Moser FG, Wang WC, Bello JA, Miller ST, et al. Longitudinal changes in brain magnetic resonance imaging findings in children with sickle cell disease. *Blood.* (2002) 99:3014–8. doi: 10.1182/blood.V99.8.3014

6. Bernaudin F, Verlhac S, Fréard F, Roudot-Thoraval F, Benkerrou M, Thuret I, et al. Multicenter prospective study of children with sickle cell disease: Radiographic and psychometric correlation. *J Child Neurol.* (2000) 15:333–43. doi: 10.1177/088307380001500510

7. Prussien K V, Jordan LC, Debaun MR, Compas BE. Cognitive function in sickle cell disease across domains, cerebral infarct status, and the lifespan: a meta-analysis. *J Pediatr Psychol.* (2019) 44:948–58. doi: 10.1093/jpepsy/jsz031

8. Kawadler JM, Clayden JD, Clark CA, Kirkham FJ. Intelligence quotient in paediatric sickle cell disease: a systematic review and meta-analysis. *Dev Med Child Neurol.* (2016) 58:672–9. doi: 10.1111/dmcn.13113

9. Jorgensen DR, Rosano C, Novelli EM. Can neuroimaging markers of vascular pathology explain cognitive performance in adults with sickle cell anemia? A review of the literature. *Hemoglobin.* (2016) 40:381–7. doi: 10.1080/03630269.2016.1242493

10. Wardlaw JM, Debette S, Jokinen H, De Leeuw FE, Pantoni L, Chabriat H, et al. ESO Guideline on covert cerebral small vessel disease. Vol 6. *Eur Stroke J.* (2021). doi: 10.1177/23969873211012132

11. Ford AL, Ragan DK, Fellah S, Binkley MM, Fields ME, Guilliams KP, et al. Silent infarcts in sickle cell disease occur in the border zone region and are associated with low cerebral blood flow. *Blood.* (2018) 132:1714–23. doi: 10.1182/blood-2018-04-841247

12. Kimmelsteil P. Vascular occlusion and ischemic infarction in sickle cell disease. *Am J Med Sci.* (1948) 216:11. doi: 10.1097/00000441-194807000-00002

13. Rothman SM, Fulling KH, Nelson JS. Sickle cell anemia and central nervous system infarction: a neuropathological study. *Ann Neurol.* (1986) 20:684–90. doi: 10.1002/ana.410200606

14. Schlotman AA, Donahue MJ, Kassim AA, Lee CA, Waddle SL, Pruthi S, et al. Intracranial and extracranial vascular stenosis as risk factors for stroke in sickle cell disease. *Pediatr Neurol.* (2021) 114:29–34. doi: 10.1016/j.pediatrneurol.2020.10.006

15. Stotesbury H, Kawadler JM, Clayden JD, Saunders DE, Hood A, Koelbel M, et al. Quantification of silent cerebral infarction on high-resolution FLAIR and cognition in sickle cell anemia. *Front Neurol.* (2022) 13:1–24. doi: 10.3389/fneur.2022.867329

16. Choi S, O'Neil SH, Joshi AA Li J, Bush AM, Coates TD, et al. Anemia predicts lower white matter volume and cognitive performance in sickle and non-sickle cell anemia syndrome. *Am J Hematol.* (2019) 94:1055–65. doi: 10.1002/ajh.25570

17. Matondo LO, Kija E, Manji KP. Neurocognitive functioning among children with sickle cell anemia attending SCA clinic at MNH, Dar es Sealaam, Tanzania. *Neurol Res Int.* (2020) 2020:1–8. doi: 10.1155/2020/3636547

18. Shmueli K. Chapter 31—quantitative susceptibility mapping. In: *Quantitative Magnetic Resonance Imaging*. Cambridge: Academic Press (2020). p. 819–38. doi: 10.1016/B978-0-12-817057-1.00033-0

19. Duyn JH, Schenck J. Contributions to magnetic susceptibility of brain tissue. *NMR Biomed.* (2017) 30:e3546. doi: 10.1002/nbm.3546

20. Shin HG, Lee J, Yun YH, Yoo SH, Jang J, Oh SH, et al. χ-separation: magnetic susceptibility source separation toward iron and myelin mapping in the brain. *Neuroimage.* (2021) 240:118371. doi: 10.1016/j.neuroimage.2021.118371

21. Chen J, Gong NJ, Chaim KT, Otaduy MCG, Liu C. Decompose quantitative susceptibility mapping (QSM) to sub-voxel diamagnetic and paramagnetic components based on gradient-echo MRI data. *Neuroimage.* (2021) 242:118477. doi: 10.1016/j.neuroimage.2021.118477

22. Rosen CL, Debaun MR, Strunk RC, Redline S, Seicean S, Craven DI, et al. Obstructive sleep apnea and sickle cell anemia. *Pediatrics.* (2014) 134:273–81. doi: 10.1542/peds.2013-4223

23. Howard J, Slee AE, Skene S, Inusa B, Kawadler J, Downes M, et al. Overnight auto-adjusting continuous airway pressure+standard care compared with standard care alone in the prevention of morbidity in sickle cell disease phase II (POMS2b): study protocol for a randomised controlled trial. *Trials.* (2018) 19:1–10. doi: 10.1186/s13063-017-2419-0

24. Stotesbury H, Hales PW, Koelbel M, Hood AM, Kawadler JM, Saunders DE, et al. Venous cerebral blood flow quantification and cognition in patients with sickle cell anemia. *J Cereb Blood Flow Metab.* (2022) 42:1061–77. doi: 10.1177/0271678X211072391

25. van der Land V, Hijmans CT, de Ruiter M, Mutsaerts HJMM, Cnossen MH, Engelen M, et al. Volume of white matter hyperintensities is an independent predictor of intelligence quotient and processing speed in children with sickle cell disease. *Br J Haematol.* (2015) 168:553–6. doi: 10.1111/bjh.13179

26. Choudhury NA, DeBaun MR, Rodeghier M, King AA, Strouse JJ, McKinstry RC. Silent cerebral infarct definitions and full-scale IQ loss in children with sickle cell anemia. *Neurology.* (2018) 90:E239–46. doi: 10.1212/WNL.0000000000004832

27. Robinson SD, Bredies K, Khabipova D, Dymerska B, Marques JP, Schweser F. An illustrated comparison of processing methods for MR phase imaging and QSM: combining array coil signals and phase unwrapping. *NMR Biomed.* (2017) 30:e3601. doi: 10.1002/nbm.3601

28. Liu T, Wisnieff C, Lou M, Chen W, Spincemaille P, Wang Y. Nonlinear formulation of the magnetic field to source relationship for robust quantitative susceptibility mapping. *Magn Reson Med.* (2013) 69:467–76. doi: 10.1002/mrm.24272

29. Karsa A, Shmueli K, SEGUE. A speedy region-growing algorithm for unwrapping estimated phase. *IEEE Trans Med Imaging.* (2018) 38:1347–57. doi: 10.1109/TMI.2018.2884093

30. Kiersnowski O, Karsa A, Thornton JS, Shmueli K. The effect of oblique image slices on the accuracy of quantitative susceptibility mapping and a robust tilt correction method. In: *ISMRM & SMRT Annual Meeting & Exhibition.* (2021). doi: 10.1101/2021.11.30.470544

31. Liu T, Khalidov I, de Rochefort L, Spincemaille P, Liu J, Tsiouris AJ, et al. A novel background field removal method for MRI using projection onto dipole fields (PDF). *NMR Biomed.* (2011) 24:1129–36. doi: 10.1002/nbm.1670

32. Karsa A, Punwani S, Shmueli K. An optimized and highly repeatable MRI acquisition and processing pipeline for quantitative susceptibility mapping in the head-and-neck region. *Magn Reson Med.* (2020) 84:3206–22. doi: 10.1002/mrm.28377

33. Hansen PC. The L-curve and its use in the numerical treatment of inverse problems. *Comput Inverse Probl Electrocardiol.* (2001) 5:119–42.

34. Fischl B. FreeSurfer. *Neuroimage.* (2012) 62:774–81. doi: 10.1016/j.neuroimage.2012.01.021

35. Dale AM, Fischl B, Sereno MI. Cortical surface-based analysis. *Neuroimage.* (1999) 9:179–94. doi: 10.1006/nimg.1998.0395

36. Jenkinson M, Bannister P, Brady M, Smith S. Improved optimization for the robust and accurate linear registration and motion correction of brain images. *Neuroimage.* (2002) 17:825–41. doi: 10.1006/nimg.2002.1132

37. Jenkinson M, Smith S, A. global optimisation method for robust affine registration of brain images. *Med Image Anal.* (2001) 5:143–56. doi: 10.1016/S1361-8415(01)00036-6

38. Wechsler D. Wechsler adult intelligence scale–Fourth Edition (WAIS–IV). *San Antonio, TX NCS Pearson.* (2008) 22:1. doi: 10.1037/t15169-000

39. Wechsler D. WISC-IV: Wechsler intelligence scale for children: technical and interpretive manual. *Psychol Corp.* (2004). doi: 10.1037/t15174-000

40. Wechsler D. Wechsler abbreviated scale of intelligence–. (1999). doi: 10.1037/t15170-000

41. McCrimmon AW, Smith AD. Review of the wechsler abbreviated scale of intelligence, second edition (WASI-II). *J Psychoeduc Assess.* (2013) 31:337–41. doi: 10.1177/0734282912467756

42. Li W, Wu B, Batrachenko A, Bancroft-Wu V, Morey RA, Shashi V, et al. Differential developmental trajectories of magnetic susceptibility in human brain gray and white matter over the lifespan. *Hum Brain Mapp.* (2014) 35:2698–713. doi: 10.1002/hbm.22360

43. DeBaun MR, Armstrong FD, McKinstry RC, Ware RE, Vichinsky E, Kirkham FJ. Silent cerebral infarcts: A review on a prevalent and progressive cause of neurologic injury in sickle cell anemia. *Blood.* (2012) 119:4587–96. doi: 10.1182/blood-2011-02-272682

44. Van Der Land V, Zwanenburg JJM, Fijnvandraat K, Biemond BJ, Hendrikse J, Mutsaerts HJMM, et al. Cerebral lesions on 7 tesla MRI in patients with sickle cell anemia. *Cerebrovasc Dis.* (2015) 39:181–9. doi: 10.1159/000373917

45. Azuma M, Hirai T, Yamada K, Yamashita S, Ando Y, Tateishi M, et al. Lateral asymmetry and spatial difference of iron deposition in the substantia nigra of patients with Parkinson disease measured with quantitative susceptibility mapping. *Am J Neuroradiol.* (2016) 37:782–8. doi: 10.3174/ajnr.A4645

46. Langkammer C, Pirpamer L, Seiler S, Deistung A, Schweser F, Franthal S, et al. Quantitative susceptibility mapping in Parkinson's disease. *PLoS ONE.* (2016) 11:1–13. doi: 10.1371/journal.pone.0162460

47. Thomas GEC, Leyland LA, Schrag AE, Lees AJ, Acosta-Cabronero J, Weil RS. Brain iron deposition is linked with cognitive severity in Parkinson's disease. *J Neurol Neurosurg Psychiatry.* (2020) 91:418–25. doi: 10.1136/jnnp-2019-322042

48. Acosta-Cabronero J, Williams GB, Cardenas-Blanco A, Arnold RJ, Lupson V, Nestor PJ. *In vivo* quantitative susceptibility mapping (QSM) in Alzheimer's disease. *PLoS ONE.* (2013) 8:e81093. doi: 10.1371/journal.pone.0081093

49. Moon Y, Han SH, Moon WJ. Patterns of brain iron accumulation in vascular dementia and alzheimer's dementia using quantitative susceptibility mapping imaging. *J Alzheimer's Dis.* (2016) 51:737–45. doi: 10.3233/JAD-151037

50. Palmer C, Menzies SL, Roberts RL, Pavlick G, Connor JR. Changes in iron histochemistry after hypoxic-ischemic brain injury in the neonatal rat. *J Neurosci Res.* (1999) 56:60–71. doi: 10.1002/(SICI)1097-4547(19990401)56:1<60::AID-JNR8>3.0.CO;2-A

51. Ovbiagele B, Saver JL. Cerebral white matter hyperintensities on MRI: current concepts and therapeutic implications. *Cerebrovasc Dis.* (2006) 22:83–90. doi: 10.1159/000093235

52. Wen W, Sachdev P. The topography of white matter hyperintensities on brain MRI in healthy 60- to 64-year-old individuals. *Neuroimage.* (2004) 22:144–54. doi: 10.1016/j.neuroimage.2003.12.027

53. van der Land V, Mutsaerts HJMM, Engelen M, Heijboer H, Roest M, Hollestelle MJ, et al. Risk factor analysis of cerebral white matter hyperintensities in children with sickle cell disease. *Br J Haematol.* (2016) 172:274–84. doi: 10.1111/bjh.13819

54. Latchaw RE, Alberts MJ, Lev MH, Connors JJ, Harbaugh RE, Higashida RT, et al. Recommendations for imaging of acute ischemic stroke: a scientific statement from the american heart association. *Stroke.* (2009) 40:3646–78. doi: 10.1161/STROKEAHA.108.192616

55. Vert C, Parra-Fariñas C, Rovira À. MR imaging in hyperacute ischemic stroke. *Eur J Radiol.* (2017) 96:125–32. doi: 10.1016/j.ejrad.2017.06.013

56. Quinn CT, Robert CM, Dowling MM, Ball WS, Kraut MA, Casella JF, et al. Acute silent cerebral ischemic events in children with sickle cell anemia. *Arch Neurol.* (2013) 70:58–65. doi: 10.1001/jamaneurol.2013.576

57. Dowling MM, Quinn CT, Plumb P, Rogers ZR, Rollins NK, Koral K, et al. Acute silent cerebral ischemia and infarction during acute anemia in children with and without sickle cell disease. *Blood.* (2012) 120:3891–7. doi: 10.1182/blood-2012-01-406314

58. Václavu L, Petr J, Petersen ET, Mutsaerts HJMM, Majoie CBL, Wood JC, et al. Cerebral oxygen metabolism in adults with sickle cell disease. *Am J Hematol.* (2020) 95:401–12. doi: 10.1002/ajh.25727

59. Milovic C, Bilgic B, Zhao B, Langkammer C, Tejos C, Acosta-Cabronero J. Weak-harmonic regularization for quantitative susceptibility mapping. *Magn Reson Med*. (2019) 81:1399–411. doi: 10.1002/mrm.27483

60. Griffanti L, Jenkinson M, Suri S, Zsoldos E, Mahmood A, Filippini N, et al. Classification and characterization of periventricular and deep white matter hyperintensities on MRI: a study in older adults. *Neuroimage*. (2018) 170:174–81. doi: 10.1016/j.neuroimage.2017.03.024

61. Lee J, Shmueli K, Fukunaga M, van Gelderen P, Merkle H, Silva AC, et al. Sensitivity of MRI resonance frequency to the orientation of brain tissue microstructure. *Proc Natl Acad Sci*. (2010) 107:5130–5. doi: 10.1073/pnas.0910222107

62. Liu C. Susceptibility tensor imaging. *Magn Reson Med Off J Int Soc Magn Reson Med*. (2010) 63:1471–7. doi: 10.1002/mrm.22482

63. Li W. Magnetic susceptibility anisotropy of human brain *in vivo* and its molecular underpinnings. *Neuroimage*. (2013) 59:2088–97. doi: 10.1016/j.neuroimage.2011.10.038

64. Stotesbury H, Hales PW, Hood AM, Koelbel M, Kawadler JM, Saunders DE, et al. Individual watershed areas in sickle cell anemia: an arterial spin labeling study. *Front Physiol*. (2022) 13:1–16. doi: 10.3389/fphys.2022.865391

65. Roseborough AD, Saad L, Goodman M, Cipriano LE, Hachinski VC, Whitehead SN. White matter hyperintensities and longitudinal cognitive decline in cognitively normal populations and across diagnostic categories: a meta-analysis, systematic review, and recommendations for future study harmonization. *Alzheimer's Dement*. (2022) 2022:1–14. doi: 10.1002/alz.12642

66. Erten-Lyons D, Woltjer R, Kaye J, Mattek N, Dodge HH, Green S, et al. Neuropathologic basis of white matter hyperintensity accumulation with advanced age. *Neurology*. (2013) 81:977–83. doi: 10.1212/WNL.0b013e3182a43e45

67. Arfanakis K, Evia AM, Leurgans SE, Cardoso LFC, Kulkarni A, Alqam N, et al. Neuropathologic correlates of white matter hyperintensities in a community-based cohort of older adults. *J Alzheimer's Dis*. (2020) 73:333–45. doi: 10.3233/JAD-190687

68. Gowland PA, Bowtell R. Theoretical optimization of multi-echo fMRI data acquisition. *Phys Med Biol*. (2007) 52:1801–13. doi: 10.1088/0031-9155/52/7/003

69. Kawadler JM, Kirkham FJ, Clayden JD, Hollocks MJ, Seymour EL, Edey R, et al. White matter damage relates to oxygen saturation in children with sickle cell anemia without silent cerebral infarcts. *Stroke*. (2015) 46:1793–9. doi: 10.1161/STROKEAHA.115.008721

frontiers | Frontiers in Neurology

TYPE Original Research
PUBLISHED 07 December 2022
DOI 10.3389/fneur.2022.976063

OPEN ACCESS

EDITED BY
Fenella Jane Kirkham,
University College London,
United Kingdom

REVIEWED BY
Mariel Kozberg,
Massachusetts General Hospital and Harvard Medical School, United States
Andria Ford,
Washington University in St. Louis, United States

*CORRESPONDENCE
Hyacinth I. Hyacinth
hyacinhi@ucmail.uc.edu

SPECIALTY SECTION
This article was submitted to Stroke, a section of the journal Frontiers in Neurology

RECEIVED 23 June 2022
ACCEPTED 21 November 2022
PUBLISHED 07 December 2022

CITATION
Abi Rached NM, Gbotosho OT, Archer DR, Jones JA, Sterling MS and Hyacinth HI (2022) Adhesion molecules and cerebral microvascular hemodynamic abnormalities in sickle cell disease. *Front. Neurol.* 13:976063. doi: 10.3389/fneur.2022.976063

Adhesion molecules and cerebral microvascular hemodynamic abnormalities in sickle cell disease

Noor Mary Abi Rached[1], Oluwabukola T. Gbotosho[2], David R. Archer[3], Jayre A. Jones[3], Morgan S. Sterling[3] and Hyacinth I. Hyacinth[2]*

[1]Neuroscience and Behavioral Biology Undergraduate Program, Emory University, Atlanta, GA, United States, [2]Department of Neurology and Rehabilitation Medicine, University of Cincinnati College of Medicine, Cincinnati, OH, United States, [3]Aflac Cancer and Blood Disorders Center, Emory University Department of Pediatrics and Children's Healthcare of Atlanta, Atlanta, GA, United States

Cerebrovascular abnormalities are a common feature of sickle cell disease that may be associated with risk of vaso-occlusive pain crises, microinfarcts, and cognitive impairment. An activated endothelium and adhesion factors, VCAM-1 and P-selectin, are implicated in sickle cell vasculopathy, including abnormal hemodynamics and leukocyte adherence. This study examined the association between cerebral expression of these adhesion factors and cortical microvascular blood flow dynamics by using *in-vivo* two-photon microscopy. We also examined the impact of blood transfusion treatment on these markers of vasculopathy. Results showed that sickle cell mice had significantly higher maximum red blood cell (RBC) velocity (6.80 ± 0.25 mm/sec, $p \leq 0.01$ vs. 5.35 ± 0.35 mm/sec) and more frequent blood flow reversals (18.04 ± 0.95 /min, $p \leq 0.01$ vs. 13.59 ± 1.40 /min) in the cortical microvasculature compared to controls. In addition, sickle cell mice had a 2.6-fold (RFU/mm^2) increase in expression of VCAM-1 and 17-fold (RFU/mm^2) increase in expression of P-selectin compared to controls. This was accompanied by an increased frequency in leukocyte adherence (4.83 ± 0.57 /100 μm/min vs. 2.26 ± 0.37 /100 μm/min, $p \leq 0.001$). We also found that microinfarcts identified in sickle cell mice were 50% larger than in controls. After blood transfusion, many of these parameters improved, as results demonstrated that sickle cell mice had a lower post-transfusion maximum RBC velocity (8.30 ± 0.98 mm/sec vs. 11.29 ± 0.95 mm/sec), lower frequency of blood flow reversals (12.80 ± 2.76 /min vs. 27.75 ± 2.09 /min), and fewer instances of leukocyte adherence compared to their pre-transfusion imaging time point (1.35 ± 0.32 /100 μm/min vs. 3.46 ± 0.58 /100 μm/min). Additionally, we found that blood transfusion was associated with lower expression of adhesion factors. Our results suggest that blood transfusion and adhesion factors, VCAM-1 and P-selectin, are potential therapeutic targets for addressing cerebrovascular pathology, such as vaso-occlusion, in sickle cell disease.

KEYWORDS

sickle cell disease, adhesion molecules, microvascular hemodynamics, cerebral microinfarct, two-photon microscope

Introduction

Sickle cell disease (SCD) is caused by a single point mutation in the beta-globin gene, resulting in the substitution of valine for glutamic acid in the resulting β-globin peptide. In turn, this leads to polymerization of deoxyhemoglobin, forming sickled erythrocytes (1). SCD impacts an estimated 100,000 individuals in the United States, and the incidence of SCD among African Americans is approximately 1 in 360 newborns (2, 3). SCD involves clinical complications that impact multiple organ systems, with pain being the most frequent and results from vaso-occlusion. As a common symptom of SCD, vaso-occlusive pain crises result from sickle shaped erythrocytes and leukocytes blocking blood flow, particularly in small vessels, resulting in ischemia of organs and thus, pain (4, 5). Additionally, these vaso-occlusive events (VOEs) can be triggered by processes such as inflammation, thrombosis, increased aggregation of cells, and adhesion of blood cells to the vascular endothelium, ultimately leading to blockages that deprive the tissues of nutrients and oxygen (6–9). This results in tissue death and infarction in several organ systems including the spleen, liver, kidney, and lungs (4, 10). Another possible consequence of VOEs is silent cerebral infarction (SCI) or cerebral microinfarctions, which are small ischemic lesions that may occur without overt neurological symptoms. Studies have shown that SCIs or cerebral microinfarcts may be linked to the development of cognitive decline, and are associated with vascular and hemodynamic abnormalities such as cerebral macro and/or micro-vasculopathy, hypoperfusion, or obstructions to blood flow (11–13). Magnetic resonance imaging (MRI) studies from clinical cohorts in the Cooperative Study of Sickle Cell Disease showed that children with silent cerebral microinfarcts also have a higher risk of stroke (14, 15). Further studies from this cohort showed that school-aged children with SCD and silent infarcts experienced difficulties with neuropsychological functions (16). In mouse models, a recent study from our laboratory showed that sickle cell (SS) mice had 2.5 times more cortical microinfarcts than controls. Additionally, these mice had significantly higher prevalence of evidence of spontaneous cerebral vasculopathies, such as higher red blood cell (RBC) velocity, more tortuous capillary vessels, and more frequent capillary occlusive events (12).

Vaso-occlusive events occur more frequently in the low flow "micro" vessels such as, the pre-capillary arterioles, capillaries, and post-capillary venules (17, 18). Studies in non-sickle cell mouse models showed that blockage or experimental occlusion of the principal (penetrating) cortical venules (PCV) lead to stagnant flow in the upstream arterioles. This impairs blood flow into the cortex, thus, highlighting the importance of the entire cerebral microvascular tree, in the etiology of cerebral microinfarcts (11). However, it is important to note that SS RBCs typically pass through the capillary bed prior to hemoglobin polymerization, suggesting that additional factors may also be involved in the pathology of VOEs in SCD (19, 20). For instance, endothelial activation has been shown to play a role in the pathogenesis of VOEs in SCD (8, 18, 21). This is shown by the demonstration of significantly higher levels of circulating endothelial cells in patients with SCD compared with matched controls (21). *In vitro* studies also demonstrated the capacity for the more rigid sickle erythrocytes to mechanically activate endothelial cells, leading to an increase in expression of cellular adhesion molecules (markers of endothelial activation) which in turn propagates further adhesion and vaso-occlusion in a vicious cycle (8, 22, 23). The role of these cellular adhesion molecules [such as Intercellular Adhesion Molecule 1 (ICAM-1), P- and E-selectins and vascular cell adhesion molecule 1 (VCAM-1)] and thus endothelial activation in VOEs is further supported by a recent report of higher levels of E-selectin, VCAM-1, and ICAM-1 in SCD patients compared to controls (18). One study demonstrated significantly higher levels of these molecules, suggesting endothelial activation among SCD patients presenting with complications and even higher levels among those presenting with an active vaso-occlusive pain crises, compared to steady state (24). It is already well demonstrated that individuals with SCD have elevated leukocyte counts, which in the setting of increased VCAM-1 expression, results in increased endothelial interaction and thus arrest (25, 26). Our lab reported a strong relationship between serum soluble VCAM-1 (sVCAM-1), P-selectin and ICAM-1 levels, and risk of stroke in patients (children) with SCD. In the same study, we also demonstrated that lower levels of these cell adhesion markers were associated with stroke free survival as well as use of blood transfusion therapy for stroke prevention in patients with SCD (27). A paradigm proposed by Frenette et al. (28) suggests a multi-step model of vaso-occlusion whereby sickle cells induce endothelial activation, creating an environment where adherent leukocytes can interact with both RBCs and the endothelium to hinder blood flow, and subsequently create blockages (28, 29). In addition, it has been documented that among patients with sickle cell disease, those with higher cerebral blood flow as a compensatory mechanism for lack of brain oxygenation, performed more poorly on tests of cognitive function (30). This highlights the importance of exploring how adhesion factors may relate to this abnormal blood flow in SCD. Taken together we reasoned that the level of expression (or deposition) of cellular adhesion molecules in the cerebral microvascular endothelium will play a role in cerebral microvascular hemodynamics and could be a physiological mechanism for the cortical microinfarct or SCIs observed in mouse models of SCD and adults/children with SCD respectively (12, 25, 31, 32).

Vascular cell adhesion molecule-1 (i.e., VCAM-1), is expressed on blood vessels (endothelial cells) after activation by chemical (such as cytokines and chemokines) and/or mechanical stimulation, resulting in cytokine release. It is involved in

adhesion of lymphocytes, monocytes, eosinophils, and basophils to the endothelium (33). According to Stuart and Setty (34), in a state of hypoxia, sickled red blood cells adhere to endothelial VCAM-1 using the very late activation antigen-4 (VLA4) ligand (34). P-selectin has also been implicated in the pathophysiology of SCD and is currently the target of a newly developed anti-vaso-occlusive crisis (VOC) drug (35–42). The chronic inflammatory milieu of SCD results in a persistently elevated serum level as well as endothelial expression of P-selectin, which is necessary for the initial binding of leukocytes to the vascular endothelium (43, 44). However, in the setting of SCD, P- selectin expression is likely to result in disruption of normal hemodynamics from excess/aberrant leukocyte-endothelial interaction (45, 46). While studies have shown that excessive endothelial expression of VCAM-1 and P-selectin results in VOEs and therefore pain in the periphery, the impact of such excess adhesion molecule expression on leukocyte-endothelial interaction and therefore cerebral microvascular hemodynamics is not known. Our study investigated this relationship given its potential implication for cerebral/cortical infarction and therefore neurological complications such as cognitive impairment. We also examined the relationship between the deposition (expression) of the two most well documented cellular adhesion molecules, VCAM-1, and P-selectin, associated with SCD complications and cerebral microvascular hemodynamics. Finally, we examined the impact of packed red blood cell transfusion on these adhesion molecules and therefore cerebral hemodynamics.

Methods

Animal preparation

The Institutional Animal Care and Use Committees (IACUC) of Emory University and the Medical University of South Carolina approved this study, and all research was conducted in accordance with the National Research Council and National Institutes of Health *Guide for the Care and Use of Laboratory Animals 8th Edition* (47). Figure 1 provides an overview of the experiments.

This study used the Townes mouse model, a humanized sickle cell mouse model (with HbSS) and corresponding humanized control group (with HbAA). Mice are male and ∼13 months old at the time of starting the experiments. They were divided into two main groups. Group one was used to examine changes in microvascular hemodynamics while group two was used to examine the impact of red blood cell transfusion on microvascular hemodynamics. Blood transfusion therapy is a primary treatment option for primary and secondary stroke prevention for children and adults with SCD and has been shown to reduce hemoglobin S concentration as well as reducing the risk of stroke and silent cerebral infarct (cerebral microinfarcts) (48–50).

In our study, all mice in both the baseline group and the transfusion experiment group underwent implantation of a cranial window (placed over the somatosensory cortex) under anesthesia to obtain optical access to the intracranial space. The procedures for anesthetizing mice and performing the cranial window surgery have been described in our prior publications (12, 51). Two to three hours following surgery, the mice in the blood transfusion group underwent pre-transfusion two-photon laser scanning microscopy (TPLSM) imaging. Afterwards (i.e., immediately following imaging and prior to recovery from anesthesia), sickle cell mice received blood transfusions with 300 μl of packed red blood cells from humanized Townes HbAA mice while control (HbAA) mice received 300 μl intravenous (IV) saline. All infusions were performed slowly over 1 min. The goal of the packed RBC transfusion was to raise the hemoglobin level by at least 1g/dL. Mice in the baseline group underwent post-surgery imaging, but they did not receive any transfusion fusions and were sacrificed immediately following the two-photon (2 Photon) imaging. The mice that received blood or saline infusion, underwent a second 2 Photon imaging 2–3 weeks after the infusion. It is important to note that, in the transfusion experiment each group of mice served as their own treatment control, thus the pre and post transfusion imaging represent the same group of mice at different time points. The AA mice also served an additional purpose of being a control for the impact of handling as well as to enable us to show that any positive effect of packed RBC transfusion observed in the HbSS mice, is not due to the passage of time and thus resolution of the initial stress from surgery.

In vivo imaging procedure

To examine hemodynamic parameters, *in-vivo* images of cortical capillaries, precapillary arterioles, and post capillary venules (low flow cortical microvascular hemodynamics) were obtained using 2 Photon microscopy based on the schedule described above. Imaging was performed with a Sutter Moveable Objective Microscope (MOM) and a Coherent Ultra II Ti: Sapphire laser source. Methods for animal preparation during imaging and for measurement of cerebral hemodynamic and microvascular parameters *via in vivo* 2 Photon blood flow imaging have been previously described and published (12, 52, 53). about 2–5 min prior to commencing imaging, fluoresceine conjugated dextran (FITC-dextran 2kD to label the plasma) and Rhodamine 6G (to label leukocytes) are administered to the mice mouse IV, in that order.

Hemodynamic analysis

To examine hemodynamic parameters, we used custom MATLAB codes to analyze line scans of cerebral capillary,

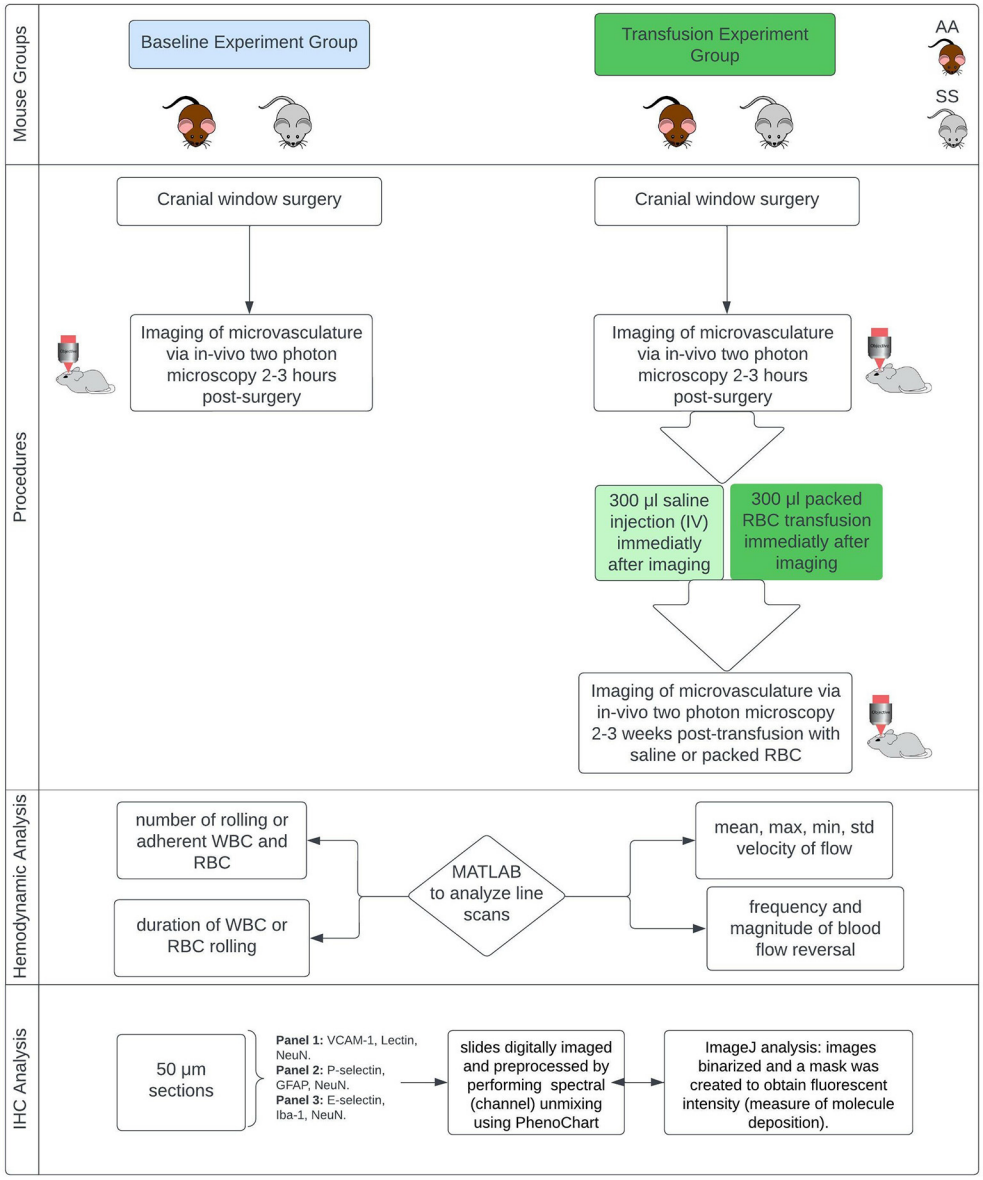


FIGURE 1
Overview of methods. Townes mouse model, a humanized sickle cell (with HbSS) and control (HbAA) mouse model (12–13 months). IHC, immunohistochemistry.

pre-capillary and post-capillary blood vessel images acquired using 2 Photon microscopy from SS and control (AA) mice. Using the MATLAB scripts, we were able to determine the following: mean and standard deviation of RBC flow velocity, maximum and minimum velocity of RBC flow, frequency (per minute) and magnitude of microvascular RBC/blood flow reversal and leukocyte (WBC) rolling on the endothelium. Microvascular RBC or blood flow reversal is a change in the original direction of blood flow relative to the direction of the line scan and could be multiple transient changes or a single change that last the duration of the line scan. A rolling or adherent WBC is defined as WBC stagnation lasting two or more seconds. We normalized the number of leukocyte rolling or leukocyte adherence events to a fixed vessel segment (100 µm) per unit time (1 min). This is to ensure reproducibility of our findings (54). All image analysis was performed by members of our laboratory who were blinded to the genotype or transfusion status of the mice.

Immunohistochemistry

At the end of 2 Photon imaging, the mice were sacrificed, and the brain extracted for immunohistochemistry (IHC) analysis to examine the microvascular deposition of adhesion factors, possible presence and size (area) of cerebral (cortical) microinfarcts, and possible presence of gliosis at the site of infarcts. The immunohistochemical protocol/approach has been previously described and published (12). For the baseline mice, the IHC was performed immediately following the imaging. For the mice that were transfused either with saline (HbAA) or packed RBC (HbSS), all IHC was performed after the post-transfusion 2 Photon imaging, and there was no pre-transfusion IHC analysis. We used the following primary antibody combinations to label 50-micron brain tissue sections from SS and AA mice. Panel 1 contained VCAM-1, Lectin (to localize and stain the vasculature), and NeuN (to localize and stain neuronal nuclei). Panel 2 contained P-selectin, GFAP (to localize and stain reactive astrocytes), and NeuN. Panel 3 contained E-selectin, Iba-1 (to localize and stain microglia), and NeuN. These slides are digitally imaged using a PerkinElmer digital slide scanner (Akoya Biosciences), and then images were preprocessed by performing spectral (channel) unmixing using PhenoChart (Akoya Biosciences). IHC Images were analyzed using ImageJ, with standard parameters for each fluorophore. Briefly, after spectral unmixing, the images from each fluorescent channel were transferred to image J, where they were binarized and a mask was created. The mask was then applied to the source images to obtain fluorescent intensity as an indication of the level of deposition (expression) of the molecule e.g., VCAM-1 of interest. The threshold values for creating the masks as well as analysis parameters were kept constant between images and between genotype (i.e., HbSS and HbAA) mice for each fluorophore/fluorescent channel and their adhesion molecule of interest. The resulting intensity was then normalized to a unit (mm^2) image size and expressed in relative fluorescence unit (RFU) per mm^2.

Statistical analysis

We performed data analysis for comparison between sickle cell and control mice using GraphPad Prism software (GraphPad Software Inc, La Jolla, CA). We checked our data for normality using the Shapiro-Wilk test, and then we used the Welch corrected *t*-test for comparison of differences between sickle cell and control mice because of the heteroscedasticity in our data based on Levene's *F*-test for equality of variance. Our study minimum sample size was 3 mice per genotype group and was based on our prior experiments and publications using this mouse model (55–57). Quantitative results are presented using bar plots with means and standard error of means (SEM), comparing sickle to control mice and with a p-value of < 0.05 considered statistically significant. Qualitative data are presented as representative array of histochemical images.

Results

Cerebral hemodynamic properties at baseline for sickle cell mice compared to age-matched controls

Analysis of the 2 Photon microscopy imaging data for cerebral microvascular hemodynamic measurements revealed that sickle cell mice had significantly higher maximum RBC velocity (6.80 ± 0.25 mm/sec vs. 5.35 ± 0.35 mm/sec, $p = 0.0009$) compared to age-matched controls (Figure 2A). In addition, we noted that that sickle mice have a higher frequency of cerebral microvascular blood flow reversal (18.04/min vs. 13.59/min, $p = 0.008$) compared to controls (Figure 2B). Further analysis revealed that the velocity of blood flow reversal was also significantly higher among sickle cell mice (0.84 ± 0.14 mm/sec vs. 0.52 ± 0.06 mm/sec, $p = 0.03$) compared to controls (Figure 2C). Also, blood flow reversal parameters are used to identify how often and at what velocity blood flow in a vessel is disturbed (58–60). This suggests that blood flow is abnormal in sickle cell mice, and this may be related to VOEs.

Sickle cell mice have elevated expression/deposition of endothelial adhesion factors (VCAM-1 and P-selectin) compared to controls

To better understand the potential underlying factors responsible for the disturbed hemodynamics observed above, we performed immunohistochemical (IHC) analysis of the brains from the sickle cell and control mice, after completion of 2 Photon imaging. The IHC analysis focused mainly on VCAM-1 and P-selectin for reasons already mentioned in the background section (25, 27, 36, 37). Our analysis showed that sickle cell mice had significantly larger area of VCAM-1 coverage expressed per μm^2 ($p < 0.0001$), as well as expression/deposition (intensity) measured in RFU/mm^2 ($p < 0.0001$) in the cerebral microvasculature compared to controls at baseline (Figures 3A–C). Considering both the intensity and coverage parameters for expression, high intensity areas of fluorescence are not necessarily localized to one specific brain area. Similarly, and to large extent not surprising, we also noted that sickle cell mice had significantly larger

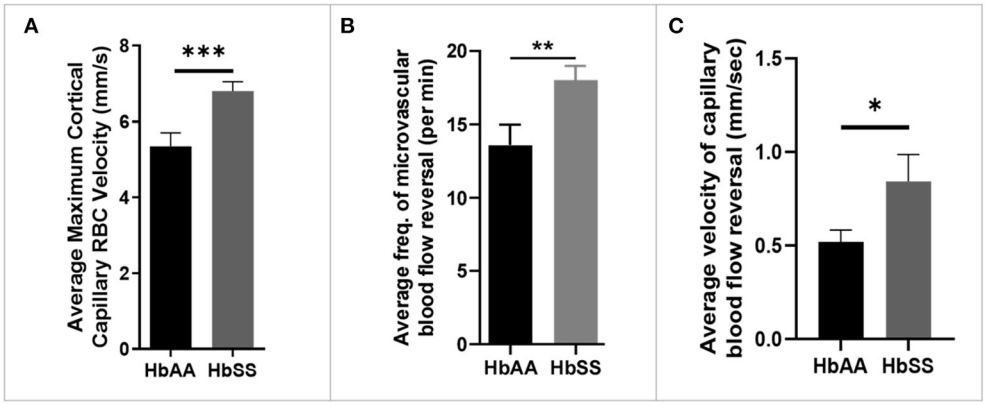


FIGURE 2
Altered capillary hemodynamics in 13-month-old sickle cell mice compared to age-matched controls. **(A)** average maximum capillary RBC velocity in millimeters per second. **(B)** average frequency of capillary blood flow reversal per minute **(C)** average velocity of capillary blood flow reversal in millimeters per second (AA: $n = 5$, average of ~194 vessel segments; SS: $n = 7$, average of ~390 vessel segments). Error bars are standard error of means (SEM). *$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$. Mean comparisons done using Welch's corrected t-test. Results here are only from the mice in the baseline group.

area of P-selectin coverage per μm^2 ($p < 0.0001$) and expression/deposition (intensity) measured in RFU/mm^2 ($p < 0.0001$) in the cerebral microvasculature compared to controls (Figures 3D–F). Using images from 2 Photon microscopy and custom MATLAB scripts as already described, we quantified leukocyte adherence in the cortical microvasculature. The result indicates a significantly higher frequency of leukocyte adherence events in sickle cell mice (4.83 ± 0.57 /100μm/min vs. 2.26 ± 0.37 /100μm/min; $p = 0.002$) compared to controls at baseline (Figure 3G). This result is unsurprising given the well-established role of elevated P-selectin and VCAM-1 in as markers of endothelial activation as well as mediators of leukocytes rolling (37, 61–65).

Microinfarct frequency and area in sickle cell mice compared to age matched controls at baseline

Using a separate cohort of mice (male AA and SS mice that were 13 months old) that were neither transfused with packed red blood cells (pRBC) nor had cranial window implanted, we assessed the frequency and size (area) of microinfarcts (≥ 50 μM in diameter) in brain slices of sickle cell and control mice by examining all brain sections from sickle cell ($N = 7$) and control ($N = 6$) mice, encompassing ~20 sections per mouse spanning the entire cerebrum. Surprisingly, our analysis revealed that there was no significant difference in the frequency (22.60 ± 5.53 vs. 21.14 ± 4.0) for every 20 brain slices (Figure 4A). On the other hand, we noted that the cortical microinfarcts were significantly larger in sickle cell [0.2 ± 0.03 cm^2 vs. 0.1 ± 0.02 cm^2, $p = 0.02$] compared to the control mice (Figures 4B–D). This recapitulates data in our previous study (12).

A single packed red blood cell transfusion improved cerebral microvascular hemodynamic measures in sickle cell mice

Given that blood transfusion therapy is still one of the most effective ways to prevent cerebrovascular complications such as stroke or recurrent stroke in SCD, (66–69) we decided to offer a single packed RBC transfusion to the sickle cell mice as described earlier. To accomplish this, we obtained whole blood from Townes humanized HbAA mice, spun it down, removed the plasma, resuspended the pellet in sterile cold PBS, centrifuged again (to wash), and then resuspended in sterile PBS at room temperature before proceeding immediately to transfuse. Sickle cell mice received 300 μL of packed RBC IV (with a goal of raising the hemoglobin by 1 g/dL), immediately following the pre-transfusion (PrT) 2 Photon microscopy session as described above. Control (HbAA) mice received 300 μL of normal saline about the same as the sickle cell mice. 2–3 weeks post transfusion, the mice underwent a second (post-transfusion) 2 Photon microscopy imaging. It is important to note, that while control mice received saline infusion, they did not receive blood transfusions and the PrT and PT designations are meant to indicate the fact that they were imaged at two time points that align

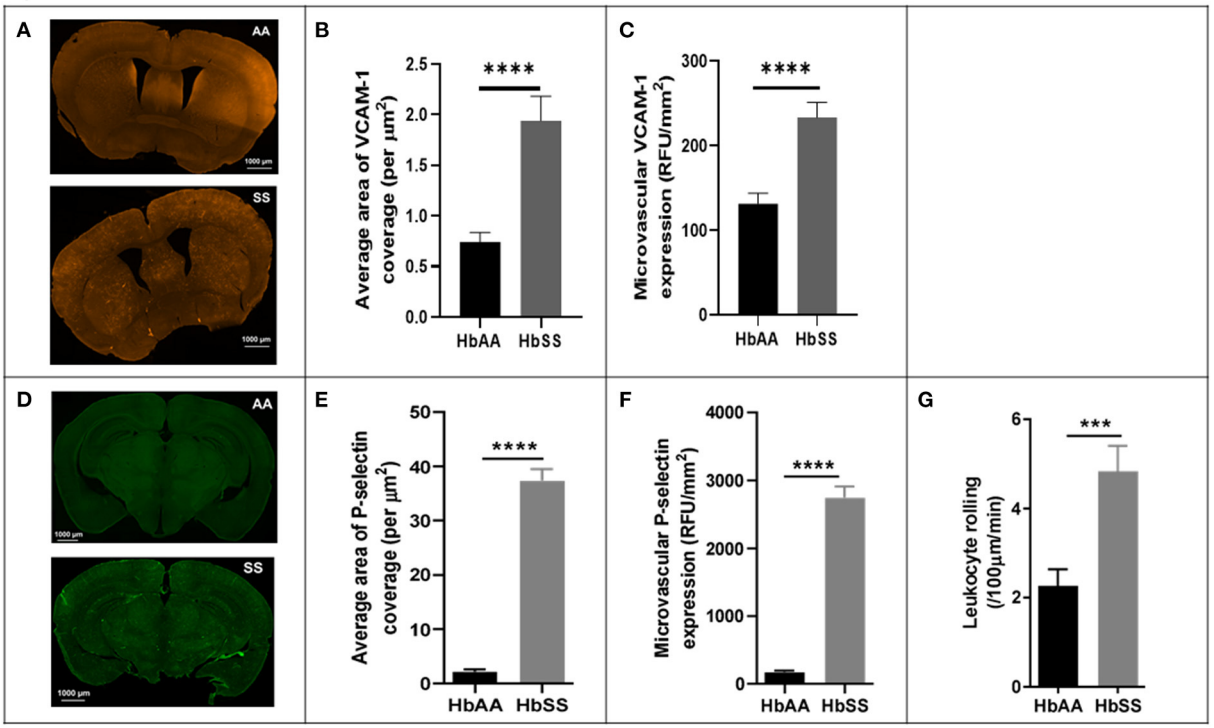


FIGURE 3
Adhesion factor expression and the rate of leukocyte adherence are elevated in the brains of sickle cell mice. Fluorophore of interest was isolated to show fluorescent areas representing VCAM-1 deposition along the microvasculature. **(A)** Representative image of stained tissue sections (50 um thick) from SS (bottom) and AA (top) mice. Areas of fluorescence indicate VCAM-1 deposition. These images were taken after spectral unmixing but prior to transferring to ImageJ for binarizing and overlaying masks. Adhesion factor expression was measured by analyzing fluorescence intensity and reported as relative fluorescence units (RFU) per millimeter squared of brain tissue. **(B)** Average area of VCAM-1 coverage per μm^2 **(C)** VCAM-1 expression compared to controls. **(D)** Representative image of stained tissue sections (50 um thick) from SS (bottom) and AA (top) mice. Fluorophore of interest was isolated to show fluorescent areas representing P-selectin deposition along the microvasculature. Areas of fluorescence indicate P-selectin deposition. **(E)** Average area of P-selectin coverage per μm^2. **(F)** microvascular P-selectin expression (RFU/mm^2). **(G)** Leukocyte adherence (defined as lasting two seconds or more) per 100 μm length of vessel per minute was higher in sickle cell mice ($p < 0.001$) (AA: $n = 5$; SS: $n = 7$). Error bars are standard error of means (SEM). ***$p \leq 0.001$; ****$p \leq 0.0001$. Mean comparisons done using Welch's corrected t-test. Results here are only from the mice in the baseline group. For VCAM-1 analysis, AA = 184 brain slices and SS = 215 brain slices, while for P-selectin analysis, AA = 189 brain slices and SS = 165 brain slices. For leukocyte rolling, AA = average of ~194 vessel segments; SS = average of ~390 vessel segments.

with pre- and post-transfusion imaging time points in sickle cell mice.

The 2 photon microscopy images generated from the pre- and post-transfusion images were analyzed as described earlier. The result from our analysis showed that sickle cell mice that received packed RBC transfusion had a 36% ($p = 0.03$) reduction in maximum cortical microvascular RBC velocity, compared to their pre-transfusion values. On the other hand, we noted a 43% increase ($p = 0.04$) in maximum cortical microvascular RBC velocity in the control mice compared to their pre-transfusion values (Figure 5A). Also, compared to pre-transfusion levels, we noted significant lower frequency of microvascular blood flow reversal (Figure 4B, $p < 0.0001$) as well as decreased velocity (0.62 ± 0.06 mm/sec vs. 0.46 ± 0.06 mm/sec, $p = 0.04$) of cerebral microvascular blood flow reversal (Figure 4C) in sickle cell mice that were transfused with packed RBC. This suggests that blood transfusion treatment significantly improves hemodynamic abnormalities in sickle cell mice.

Packed RBC transfusion decreases endothelial activation in cerebral microvasculature by decreasing expression/deposition of VCAM-1 and P-selectin, and leukocyte adherence in sickle cell mice

As stated earlier (Figure 3B), packed RBC transfusion is one of the mainstays for the prevention of stroke and neurovascular complications of SCD. Thus, as reported earlier, we used IHC to examine whether the single bolus of packed RBC transfusion had any impact on endothelial activation and

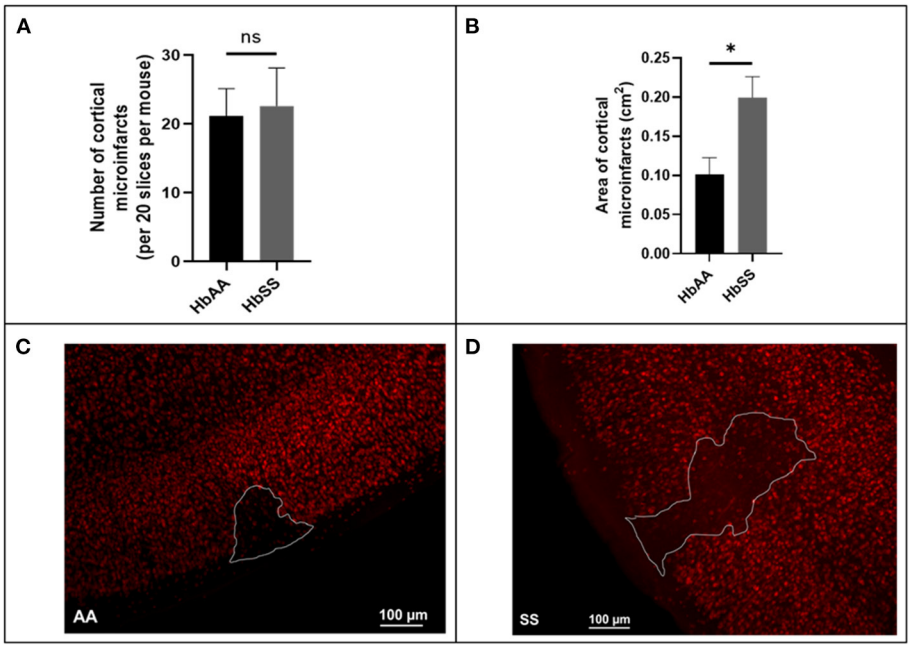


FIGURE 4
Baseline microinfarct data from 13-month-old mice. **(A)** Frequency of cortical infarcts per was not significantly different between HbAA and HbSS. **(B)** area of cortical infarcts in cm^2. Error bars are standard error of means (SEM) (AA: $n = 5$; SS: $n = 5$). *$p < 0.05$. **(C)** Representative image of AA mouse microinfarct area. **(D)** Representative image of SS mouse microinfarct area. Mean comparisons done using Welch's corrected *t*-test.

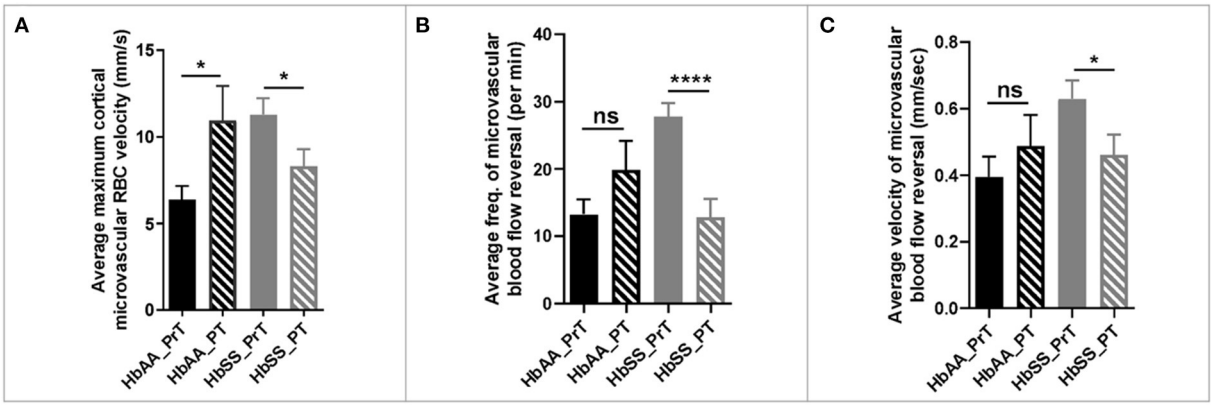


FIGURE 5
Hemodynamic analysis in sickle cell and control mice before and after blood or saline transfusion. **(A)** average maximum capillary RBC velocity in millimeters per second. **(B)** average frequency of capillary blood flow reversal per minute **(C)** average velocity of capillary blood flow reversal in millimeters per second (AA: $n = 3$–5; SS: $n = 5$–7). Error bars are standard error of means (SEM). NS, Not significant; *$p \leq 0.05$; ****$p \leq 0.0001$. Mean comparisons done using Welch's corrected *t*-test (HbAA_PrT was compared to HbAA_PT and HbSS_PrT was compared to HbSS_PT. There was no cross-genotype comparison since AA mice were not transfused with packed RBC. Prt, Pre-transfusion; PT, Post-transfusion. AA = average of ~73 vessel segments pre-transfusion and 23 vessel segments post-transfusion; SS = average of ~90 vessel segments pre-transfusion and 54 vessel segments post-transfusion.

therefore expression/deposition of VCAM-1 and/or P-selectin. Analysis of the IHC images, showed that sickle cell mice had significantly lower coverage (0.25 ± 0.02 /µm vs. 0.91 ± 0.14 /µm, $p < 0.0001$) compared with control mice. Also, when compared to the values obtained for sickle cell mice at baseline (Figure 3B), sickle cell mice that were transfused with packed RBC had a more than 7-fold lower VCAM-1 coverage. Additionally, microvascular VCAM-1 expression/deposition was significantly lower in sickle cell mice post transfusion (33.45 ± 3.44 RFU/mm^2 vs. 168.90 ± 20.71 RFU/mm^2, $p < 0.0001$)

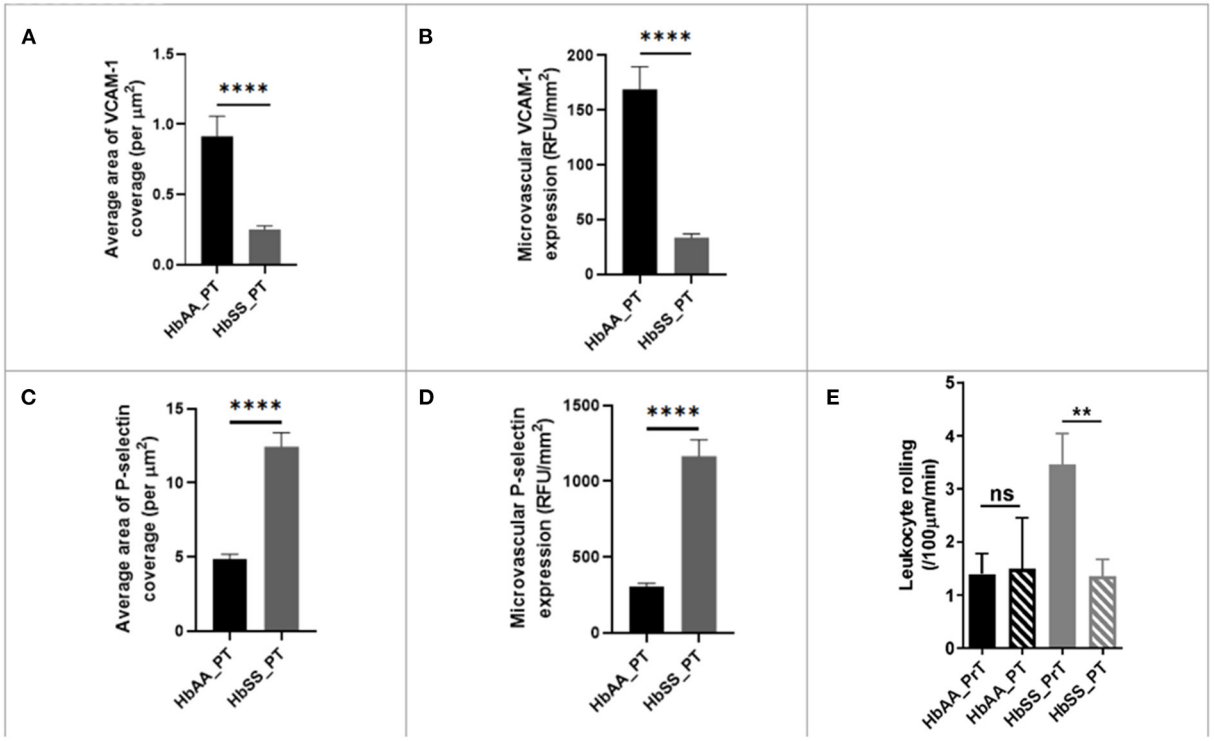


FIGURE 6
Blood transfusion improves cerebral vascular endothelium in sickle cell mice. **(A)** Average area of VCAM-1 coverage per μm^2 in AA mice compared to SS. **(B)** Microvascular VCAM-1 expression in RFU/mm^2 in AA mice compared to SS. **(C)** Average area of P-selectin coverage per μm^2 ($p < 0.0001$). **(D)** Microvascular P-selectin expression (RFU/mm^2). **(E)** Leukocyte adherence in AA and SS mice pre- and post-transfusion. AA: $n = 3–5$; SS: $n = 5$-7. Error bars are SEM. NS, Not significant; **$p \leq 0.01$; ****$p \leq 0.0001$. Mean comparisons done using Welch's corrected *t*-test. For VCAM-1 analysis, AA = 73 brain slices and SS = 147 brain slices, while for P-selectin analysis, AA = 162 brain slices and SS = 164 brain slices. For leukocyte rolling, AA = average of ~73 vessel segments pre-transfusion and 23 vessel segments post-transfusion; SS = average of ~90 vessel segments pre-transfusion and 54 vessel segments post-transfusion.

compared with control mice. And, when compared with values measured at baseline for sickle cell mice that were not transfused (Figure 3C), sickle cell mice that were transfused with packed RBC had an ~7-fold lower VCAM-1 expression/deposition in the cerebral microvasculature (Figures 6A,B). We also examined P-selectin coverage and expression/deposition as described earlier and observed that microvascular P-selectin coverage (12.45 ± 0.94 /µm vs. 4.85 ± 0.33 /µm, $p < 0.0001$) and expression/deposition (1165.00 ± 109.20 RFU/mm^2 vs. 302.30 ± 24.87 RFU/mm^2, $p < 0.0001$) were higher among sickle cell mice that were transfused with packed RBC, compared with controls (Figures 3C,D). Notwithstanding, when compared to values measured at baseline for sickle cell mice (Figures 3E,F), sickle cell mice that received packed RBC transfusion, had an approximately 3-fold (12.45 ± 0.94 /µm vs. 37.34 ± 2.16 /µm) and 2.4-fold (1165.00 ± 109.20 RFU/mm^2 vs. 2742.00 ± 169.70 RFU/mm^2) lower P-selectin coverage and expression/deposition respectively (Figures 6C,D). Finally, we examined pre- and post-packed RBC (for sickle cell mice) and saline (for controls) transfusion leukocyte adherence events. We noted that there was no significant difference in leukocyte adherence events between both time points for the control mice. However, sickle cell mice transfused with packed RBC had a significant reduction in leukocyte adherence events (1.35 ± 0.32 /100µm/min vs. 3.46 ± 0.58 /100µm/min; $p = 0.0017$) compared to pre-transfusion levels (Figure 6E). The data here suggests that a potential underlying benefit of transfusion for reduction of VOE could be *via* mitigating endothelial activation and therefore leukocyte adherence events.

Discussion

Cerebrovascular abnormalities, including strokes and microinfarcts, have been well-documented in sickle cell disease and are associated with cognitive impairment (14, 70). In addition, high levels of adhesion factors have been implicated in SCD related complications, where they act as mediators of cellular (especially leukocyte) endothelial interactions and therefore vaso-occlusion (71, 72). Taken together, these events are crucial in the understanding of neurological pathology in SCD. The goal of this study was to examine

the role of endothelial adhesion molecules (VCAM-1 and P-selectin) in cerebral microvascular hemodynamics, as well as the impact of blood transfusion treatment on these parameters (hemodynamics and expression of adhesion molecules). This study showed that compared to age-matched controls, aged Townes sickle cell mice have an abnormal cerebral microvascular hemodynamic profile, which is also associated with increased leukocyte adherence that seems to be mediated by a higher expression of adhesion factors (VCAM-1 and P-selectin). We also observed that these abnormalities were reduced, and in some instances, reversed, by blood transfusion treatment.

We observed a significantly higher average maximum microvascular RBC velocity in sickle cell mice compared to controls (Figure 2A). This finding corresponds with that from a previous study that showed higher capillary RBC velocity in aged sickle cell mice (12) as well as with observations in children and adults with SCD (25). The average maximum cortical blood flow velocity may be an indication of a compensatory mechanism for poor cerebral perfusion from downstream narrowing or obstruction and/or anemia. It is important to note that we are unable to precisely report capillary RBC velocity because we did not stain for smooth muscle actin, which would have enabled us to discriminate capillaries from pre-capillary arterioles and/or post capillary venules. Thus, our 2 Photon imaging may have included precapillary arterioles and post-capillary venules, and we refer to this throughout the paper as "microvasculature". Notwithstanding, it is well known that besides the cortical capillaries, other segments of the cerebral vascular tree, such as the arteries and arteriole, carotid, and vertebral arteries, are affected in SCD, (73, 74) and the large vessel changes might reflect a later manifestation of microvascular abnormalities such as documented in this study. Furthermore, sickle cell mice had greater instances and higher velocity of blood flow reversal (Figures 2B,C). The exact mechanism for these reversals is unclear, however, studies (75, 76) suggest that experimental occlusion of either arterioles or venules results in cortical microvascular blood flow reversals as documented here. Thus, our finding suggests that the spontaneous blood flow reversals observed in our study could be due to spontaneous cerebral microvascular VOEs. While peripheral VOEs which are a hallmark of SCD and are well documented, cerebral microvascular VOEs have not been documented until recently (12) and are potentially manifesting in our study as blood flow reversals. These disturbances/turbulence in flow may also lead to endothelial activation. Overall, our report of a high velocity of flow, is corroborated by a recent report among patients with SCD, where using multiple-inflow-time arterial spin labeling, they showed a significantly higher cerebral blood flow in patients with SCD compared to controls (77).

Another important observation from our study is the elevated baseline expression/deposition of cerebral microvascular VCAM-1 and P-selectin in sickle cell mice compared to controls (Figures 3B–F). This data suggests that endothelial dysfunction may play a large role in SCD-related cerebral micro vasculopathy and thus neurovascular complications. The high maximum velocity and frequent flow reversals found in these mice may constitute some of the mechanical forces that trigger the expression of these endothelial adhesion factors (25, 78, 79). A recent study documented increased expression of VCAM-1 in the endothelium of aortic valve leaflets when they were exposed to shear stress (80). Thus, in concert with the prior stated mechanical damage to the endothelium, resulting from the physical contact with sickle RBC, these mechanical forces contribute to the promotion of microvascular thrombus generation from increased released of tissue factors, exposure of platelets to subendothelial tissues and therefore formation of platelet aggregates. This further promotes and increases the likelihood of cerebral microvascular VOEs and highlights the value of closely examining the role of endothelial adhesion factors in cerebral microvasculopathy (81).

Given the observations (Figures 3B–F), it is therefore no surprise for us to see a significantly higher frequency of leukocyte adherence in sickle cell mice compared to controls. This increased frequency of leukocyte adherence may also be associated with the higher expression of adhesion factors observed in Figures 3B–F. For example, VCAM-1 is the primary means by which leukocytes bind to the endothelium, using VLA-4, while P-selectin is one of the primarily means *via* which neutrophils interacts with the endothelium. Several studies in both humans and mice have shown that P-selectin and VCAM-1 are heavily implicated in SCD vascular dysfunction (26, 78, 82). These studies suggest that leukocyte-endothelial interactions (possibly mediated by VCAM-1 and/or P-selectin) are predominantly occurring in the post-capillary venules (83–86). Furthermore, recent clinical trials with crizanlizumab, a humanized P-selectin monoclonal antibody, have shown incredible promise, with patients with SCD who were treated with the drug, experiencing significantly fewer vaso-occlusive pain crises compared to patients on placebo, although these studies have no cerebral endpoints (38, 87). Another *in-vitro* study of Crizanlizumab showed inhibition of leukocyte adherence to P-selectin under physiologic blood flow conditions (44). Although the long-term benefits of crizanlizumab are yet to be determined, based on these previous studies and our results, it is tempting to predict that long-term use of crizanlizumab may attenuate some features of cerebral microvasculopathy and possibly SCD-related neurovascular pathologies, *via* reduction occurrence of VOEs in in the cerebral microvasculature (38, 44, 87).

Additionally, we noted that at baseline (without any intervention) the frequency of spontaneous microinfarct was not significantly different. The size (area) was significantly larger among sickle cell mice compared to controls (Figure 4B).

The exact mechanism behind this is not yet clear. In a prior study, Luo et al. (88) reported that following middle cerebral artery occlusion, Townes sickle cell mice had significantly larger cerebral infarct size, compared to controls (88). Based on the rest of our data, it is plausible to reason that the growth of the infarct might be a self-propagating process (89) in the background of increased endothelial expression of adhesion molecules, increased leukocyte adherence and therefore higher frequency of VOEs as reported earlier in this section. This is a mechanism that warrants further investigation, and our lab is actively looking into this as it could also represent a therapeutic target for reducing the well described SCD-related cognitive decline which occurs in children and adults in the absence of an overt cerebral injury (90, 91).

Our observation of an apparent "normalization" of hemodynamic parameters/measured with packed RBC transfusion, compared to pre-transfusion levels in sickle cell mice and compared to controls (Figures 5A–C) was an intriguing albeit unsurprising finding. This result is exciting as it represents the first-time demonstration of a possible underlying mechanism of the benefits of blood transfusion. The dramatic change in maximum cerebral microvascular blood flow velocity could imply an improved ability to effectively perfuse the brain tissue without needing additional output velocity as a compensatory mechanism. According to this data, it seems that blood transfusion might have as a benefit, the normalization of cerebral microvascular dysfunction as a mechanism for the reported benefit in stroke prevention reported in children with SCD (48). Additionally, it is also possible that the reduction in flow velocity could be due to a decreased in VOEs, as we also observed a reduction in frequency (Figure 5B) and velocity (Figure 5B) of blood flow reversal. Furthermore, we also noted that compared to pre-transfusion or baseline levels, there was a significantly less microvascular expression and deposition of VCAM-1 and P-selectin, as well as significant decrease in leukocyte adherence in the cerebral of sickle cell mice. Taken together, the significant difference in expression and deposition of adhesion molecules combined with the reduction in leukocyte adherence, might account for some if not all the improvement seen in cerebral hemodynamic parameters. Due to the short duration of the blood transfusion therapy in addition to the fact that the assessment of cortical microinfarcts were performed post-mortem, we were unable to examine the impact of blood transfusion on frequency or size of the infarct as a function of the lower expression/deposition of adhesion endothelial adhesion molecules and/or leukocyte adherence. However, this will be the subject of future investigations in our laboratory.

A limitation of our study was that the expression of adhesion factors was quantified in the whole brain while hemodynamic changes were measured in cortical microvasculature. Nevertheless, our study corroborates the findings of several *in vivo* and *in vitro* studies by showing evidence of leukocyte-endothelial interactions, likely promoted by increased expression of adhesion factors expression. As mentioned earlier, we were also not able to reliably evaluate the impact of blood transfusion on frequency or size of cortical microinfarcts due to the short duration of time (2 weeks) from packed RBC transfusion to imaging and sacrifice of the mice. Future study designs have already worked out ways around this limitation using longitudinal approach to imaging. Due to equipment availability and other logistical reasons, we were also not able to access the post-transfusion hemoglobin levels and as such we are unable to make a firm statement with regards to how much the hemoglobin levels of the sickle cell mice went up post transfusion. Finally, we are not able to directly infer from our current data, a causal relationship between the improvement of hemodynamic parameters and lower expression of adhesion factors. However, ongoing studies in our lab using bone marrow chimera as well as sickle cell mice null for P-selectin and VCAM-1, should enable us to make such inference in the future.

Conclusion

By examining hemodynamics and adhesion factors using two-photon laser microscopy and post-mortem immunohistochemistry in both pre- and post- transfusion sickle cell mice, we were able to document evidence of cerebral microvasculopathy in sickle cell mice. Additionally, we were able to show that blood transfusion might exert its benefit of preventing neurovascular complications by mitigating cerebral microvascular endothelial activation and the underlying mechanism of VOEs. Thus, our study potentially highlights one of the mechanisms that may be contributing to how blood transfusion prevents stroke and other neurovascular pathologies. The significant decrease in VCAM-1 and P-selectin expression in the brain following blood transfusion offers a particular new avenue for investigation, as well as therapeutic targets.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was reviewed and approved by Institutional Animal Care and Use Committees (IACUC) of Emory University and the Medical University of South Carolina.

Author contributions

HH designed the experiment and performed the final critical review. NA, DA, JJ, MS, and HH performed experiments and data analysis. NA, OTG, and HH wrote the manuscript. DA, JJ, and MS provided critical review. All authors endorsed the submission of this manuscript.

Funding

This study was supported by grants from the National Institutes of Health, R01HL138423, R01HL156024, and R01AG072592 to HH.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Pauling L, Itano HA, Singer SJ, Wells IC. Sickle cell anemia, a molecular disease. *Science.* (1949) 110:543–8. doi: 10.1126/science.110.2865.543

2. Wang Y, Kennedy J, Caggana M, Zimmerman R, Thomas S, Berninger J, et al., Grosse SD. Sickle cell disease incidence among newborns in New York State by maternal race/ethnicity and nativity. *Gen Med.* (2013) 15:222–8. doi: 10.1038/gim.2012.128

3. Hassell K. Population estimates of sickle cell disease in the US. *Am J Prev Med.* 38:S512–21. 2010. doi: 10.1016/j.amepre.2009.12.022

4. Kato GJ, Piel FB, Reid CD, Gaston MH, Ohene-Frempong K, Krishnamurti L, et al., Costa FF. Sickle cell disease nature reviews disease. *Primers.* (2018) 4:1–22. doi: 10.1038/nrdp.2018.10

5. Manwani D, Frenette PS. Vaso-occlusion in sickle cell disease: pathophysiology and novel targeted therapies. *Blood.* (2013) 122:3892–8. doi: 10.1182/blood-2013-05-498311

6. Sundd P, Gladwin MT, Novelli EM. Pathophysiology of sickle cell disease. *Annu Rev Pathol.* (2019) 14:263–92. doi: 10.1146/annurev-pathmechdis-012418-012838

7. Zhang D, Xu C, Manwani D, Frenette PS. Neutrophils, platelets, and inflammatory pathways at the nexus of sickle cell disease pathophysiology. *Blood.* (2016) 127:801–9. doi: 10.1182/blood-2015-09-618538

8. Telen MJ. Role of adhesion molecules and vascular endothelium in the pathogenesis of sickle cell disease. *Hematol Am Soc Hematol Educ Program.* (2007) 2007:84–90. doi: 10.1182/asheducation-2007.1.84

9. Horton RE. Microfluidics for investigating vaso-occlusions in sickle cell disease. *Microcirculation.* (2017) 24:e12373. doi: 10.1111/micc.12373

10. Ofori-Acquah SF. Sickle cell disease as a vascular disorder. *Expert Rev Hematol.* (2020) 13:645–53. doi: 10.1080/17474086.2020.1758555

11. Hartmann DA, Hyacinth HI, Liao FF, Shih AY. Does pathology of small venules contribute to cerebral microinfarcts and dementia? *J Neurochem.* (2018) 144:517–26. doi: 10.1111/jnc.14228

12. Hyacinth HI, Sugihara CL, Spencer TL, Archer DR, Shih AY. Higher prevalence of spontaneous cerebral vasculopathy and cerebral infarcts in a mouse model of sickle cell disease. *J Cereb Blood Flow Metab.* (2019) 39:342–51. doi: 10.1177/0271678X17732275

13. Guilliams KP, Fields ME, Ragan DK, Chen Y, Eldeniz C, Hulbert ML, et al., Ford AL. Large-vessel vasculopathy in children with sickle cell disease: a magnetic resonance imaging study of infarct topography and focal atrophy. *Pediatr Neurol.* (2017) 69:49–57. doi: 10.1016/j.pediatrneurol.2016.11.005

14. Ohene-Frempong K, Weiner SJ, Sleeper LA, Miller ST, Embury S, Moohr JW, et al., Gill FM. Cerebrovascular accidents in sickle cell disease: rates and risk factors. *Blood.* (1998) 91:288–94.

15. Pegelow CH, Macklin EA, Moser FG, Wang WC, Bello JA, Miller ST, et al., Kinney TR. Longitudinal changes in brain magnetic resonance imaging findings in children with sickle cell disease. *Blood.* (2002) 99:3014–8. doi: 10.1182/blood.V99.8.3014

16. Wang W, Enos L, Gallagher D, Thompson R, Guarini L, Vichinsky E, et al. Neuropsychologic performance in school-aged children with sickle cell disease: a report from the cooperative study of sickle cell disease. *J Pediatr.* (2001) 139:391–7. doi: 10.1067/mpd.2001.116935

17. Shih AY, Hyacinth HI, Hartmann DA, and Van Veluw SJ. Rodent models of cerebral microinfarct and microhemorrhage. *Stroke.* (2018) 49:803–10. doi: 10.1161/STROKEAHA.117.016995

18. Kato GJ, Martyr S, Blackwelder WC, Nichols JS, Coles WA, Hunter LA, et al. Levels of soluble endothelium-derived adhesion molecules in patients with sickle cell disease are associated with pulmonary hypertension, organ dysfunction, and mortality. *Br J Haematol.* (2005) 130:943–53. doi: 10.1111/j.1365-2141.2005.05701.x

19. Pathare A, Kindi SA, Daar S, Dennison D. Cytokines in sickle cell disease. *Hematology.* (2003) 8:329–37. doi: 10.1080/1024533031000160471 9

20. Hofrichter J, Ross PD, Eaton WA. Kinetics and mechanism of deoxyhemoglobin S gelation: a new approach to understanding sickle cell disease. *Proc Natl Acad Sci U S A.* (1974) 71:4864–8. doi: 10.1073/pnas.71.12.4864

21. Solovey A, Lin Y, Browne P, Choong S, Wayner E, Hebbel RP. Circulating activated endothelial cells in sickle cell anemia. *N Engl J Med.* (1997) 337:1584–90. doi: 10.1056/NEJM199711273372203

22. Brown MD, Wick TM, Eckman JR. Activation of vascular endothelial cell adhesion molecule expression by sickle blood cells. *Pediatr Pathol Mol Med.* (2001) 20:47–72. doi: 10.3109/15513810109168817

23. Shiu YT, Udden MM, McIntire LV. Perfusion with sickle erythrocytes up-regulates ICAM-1 and VCAM-1 gene expression in cultured human endothelial cells. *Blood.* (2000) 95:3232–41. doi: 10.1182/blood.V95.10.3232.010k16_3232_3241

24. Antwi-Boasiako C, Donkor ES, Sey F, Dzudzor B, Dankwah GB, Otu KH, et al. Levels of soluble endothelium adhesion molecules and complications among sickle cell disease patients in Ghana. *Diseases.* (2018) 6:29. doi: 10.3390/diseases6020029

25. Connes P, Verlhac S, Bernaudin F. Advances in understanding the pathogenesis of cerebrovascular vasculopathy in sickle cell anaemia. *Br J Haematol.* (2013) 161:484–98. doi: 10.1111/bjh.12300

26. White J, Lancelot M, Gao XF, McGraw BJ, Tabb C, Hines P. Cross-sectional analysis of adhesion in individuals with sickle cell disease using a standardized whole blood adhesion bioassay to VCAM-1. *Blood Cells Mol Dis.* (2020) 81:102397. doi: 10.1016/j.bcmd.2019.102397

27. Hyacinth HI, Adams RJ, Voeks JH, Hibbert JM, Gee BE. Frequent red cell transfusions reduced vascular endothelial activation and thrombogenicity in children with sickle cell anemia and high stroke risk. *Am J Hematol.* (2014) 89:47–51. doi: 10.1002/ajh.23586

28. Frenette PS. Sickle cell vaso-occlusion: multistep and multicellular paradigm. *Curr Opin Hematol.* (2002) 9:101–6. doi: 10.1097/00062752-200203000-00003

29. Barabino GA, Platt MO, Kaul DK. Sickle cell biomechanics. *Annu Rev Biomed Eng.* (2010) 12:345–67. doi: 10.1146/annurev-bioeng-070909-105339

30. Prussien KV, Compas BE, Siciliano RE, Ciriegio AE, Lee CA, Kassim AA, et al., Jordan LC. Cerebral hemodynamics and executive function in sickle cell anemia. *Stroke.* (2021) 52:1830–4. doi: 10.1161/STROKEAHA.120.032741

31. Jordan LC, Casella JF, DeBaun MR. Prospects for primary stroke prevention in children with sickle cell anaemia. *Br J Haematol.* (2012) 157:14–25. doi: 10.1111/j.1365-2141.2011.09005.x

32. Kassim AA, DeBaun MR. The case for and against initiating either hydroxyurea therapy, blood transfusion therapy or hematopoietic stem cell transplant in asymptomatic children with sickle cell disease. *Expert Opin Pharmacother.* (2014) 15:325–36. doi: 10.1517/14656566.2014.868435

33. Barreiro O, Yanez-Mo M, Serrador JM, Montoya MC, Vicente-Manzanares M, Tejedor R, et al. Dynamic interaction of VCAM-1 and ICAM-1 with moesin and ezrin in a novel endothelial docking structure for adherent leukocytes. *J Cell Biol.* (2002) 157:1233–45. doi: 10.1083/jcb.200112126

34. Stuart MJ, Setty BN. Sickle cell acute chest syndrome: pathogenesis and rationale for treatment. *Blood.* (1999) 94:1555–60. doi: 10.1182/blood.V94.5.1555.417k36_1555_1560

35. Kutlar A, Ataga KI, McMahon L, Howard J, Galacteros F, Hagar W, et al., Embury SH. A potent oral P-selectin blocking agent improves microcirculatory blood flow and a marker of endothelial cell injury in patients with sickle cell disease. *Am J Hematol.* (2012) 87:536–9. doi: 10.1002/ajh.23147

36. Wood K, Russell J, Hebbel RP, Granger DN. Differential expression of E- and P-selectin in the microvasculature of sickle cell transgenic mice. *Microcirculation.* (2004) 11:377–85. doi: 10.1080/10739680490437559

37. Wood KC, Hebbel RP, Granger DN. Endothelial cell P-selectin mediates a proinflammatory and prothrombogenic phenotype in cerebral venules of sickle cell transgenic mice. *Am J Physiol Heart Circ Physiol.* (2004) 286:H1608–14. doi: 10.1152/ajpheart.01056.2003

38. Ataga KI, Kutlar A, Kanter J, Liles D, Cancado R, Friedrisch J, et al. Crizanlizumab for the prevention of pain crises in sickle cell disease. *N Engl J Med.* (2017) 376:429–39. doi: 10.1056/NEJMoa1611770

39. Blair HA. Crizanlizumab: first approval. *Drugs.* (2020) 80:79–84. doi: 10.1007/s40265-019-01254-2

40. Slomski A. Crizanlizumab prevents sickle cell pain crises. *JAMA.* (2017) 317:798. doi: 10.1001/jama.2017.0355

41. Stevens DL, Hix M, Gildon BL. Crizanlizumab for the prevention of vaso-occlusive pain crises in sickle cell disease. *J Pharm Technol.* (2021) 37:209–15. doi: 10.1177/87551225211008460

42. Yu Z, Blankenship L, Jaiyesimi I. Crizanlizumab in sickle cell disease. *N Engl J Med.* (2017) 376:1795–6. doi: 10.1056/NEJMc1703162

43. Matsui NM, Borsig L, Rosen SD, Yaghmai M, Varki A, Embury SH. P-selectin mediates the adhesion of sickle erythrocytes to the endothelium. *Blood.* (2001) 98:1955–62. doi: 10.1182/blood.V98.6.1955

44. Man Y, Goreke U, Kucukal E, Hill A, An R, Liu S, et al. Leukocyte adhesion to P-selectin and the inhibitory role of Crizanlizumab in sickle cell disease: A standardized microfluidic assessment. *Blood Cells Mol Dis.* (2020) 83:102424. doi: 10.1016/j.bcmd.2020.102424

45. Lawrence MB, Springer TA. Leukocytes roll on a selectin at physiologic flow rates: distinction from and prerequisite for adhesion through integrins. *Cell.* (1991) 65:859–73. doi: 10.1016/0092-8674(91)90393-D

46. Robinson SD, Frenette PS, Rayburn H, Cummiskey M, Ullman-Cullere M, Wagner DD, et al. Multiple, targeted deficiencies in selectins reveal a predominant role for P-selectin in leukocyte recruitment. *Proc Natl Acad Sci U S A.* (1999) 96:11452–7. doi: 10.1073/pnas.96.20.11452

47. Clark JD, Gebhart GF, Gonder JC, Keeling ME, Kohn DF. The 1996 guide for the care and use of laboratory animals. *ILAR J.* (1997) 38:41–8. doi: 10.1093/ilar.38.1.41

48. Adams RJ, McKie VC, Hsu L, Files B, Vichinsky E, Pegelow C, et al. Prevention of a first stroke by transfusions in children with sickle cell anemia and abnormal results on transcranial Doppler ultrasonography. *N Engl J Med.* (1998) 339:5–11. doi: 10.1056/NEJM199807023390102

49. DeBaun MR, Gordon M, McKinstry RC, Noetzel MJ, White DA, Sarnaik SA, et al. Controlled trial of transfusions for silent cerebral infarcts in sickle cell anemia. *N Engl J Med.* (2014) 371:699–710. doi: 10.1056/NEJMc1411133

50. Beverung LM, Strouse JJ, Hulbert ML, Neville K, Liem RI, Inusa B, et al. Health-related quality of life in children with sickle cell anemia: impact of blood transfusion therapy. *Am J Hematol.* (2015) 90:139–43. doi: 10.1002/ajh.23877

51. Shih AY, Mateo C, Drew PJ, Tsai PS, Kleinfeld D. A polished and reinforced thinned-skull window for long-term imaging of the mouse brain. *J Vis Exp.* (2012) 61:e3742. doi: 10.3791/3742

52. Shih AY, Driscoll JD, Drew PJ, Nishimura N, Schaffer CB, Kleinfeld D. Two-photon microscopy as a tool to study blood flow and neurovascular coupling in the rodent brain. *J Cereb Blood Flow Metab.* (2012) 32:1277–309. doi: 10.1038/jcbfm.2011.196

53. Driscoll JD, Shih AY, Drew PJ, Cauwenberghs G, Kleinfeld D. Two-photon imaging of blood flow in the rat cortex. *Cold Spring Harb Protoc.* (2013) 2013:759–67. doi: 10.1101/pdb.prot076513

54. Drew PJ, Blinder P, Cauwenberghs G, Shih AY, Kleinfeld D. Rapid determination of particle velocity from space-time images using the Radon transform. *J Comput Neurosci.* (2010) 29:5–11. doi: 10.1007/s10827-009-0159-1

55. Capers PL, Hyacinth HI, Cue S, Chappa P, Vikulina T, Roser-Page S, et al. Body composition and grip strength are improved in transgenic sickle mice fed a high-protein diet. *J Nutr Sci.* (2015) 4:e6. doi: 10.1017/jns.2014.63

56. Hyacinth HI, Capers PL, Archer DR, Hibbert JM. *TNF-alpha, IFN-gamma, IL-10, and IL-4 levels were elevated in a murine model of human sickle cell anemia maintained on a high protein/calorie diet Experimental biology and medicine (Maywood, NJ).* (2014) 239:65–70. doi: 10.1177/1535370213508357

57. Manci EA, Hyacinth HI, Capers PL, Archer DR, Pitts S, Ghosh S, et al. High protein diet attenuates histopathologic organ damage and vascular leakage in transgenic murine model of sickle cell anemia. *Exp Biol Med.* (2014) 239:966–74. doi: 10.1177/1535370214531863

58. Hashimoto J, Ito S. Aortic stiffness determines diastolic blood flow reversal in the descending thoracic aorta: potential implication for retrograde embolic stroke in hypertension. *Hypertension.* (2013) 62:542–9. doi: 10.1161/HYPERTENSIONAHA.113.01318

59. Hashimoto J, Ito S. Aortic blood flow reversal determines renal function: potential explanation for renal dysfunction caused by aortic stiffening in hypertension. *Hypertension.* (2015) 66:61–7. doi: 10.1161/HYPERTENSIONAHA.115.05236

60. Tsai CL, Lee JT, Cheng CA, Liu MT, Chen CY, Hu HH, Peng GS. Reversal of ophthalmic artery flow as a predictor of intracranial hemodynamic compromise: implication for prognosis of severe carotid stenosis. *Eur J Neurol.* (2013) 20:564–70. doi: 10.1111/ene.12038

61. Panes J, Perry M, Granger DN. Leukocyte-endothelial cell adhesion: avenues for therapeutic intervention. *Br J Pharmacol.* (1999) 126:537–50. doi: 10.1038/sj.bjp.0702328

62. Gavins F, Yilmaz G, Granger DN. The evolving paradigm for blood cell-endothelial cell interactions in the cerebral microcirculation. *Microcirculation.* (2007) 14:667–81. doi: 10.1080/10739680701404903

63. Henninger DD, Panes J, Eppihimer M, Russell J, Gerritsen M, Anderson DC, Granger DN. Cytokine-induced VCAM-1 and ICAM-1 expression in different organs of the mouse. *J Immunol.* (1997) 158:1825–32.

64. Ishikawa M, Stokes KY, Zhang JH, Nanda A, Granger DN. Cerebral microvascular responses to hypercholesterolemia: roles of NADPH oxidase and P-selectin. *Circ Res.* (2004) 94:239–44. doi: 10.1161/01.RES.0000111524.05779.60

65. Wood KC, Hebbel RP, Granger DN. Endothelial cell NADPH oxidase mediates the cerebral microvascular dysfunction in sickle cell transgenic mice. *FASEB J.* (2005) 19:989–91. doi: 10.1096/fj.04-3218fje

66. Russell MO, Goldberg HI, Hodson A, Kim HC, Halus J, Reivich M, Schwartz E. Effect of transfusion therapy on arteriographic abnormalities and on recurrence of stroke in sickle cell disease. *Blood.* (1984) 63:162–9. doi: 10.1182/blood.V63.1.162.bloodjournal631162

67. Pegelow CH, Adams RJ, McKie V, Abboud M, Berman B, Miller ST, et al. Risk of recurrent stroke in patients with sickle cell disease treated with erythrocyte transfusions. *J Pediatr.* (1995) 126:896–9. doi: 10.1016/S0022-3476(95)70204-0

68. Scothorn DJ, Price C, Schwartz D, Terrill C, Buchanan GR, Shurney W, et al. Risk of recurrent stroke in children with sickle cell disease receiving blood transfusion therapy for at least five years after initial stroke. *J Pediatr.* (2002) 140:348–54. doi: 10.1067/mpd.2002.122498

69. Wang WC, Kovnar EH, Tonkin IL, Mulhern RK, Langston JW, Day SW, et al. High risk of recurrent stroke after discontinuance of five to twelve years of transfusion therapy in patients with sickle cell disease. *J Pediatr.* (1991) 118:377–82. doi: 10.1016/S0022-3476(05)82150-X

70. Hirtz D, Kirkham FJ. Sickle cell disease and stroke. *Pediatr Neurol.* (2019) 95:34–41. doi: 10.1016/j.pediatrneurol.2019.02.018

71. Darbari DS, Sheehan VA, Ballas SK. The vaso-occlusive pain crisis in sickle cell disease: definition, pathophysiology, and management. *Eur J Haematol.* (2020) 105:237–46. doi: 10.1111/ejh.13430

72. Hebbel RP. Perspectives series: cell adhesion in vascular biology. Adhesive interactions of sickle erythrocytes with endothelium. *J Clin Invest.* (1997) 99:2561–4. doi: 10.1172/JCI119442

73. Detterich JA, Kato R, Bush A, Chalacheva P, Ponce D, De Zoysa M, et al. Sickle cell microvascular paradox-oxygen supply-demand mismatch. *Am J Hematol.* (2019) 94:678–88. doi: 10.1002/ajh.25476

74. Bush AM, Borzage MT, Choi S, Vaclavu L, Tamrazi B, Nederveen AJ, et al. Determinants of resting cerebral blood flow in sickle cell disease. *Am J Hematol.* (2016) 91:912–7. doi: 10.1002/ajh.24441

75. Blinder P, Shih AY, Rafie C, Kleinfeld D. Topological basis for the robust distribution of blood to rodent neocortex. *Proc Nat Acad Sci U S A.* (2010) 107:12670–5. doi: 10.1073/pnas.1007239107

76. Nguyen J, Nishimura N, Fetcho RN, Iadecola C, Schaffer CB. Occlusion of cortical ascending venules causes blood flow decreases, reversals in flow direction, and vessel dilation in upstream capillaries. *J Cereb Blood Flow Metab.* (2011) 31:2243–54. doi: 10.1038/jcbfm.2011.95

77. Kawadler JM, Hales PW, Barker S, Cox TCS, Kirkham FJ, Clark CA. Cerebral perfusion characteristics show differences in younger versus older children with sickle cell anaemia: Results from a multiple-inflow-time arterial spin labelling study. *NMR Biomed.* (2018) 31:e3915. doi: 10.1002/nbm.3915

78. Kaul DK, Finnegan E, Barabino GA. Sickle red cell-endothelium interactions. *Microcirculation.* (2009) 16:97–111. doi: 10.1080/10739680802279394

79. Breen LT, McHugh PE, Murphy BP. HUVEC ICAM-1 and VCAM-1 synthesis in response to potentially athero-prone and athero-protective mechanical and nicotine chemical stimuli. *Ann Biomed Eng.* (2010) 38:1880–92. doi: 10.1007/s10439-010-9959-8

80. Sucosky P, Balachandran K, Elhammali A, Jo H, Yoganathan AP. Altered shear stress stimulates upregulation of endothelial VCAM-1 and ICAM-1 in a BMP-4–and TGF-β1–dependent pathway. *Arterioscler Throm Vas Biol.* (2009) 29:254–60. doi: 10.1161/ATVBAHA.108.176347

81. Hollopeter G, Jantzen HM, Vincent D, Li G, England L, Ramakrishnan V, et al. Identification of the platelet ADP receptor targeted by antithrombotic drugs. *Nature.* (2001) 409:202–7. doi: 10.1038/35051599

82. Embury SH, Matsui NM, Ramanujam S, Mayadas TN, Noguchi CT, Diwan BA, et al. The contribution of endothelial cell P-selectin to the microvascular flow of mouse sickle erythrocytes *in vivo*. *Blood.* (2004) 104:3378–85. doi: 10.1182/blood-2004-02-0713

83. Kaul DK, Fabry ME, Nagel RL. Microvascular sites and characteristics of sickle cell adhesion to vascular endothelium in shear flow conditions: pathophysiological implications. *Proc Natl Acad Sci U S A.* (1989) 86:3356–60. doi: 10.1073/pnas.86.9.3356

84. Kaul DK, Fabry ME, Costantini F, Rubin EM, Nagel RL. *In vivo* demonstration of red cell-endothelial interaction, sickling and altered microvascular response to oxygen in the sickle transgenic mouse. *J Clin Invest.* (1995) 96:2845–53. doi: 10.1172/JCI118355

85. Kaul DK, Kollander R, Mahaseth H, Liu XD, Solovey A, Belcher J, et al. Robust vascular protective effect of hydroxamic acid derivatives in a sickle mouse model of inflammation. *Microcirculation.* (2006) 13:489–97. doi: 10.1080/10739680600778456

86. Zennadi R, Moeller BJ, Whalen EJ, Batchvarova M, Xu K, Shan S, et al. Epinephrine-induced activation of LW-mediated sickle cell adhesion and vaso-occlusion *in vivo*. *Blood.* (2007) 110:2708–17. doi: 10.1182/blood-2006-11-056101

87. Kutlar A, Kanter J, Liles DK, Alvarez OA, Cancado RD, Friedrisch JR, et al. Effect of crizanlizumab on pain crises in subgroups of patients with sickle cell disease: A SUSTAIN study analysis. *Am J Hematol.* (2019) 94:55–61. doi: 10.1002/ajh.25308

88. Luo W, Su EJ, Wang J, Wang H, Guo C, Pawar A, et al. Increased stroke size following MCA occlusion in a mouse model of sickle cell disease. *Blood.* (2014) 123:1965–7. doi: 10.1182/blood-2014-01-549717

89. Taylor ZJ, Hui ES, Watson AN, Nie X, Deardorff RL, Jensen JH, et al. Microvascular basis for growth of small infarcts following occlusion of single penetrating arterioles in mouse cortex. *J Cereb Blood Flow Metab.* (2016) 36:1357–73. doi: 10.1177/0271678X15608388

90. Vichinsky EP, Neumayr LD, Gold JI, Weiner MW, Rule RR, Truran D, et al. Neuropsychological dysfunction and neuroimaging abnormalities in neurologically intact adults with sickle cell anemia. *JAMA.* (2010) 303:1823–31. doi: 10.1001/jama.2010.562

91. Andreotti C, King AA, Macy E, Compas BE, DeBaun MR. The association of cytokine levels with cognitive function in children with sickle cell disease and normal mri studies of the brain. *J Child Neurol.* (2015) 30:1349–53. doi: 10.1177/0883073814563140

frontiers | Frontiers in Neurology

TYPE Original Research
PUBLISHED 13 February 2023
DOI 10.3389/fneur.2023.1101223

OPEN ACCESS

EDITED BY
Alessandro Simonati,
University of Verona, Italy

REVIEWED BY
Sara Mazzucco,
University of Oxford, United Kingdom
Hyacinth Idu Hyacinth,
University of Cincinnati, United States
Giulia Turri,
University of Verona, Italy

*CORRESPONDENCE
Fenella Jane Kirkham
✉ fenella.kirkham@ucl.ac.uk

SPECIALTY SECTION
This article was submitted to Pediatric Neurology, a section of the journal Frontiers in Neurology

RECEIVED 17 November 2022
ACCEPTED 18 January 2023
PUBLISHED 13 February 2023

CITATION
Hamdule S, Kölbel M, Stotesbury H, Murdoch R, Clayden JD, Sahota S, Hood AM, Clark CA and Kirkham FJ (2023) Effects of regional brain volumes on cognition in sickle cell anemia: A developmental perspective.
Front. Neurol. 14:1101223.
doi: 10.3389/fneur.2023.1101223

Effects of regional brain volumes on cognition in sickle cell anemia: A developmental perspective

Shifa Hamdule[1], Melanie Kölbel[1,2], Hanne Stotesbury[1], Russell Murdoch[3], Jonathan D. Clayden[1], Sati Sahota[1], Anna Marie Hood[1,4], Christopher A. Clark[1] and Fenella Jane Kirkham[1,5]*

[1]Developmental Neurosciences Section, UCL Great Ormond Street Institute of Child Health, London, United Kingdom, [2]Sleep Education and Research Laboratory, UCL Institute of Education, London, United Kingdom, [3]Department of Medical Physics and Biomedical Engineering, University College London, London, United Kingdom, [4]Division of Psychology and Mental Health, Manchester Centre for Health Psychology, University of Manchester, Manchester, United Kingdom, [5]Clinical and Experimental Sciences, University of Southampton, Southampton, United Kingdom

Background and objectives: Cognitive difficulties in people with sickle cell anemia (SCA) are related to lower processing speed index (PSI) and working memory index (WMI). However, risk factors are poorly understood so preventative strategies have not been explored. Brain volumes, specifically white matter volumes (WMV) which increases through early adulthood, have been associated with better cognition in healthy typically developing individuals. In patients with SCA, the reduced WMV and total subcortical volumes noted could explain cognitive deficits. We therefore examined developmental trajectories for regional brain volumes and cognitive endpoints in patients with SCA.

Methods: Data from two cohorts, the Sleep and Asthma Cohort and Prevention of Morbidity in SCA, were available. MRI data included T1-weighted axial images, pre-processed before regional volumes were extracted using Free-surfer. PSI and WMI from the Weschler scales of intelligence were used to test neurocognitive performance. Hemoglobin, oxygen saturation, hydroxyurea treatment and socioeconomic status from education deciles were available.

Results: One hundred and twenty nine patients (66 male) and 50 controls (21 male) aged 8–64 years were included. Brain volumes did not significantly differ between patients and controls. Compared with controls, PSI and WMI were significantly lower in patients with SCA, predicted by increasing age and male sex, with lower hemoglobin in the model for PSI but no effect of hydroxyurea treatment. In male patients with SCA only, WMV, age and socioeconomic status predicted PSI, while total subcortical volumes predicted WMI. Age positively and significantly predicted WMV in the whole group (patients + controls). There was a trend for age to negatively predict PSI in the whole group. For total subcortical volume and WMI, age predicted decrease only in the patient group. Developmental trajectory analysis revealed that PSI only was significantly delayed in patients at 8 years of age; the rate of development for the cognitive and brain volume data did not differ significantly from controls.

Discussion: Increasing age and male sex negatively impact cognition in SCA, with processing speed, also predicted by hemoglobin, delayed by mid childhood. Associations with brain volumes were seen in males with SCA. Brain endpoints, calibrated against large control datasets, should be considered for randomized treatment trials.

KEYWORDS

sickle cell disease, brain volume, cognition, development, working memory, processing speed

1. Introduction

Sickle cell anemia (SCA) is an inherited single gene disorder affecting millions of people worldwide (1). Cognitive difficulties in SCA occur regardless of presence of stroke or silent cerebral infarction (SCI) (2). Commonly reported cognitive deficits include reduced processing speed and lower working memory (3–6) which may affect quality of life (7). There is a lack of literature investigating risk factors for cognitive difficulties over time in the SCA population. Reduced white matter volumes (WMV) and subcortical volumes have been found in both adults and children with SCA (8, 9) but any association with cognitive difficulties is under-studied in this population (10).

In healthy individuals, increase in WMV is associated with increase in general IQ (11). In young adult patients with SCA, regional white matter volumes show atrophy in frontal, temporal and parietal regions (12). Reduced IQ in patients with SCA appears to be in part related to reduced processing speed index (PSI) (13). Although PSI has been associated with white matter microstructural damage (13), there are few data examining any association between WMV and PSI, although one study reported reduced WMV associated with IQ loss in male patients with SCA (12). It is therefore reasonable to explore the possibility that reduced WMV in patients with SCA is associated with reduced PSI.

There are few studies on subcortical volumes and cognitive function in the general population although the available evidence suggests that an association after hypoxic-ischemic exposure but not in healthy children (14, 15). Total subcortical volumes are reduced in children with SCA (16) and smaller volumes have been noted in individual subcortical regions including the hippocampal subfields (17), amygdala, pallidum, caudate, putamen and thalamus in adults (9) and children with SCA (16). In adults with SCA, reduced basal ganglia and thalamus volumes were associated with lower working memory index (WMI) (9) but there are no equivalent data in children with SCA.

Studies looking at developmental trajectories of volumetric growth and cognitive development in SCA have reported mixed results. Longitudinal data studying cognitive development showed reduction in verbal IQ and coding, a measure of processing speed, over time in patients with SCA (18). A cross-sectional study plotting trajectories suggested reduced processing speed and working memory in children with SCA with increasing age (14) but there are few data examining any association with brain volume. A longitudinal study of children with SCA enrolled in the Silent Infarct Transfusion (SIT) trial showed reduction in global brain volume over time, but no association with change in IQ (19, 20). The cross-sectional data of Steen et al. (20) did not find any reduction in regional brain volumes but suggested maturational delay in gray matter volume growth in children with SCA but no differences in the WMV development between patients and controls. Chen et al. (21) on the other hand found that WMV in children with SCA was increasing at half the rate of controls (22). However, most studies looking at brain volume or cognitive developmental trajectories lacked control groups, were underpowered and reported failure to follow-up or mixed findings, warranting further investigation of brain volume and cognitive developmental trajectories in children with SCA (20).

In summary, research suggests that volumes of different brain regions might predict cognitive outcomes in children with SCA. Moreover, developmental trajectories may differ between patients with SCA and controls. Therefore, the purpose of this cross-sectional study was to examine the relationship between regional brain volumes (WMV and total subcortical volume) and cognitive variables (PSI and WMI). Hence, we hypothesized:

1. WMV would predict PSI in patients with SCA and controls.
2. Total subcortical volumes would predict WMI in patients with SCA and controls.
3. Sex differences would be observed in patients for WMV predicting PSI and total subcortical volumes predicting WMI.
4. Developmental trajectories for cognitive variables, as well as regional volumes, would differ between patients with SCA and controls.

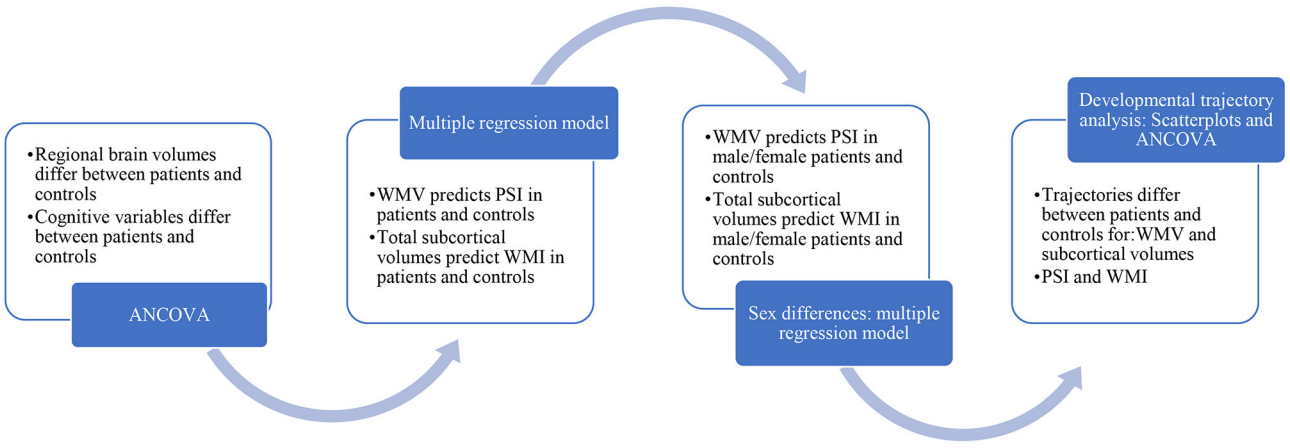


FIGURE 1
Statistical analysis.

2. Methods

2.1. Participants

Participants were enrolled in two studies (23): the Sleep Asthma cohort study (SAC) and the Prevention of Morbidity in SCA 2b (POMS) trial, and were aged >8 years at recruitment and assessment (2015–2019). Participants were ineligible if they were participating in blood transfusion or oxygen therapy trials, receiving nocturnal oxygen support, had chronic lung diseases (except asthma) or respiratory failure. Additional exclusion criteria for the POMS study included hospital admissions for acute sickle cell within 1 month of enrolment, more than 6 hospital admissions within 12 months of enrolment, mean overnight oxygen saturation of <90% for more than 30% total sleep time as measured on overnight oximetry, severe sleep apnea determined by 4% oxygen desaturation index > 15/h, and blood transfusion within 3 months of enrolment or chronic transfusion. The SAC study included patients regardless of sickle or sleep related morbidity or transfusion status. Healthy ethnically matched controls with no history of neurologic or psychiatric conditions were recruited from the community.

2.2. Ethical considerations

West London and South Yorkshire research ethics committees granted approval for the studies. Fully informed consent was obtained from adults and from parents/guardians with assent for children. Procedures were conducted in accordance with the Declaration of Helsinki.

2.3. Neurocognitive variables

The Weschler Scales were used to measure cognitive variables. For FSIQ, the Weschler Intelligence Scale for Children (WISC-IV) was used in SAC patients and controls under 16 years, the Weschler Adult Intelligence Scale (WAIS-IV) for adult SAC patients and controls, and the Weschler Abbreviated Scale of Intelligence (WASI-II) for POMS patients. The primary cognitive outcomes were PSI and WMI which were derived from the WISC-IV and WAIS-IV, with subtests administered separately for the POMS patients. PSI was calculated from Symbol Search and Coding subtests while WMI was calculated from the Arithmetic and Digit span subtests on the WISC-IV and WAIS-IV, respectively. There are strong correlations between editions of the Wechsler scales (WASI, WISC and WAIS) and between the adult and child versions (WISC and WAIS), justifying their inclusion in the same analyses (13, 24). Trained assessors double scored each test and were blinded to participant disease status (13). In case of disagreement or ambiguity, an independent assessor's opinion was sought.

2.4. Socioeconomic variables

Education deciles (25), obtained from UK postcodes, were used as an indicator of socioeconomic status (SES). Education deciles rank residential areas from 1 (most deprived) to 10 (least deprived) based on several indicators, including average scores for students aged

TABLE 1 Demographic details.

Variables	SCA									Control						T score	*P*-value
		Male			Female			Total			Total							
	Mean	SD	Std error	Mean	SD	Std error	Mean	SD	Std error	Mean	SD	Std error	Mean	SD	Std error		
Patient group	129									50 (33 siblings)						-	-
Male (%)	66 (51.1)									21 (42)						-	-
Genotype (SS, other)	125, 4									0, 50						-	-
Age	17.94	8.93	1.18	19.84	9.11	1.18	19.18	9.45	.84	17.25	8.44	1.19	1.255	0.276			
SES	5.21	2.43	.322	5.27	1.91	.25	5.39	2.12	.213	4.92	2.00	.28	1.301	0.255			
Hemoglobin (g/l)	88.97	15.20	2.01	88.14	13.65	1.77	88.13	15.03	1.32	134.0	8.17	1.15	−26.127	<.001			
SpO_2	96.47	2.62	.346	96.18	2.76	.360	96.69	2.682	.237	98.54	1.41	.221	−3.606	<.001			

SES, socioeconomic status; SpO_2, oxygen saturation from pulse oximetry.

TABLE 2 Analysis of covariance (ANCOVA) for MRI and cognitive variable between SCA patients and controls.

Variable	Adjusted mean		*F* statistic (1,161)	*p*
	Patients	Controls		
White matter volume (mm^3)	408640.21	416643.93	3.690	0.057
Subcortical volume (mm^3)	58709.31	58475.72	0.192	0.662
Processing speed index	88.79	95.82	10.881	**0.001**
Working memory index	91.79	98.18	7.981	**0.005**

Bold values signify statistical significance.

TABLE 3 ANCOVA for sex differences: Controls.

Variable	Controls				Patients			
	Adjusted mean		F statistic (1, 46)	*p*	Adjusted mean		*F* statistic (1, 128)	*p*
	Females	Males			Females	Males		
WMV (mm^3)	404128.76	451397.24	0.398	0.532	390482.61	421954.40	2.600	0.110
Subcortical Volume (mm^3)	57542.85	61342.63	0.431	0.515	56884.11	60064.70	0.398	0.529
PSI	100.75	92.90	5.288	**0.026**[b]	92.17	84.99	10.917	**0.001**[c]
WMI	99.45	97.67	0.331	0.568	93.44	89.82	2.273	0.134

WMV, white matter volume; PSI, processing speed index; WMI, working memory index.
[a]$p > 0.05 < 0.1$; [b]$p < 0.05$; [c]$p < 0.005$.
Bold values signify statistical significance.

7–11 years and 14–16 years in state-funded schools, absence from secondary schooling, proportion of people studying beyond 16 years, entry into higher education, language proficiency and proportion of working adults with low qualifications.

2.5. Hematologic variables

Hemoglobin was acquired from patient medical records up to 6 months prior to the day of cognitive testing. Reference norms were used for controls (26). Oxygen saturation (SpO_2) was measured using a pulse oximeter on the day of the cognitive testing for SAC participants and during baseline clinic visits for POMS patients. Medication history was also recorded. Since previous studies have shown an association between hydroxyurea use and cognitive functioning (27), hydroxyurea use was included in the models.

2.6. MRI acquisition

Within 2 weeks of cognitive assessments, all participants underwent scans on a 3T Siemens Prisma (Erlangen, Germany) MRI with 80 mT/m gradients and a 64-channel receive head coil. Axial T1 weighted images were acquired with repetition time (TR) = 2,300 milliseconds (ms), echo time (TE) = 2.74 ms, TI = 909 ms, flip angle = 8° and voxel size = 1 x 1 x 1 mm^3.

The images were processed and analyzed using the "*recon-all*" pipeline in FreeSurfer version 4.5 (https://surfer.nmr.mgh.harvard.edu/) (28, 29). In summary, FreeSurfer conducts several pre-processing steps including motion correction, intensity normalization, affine transformation to the Montreal Neurological Institute (MNI) atlas, normalization to the original T1-weighted image, and skull stripping. For segmenting specific regional brain volumes, FreeSurfer uses the following steps: linear registration to Gaussian Classifier Atlas, canonical segmentation, and subcortical segmentation of individual regions (pallidum, caudate, putamen, thalamus, hippocampus, amygdala and accumbens).

2.7. Statistical analyses

IBM SPSS Statistics version 28.0.0.0 was used for statistical analyses. All variables were tested for normality using the Shapiro-Wilk and Levene's tests. ANCOVA was conducted to compare cognitive variables (PSI and WMI, adjusting for age, sex, and SES) and regional brain volumes [WMV and total subcortical volume, adjusting for age, sex, and intracranial volume (ICV)] between patients and controls. One patient with extremely high WMV (3SD above mean) was excluded from the analysis. Multiple linear regression models were generated for patients and controls, for WMV predicting PSI, and for total subcortical volumes predicting WMI. See Figure 1 for details.

Previous literature has reported an association between WMV and IQ only in male patients with SCA (12) which prompted us to look at sex differences in our sample. In an exploratory analysis, separate multiple linear regression models (WMV predicting PSI and total subcortical volumes predicting WMI) were generated for males and females for both groups-patients and controls. We also examined if there were differences in hemoglobin levels between male and female patients using an independent sample *t*-test. We wanted to examine if there were volumetric differences between patients and controls in the individual regions of the subcortex. Hence, an ANCOVA was used to compare volumes of individual regions of the subcortex (basal ganglia, thalamus, amygdala, and

TABLE 4 Regression coefficients for processing speed index in patients.

Variable	Patients			Controls		
	Unstandardised b	Standardized B	*p*	Unstandardised b	Standardized B	*p*
WMV	6.843^{E-5}	**0.243**	0.068	−4.667E-5	−0.177	0.569
Hemoglobin	0.161	**0.178**	0.047	0.391	0.272	0.360
SpO_2	0.224	0.523	0.602	1.882	0.221	0.289
Sex	−7.418	**−0.283**	0.011	−13.071	**−0.534**	**0.039**
Age	−0.469	**−0.345**	<0.001	−0.256	−0.191	0.345
SES	0.817	0.136	0.126	0.342	0.059	0.712
ICV	-1.551^{E-5}	−0.150	0.359	1.872E-5	0.205	0.496
Hydroxyurea use	1.636	0.059	0.509	-	-	-

Adjusted R^2, 0.156; WMV, white matter volume; SpO_2, oxygen saturation from pulse oximetry; SES, socioeconomic status; ICV, intracranial volume.
Bold values signify statistical significance.

TABLE 5 Regression coefficients for working memory index in patients.

Variable	Patients			Controls		
	Unstandardised b	Standardized B	*p*	Unstandardised b	Standardized B	*p*
Subcortical volume	0.001	0.201	0.103	−0.001	−0.373	0.086
Hemoglobin	0.143	0.148	0.119	0.654	0.416	0.111
SpO_2	0.290	0.057	0.538	3.254	0.349	0.063
Sex	−7.007	**−0.255**	0.027	−9.352	−0.350	0.149
Age	−0.303	**−0.198**	0.044	−0.282	−0.192	0.323
SES	0.461	0.073	0.428	−2.034	**−0.321**	**0.041**
ICV	2.902^{E-6}	0.027	0.845	3.979E-5	0.398	0.116
Hydroxyurea use	−4.563	−0.157	0.385	-	-	-

Adjusted R^2, 0.069; SpO_2, oxygen saturation from pulse oximetry; SES, socioeconomic status; ICV, intracranial volume.
Bold values signify statistical significance.

hippocampus) adding age, sex and ICV as covariates. Since total subcortical volumes were associated with WMI only in male patients with SCA, we wanted to investigate the effect of individual subcortical regions on WMI in male patients. Multiple linear regression models (adjusting for ICV and age) were generated for male patients for volumes of individual regions of the subcortex predicting WMI. Model fits were evaluated using Cook's distance and an analysis of residuals.

Developmental trajectories for cognitive variables and regional brain volumes (WMV and total subcortical volumes) were compared for patients and controls using the method outlined by Thomas et al. (30). In summary, linear regression models for cognitive variables and brain volumes were plotted by age for controls as well as patients to assess if there were any valid trajectory comparisons. For developmental trajectories to be valid, linear regression with age must be significant for the whole group (patients + controls) or it must be significant for typically developing controls. For valid trajectories (WMV and PSI), the two groups (patients and controls) were compared using analyses of covariances (ANCOVA) with regional brain volumes (adjusting for ICV and sex) or cognitive variables as response variable and age and group as covariates. An interaction term of age x group was also included in the analysis. Variables were fitted as a function of the following equation:

$$\textbf{Volume/cognitive variable} = (\textbf{Intercept for group}) + (\textbf{Age} * \textbf{gradient})$$

We examined the groups at onset at 8 years (intercept) and the slopes for rate of change in development between two groups.

Participants >30 years were excluded in the WMV trajectory analysis as, in typically-developing populations, WMV increases until the age of 28 years and then declines, creating a curved trajectory beyond that age (31).

3. Results

3.1. Demographic variables

Participant characteristics are described in Table 1. No significant differences were found between patients ($n = 129$) and controls ($n = 50$) regarding age, sex, and SES ($ps > 0.05$). The mean age of patients and controls was 19.18 years and 17.25 years, respectively. Sixty-six of 129 patients with SCA (51%) and 21 of 50 controls (were male. Mean hemoglobin and SpO_2 levels for patients with SCA (88.13 g/l and 96.69%) were significantly lower than the reference norm for controls (134 g/l and 98.54%) (Table 1).

TABLE 6 Regression coefficients for processing speed index in male and female patients.

Variables	Males			Females		
	Unstandardised b	Standardized B	Significance	Unstandardised b	Standardized B	Significance
WMV	0.000	**0.424**	0.019	3.076E-5	0.110	0.619
Hemoglobin	0.194	0.215	0.10	0.046	0.051	0.734
SpO_2	−0.245	−0.047	0.708	0.709	0.169	0.238
Age	−0.617	**−0.402**	0.003	−0.522	**−0.314**	**0.044**
SES	1.572	**0.279**	0.03	−0.557	−0.093	0.543
ICV	−2.916E-5	−0.238	0.184	−2.892E-5	−0.227	0.906

Males: Adjusted $R^2 = 0.207$; Females: Adjusted $R^2 = 0.032$; WMV, white matter volume; SpO_2, oxygen saturation from pulse oximetry; SES, socioeconomic status; ICV, intracranial volume. Bold values signify statistical significance.

TABLE 7 Regression coefficients for working memory index in male and female patients.

Variable	Males			Females		
	Unstandardised b	Standardized B	Significance	Unstandardised b	Standardized B	Significance
Subcortical volume	0.001	**0.355**[a]	0.025	0.000	−0.076	0.686
Hemoglobin	0.224	0.299	0.092	0.017	0.017	0.908
SpO_2	−0.026	−0.005	0.971	0.466	0.101	0.470
Age	−0.266	−0.160	0.262	−0.794	**−0.315**	**0.026**
SES	0.150	0.025	0.851	0.520	0.079	0.590
ICV	4.300E-6	0.032	0.834	6.147E-6	0.048	0.800

Males: Adjusted R^2, 0.126; Females: Adjusted R^2, 0.000.
SpO_2, oxygen saturation from pulse oximetry; SES, socioeconomic status; ICV, intracranial volume.

3.2. MRI and cognitive variables

The data met the statistical assumptions for ANCOVA. The details are summarized in Table 2. WMV was lower in patients with SCA with a trend toward significance ($p = 0.057$) while total subcortical volumes were lower in patients with SCA than controls but not statistically different (Table 2). PSI (mean difference = 7.03) and WMI (mean difference = 6.39) were significantly lower in patients than controls. There were no differences in WMV or total subcortical volumes between males and females in the patient or control group (Table 3). Hemoglobin was not significantly different between the sexes in the patient group ($p = 0.6$). In both groups, females had significantly higher PSI than males (mean difference in patient group = 7.18) (Table 3).

3.3. Associations between neurocognitive and MRI variables

Multiple regression analyses were conducted for patients and controls separately to predict PSI from WMV and WMI from total subcortical volumes. Both models were adjusted for age, sex, SES, ICV, hemoglobin, hydroxyurea use and SpO_2. WMV was associated with PSI with a trend toward significance ($p = 0.068$; Table 4). Total subcortical volume was not significantly associated with WMI ($p = 0.103$; Table 5). Age and sex predicted both PSI and WMI in patients (Tables 4, 5). Hemoglobin was independently associated with PSI in patients (Table 4). In controls, sex predicted PSI but age and SES did not (Table 4) while SES predicted WMI but age and sex did not (Table 5). Hydroxyurea use was not associated with PSI (Table 4) or WMI (Table 5) in the patient group.

Separate multiple linear regression models for males and females with SCA revealed that WMV predicted PSI (Table 6) and total subcortical volume predicted WMI (Table 7) in males only. Age predicted PSI in both sexes (Table 6) and predicted WMI in females (Table 7). SES independently predicted PSI in males (Table 6) but did not predict WMI in either sex (Table 7). In male patients, individual subcortical regions predicting WMI were statistically significant for left and right thalamus only (Table 8).

3.4. Developmental trajectory analysis

Developmental trajectories for WMV, PSI, total subcortical volume, and WMI are shown in Figure 2 and were compared between patients and controls (Table 9). Developmental trajectories are only valid if age significantly predicts variables in patients + controls or in the control group alone.

Age predicted WMV (significantly) ($p < 0.001$) in the whole group (patients + controls). WMV increased for both patients and controls, and the rate did not differ between patients and controls nor were they significantly different at onset (Figure 2A, Table 9).

In patients, but not controls, age significantly predicted decline in total subcortical volume ($p = .027$). Subcortical gray matter volume

TABLE 8 Regression for working memory index for individual subcortical regions for male patients.

Subcortical region	Adjusted R^2	Estimated mean volume (mm^3)	Unstandardised b	Standardized beta	*p*
Left caudate	0.090	3808.040	0.007	0.247	0.061
Right putamen	0.045	5891.307	0.001	0.064	0.614
Left thalamus	**0.117**	8110.063	0.005	0.293[a]	**0.019**
Left putamen	0.046	6100.057	0.001	0.068	0.602
Left pallidum	0.065	1474.678	0.008	0.159	0.200
Left accumbens area	0.045	654.352	0.006	0.059	0.646
Left hippocampus	0.068	4028.603	0.007	0.160	0.175
Left amygdala	0.044	1704.197	−0.003	−0.045	0.713
Right thalamus	**0.130**	7088.522	0.007	0.305[a]	**0.011**
Right caudate	0.043	3861.283	0.001	0.039	0.766
Right pallidum	0.060	1598.027	0.010	0.146	0.255
Right accumbens area	0.094	665.590	0.029	0.231	0.053
Right hippocampus	0.064	4114.112	0.007	0.148	0.209
Right amygdala	0.042	1815.090	0.001	0.014	0.912

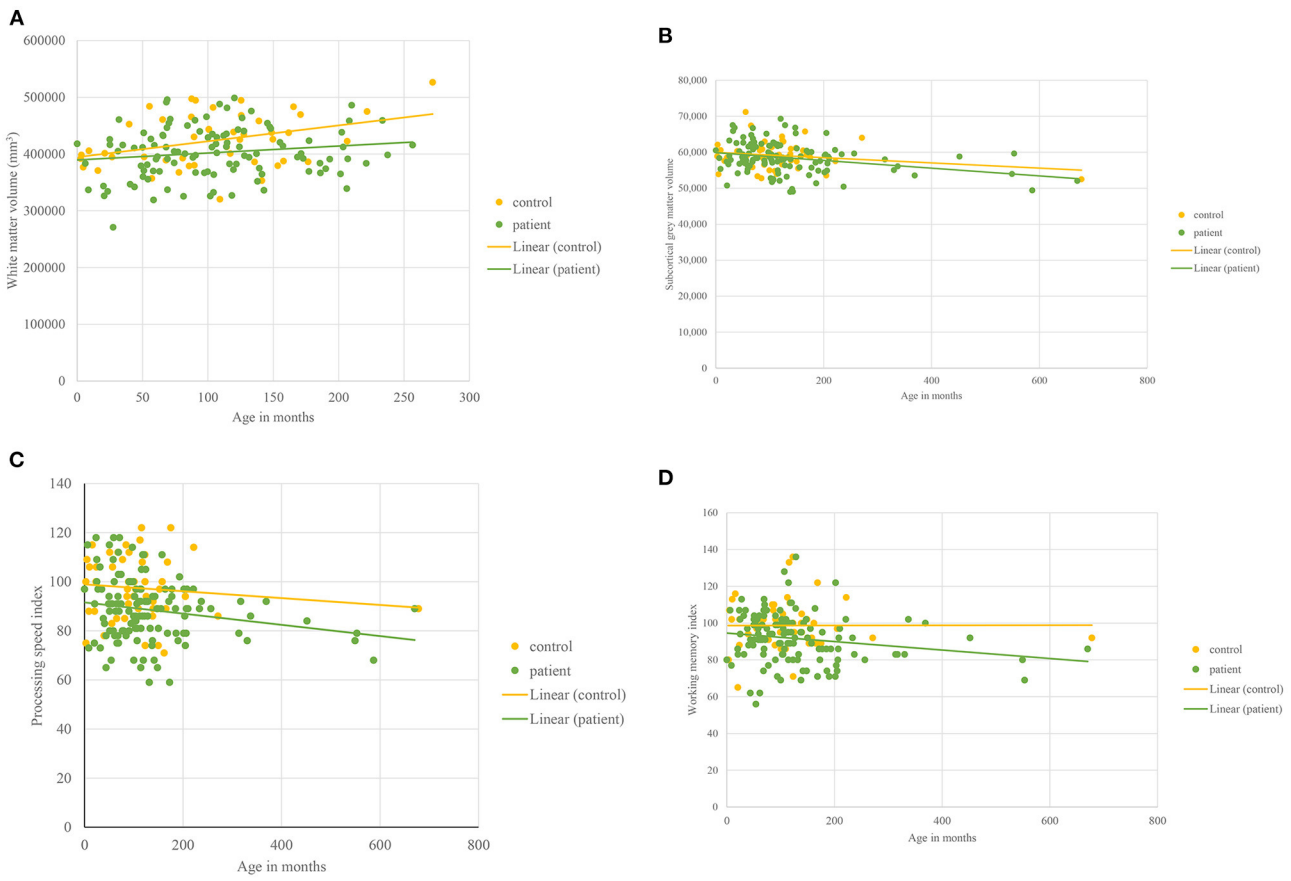


FIGURE 2
Developmental trajectories. For all figures, 0 denotes 8 years of age as it represents the earliest age at which data were collected. **(A)** White matter volume plotted against Age in patients with sickle cell disease and controls aged <30 years. **(B)** Total subcortical volume plotted against Age in patients with sickle cell disease and controls. **(C)** Processing speed index plotted against Age in patients with sickle cell disease and controls. **(D)** Working memory Index plotted against Age in patients with sickle cell disease and controls.

TABLE 9 Developmental trajectories.

Variable	Slope: Volume/cognitive variable = (Intercept for group) + (Age * gradient)		Delay at onset	*p*	Rate of development for patients	*p*
	Patients	Controls				
WMV (mm^3)	34082.693 + age * 169.871	−952.155 + age * 283.435	−35034.884	0.538	0.59	0.186
Subcortical Volume (mm^3)	31336.313 + age * −10.827	31390.132 + age * 2.27	53.819	0.627	−4.76	0.181
PSI	91.583 + age * −0.023	98.947 + age * −0.014	**7.36**[a]	0.023	1.64	0.667
WMI	94.655 + age * −0.023	96.687 + age * 0.022	2.03	0.993	−1.04	0.156

WMV, white mater volumes; PSI, processing speed index; WMI, working memory index.
Delay at onset, Intercept controls–intercept patient, Rate of development for patient, gradient patients/gradient controls.
[a]$p < 0.001$.
Bold values signify statistical significance.

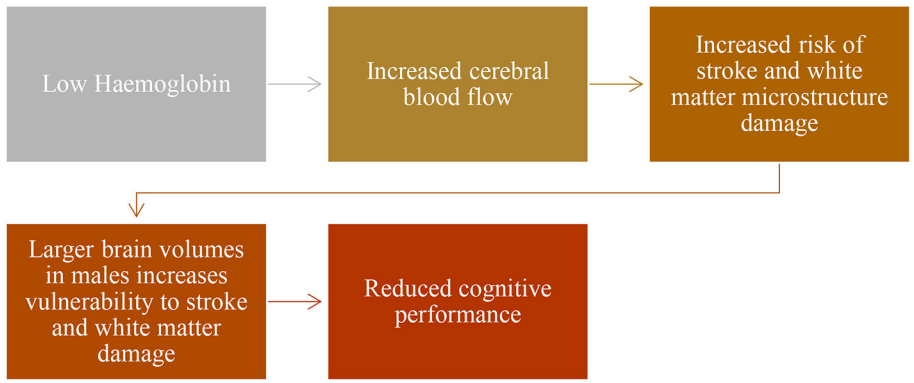


FIGURE 3
Beyond brain volume.

decreased with age (Figure 2B, Table 9) in both patients and controls with no significant difference in onset or rate of decline.

Age predicted PSI (trend level) ($p = 0.07$) in the whole group (patients + controls). At onset (8.02 years), PSI ($p < 0.001$) was significantly lower in patients as compared to controls (Figure 2C, Table 9) with PSI declining with age in patients and controls (Figure 2C) but with no significant difference in rate (Table 9).

In patients but not controls, age significantly predicted decline in WMI ($p = 0.043$). In the trajectory analysis, WMI was maintained in controls and declined in patients (Figure 2D, Table 9) but the difference was not statistically significant.

4. Discussion

The purpose of this study was to investigate the effect of WMV on processing speed and the effect of total subcortical volume on working memory. We also aimed to compare cross-sectional developmental trajectories between patients with SCA and controls. In patients with SCA (male + female), WMV predicted processing speed at a trend level, providing some evidence supporting hypothesis 1. For hypothesis 2, there was weak evidence for an association between total subcortical volumes and WMI in patients and controls although neither was significant when males and females were included. However, these relationships were significant in male patients, supporting hypothesis 3. In line with the fourth hypothesis, some developmental trajectories differed between patients and controls. In patients, PSI was significantly delayed at onset (8.02 years).

When both male and female patients with SCA were included, WMV was associated with processing speed at trend level. When the analysis was conducted only in male patients, this association was significant. We also found that in a model including age and sex, hemoglobin was independently associated with processing speed in patients. As processing speed appears compromised in childhood, cerebral hemodynamic compensation for anemia in early life may affect the development of white matter in patients, particularly in males, with a secondary effect on processing speed. Previous literature suggests that hemoglobin and cerebral blood flow velocity are associated with neurodevelopmental performance in 9-month-old infants with SCA (32). During the pre-school years, hemoglobin is lower in patients as compared to controls (32). Processing speed at this age is 1.5 SD below the mean (6). Furthermore, reduced PSI is associated with white matter microstructural damage in watershed areas in patients with SCA (33). It is likely that lower hemoglobin is compensated for by higher cerebral blood flow and higher oxygen extraction fraction (4) which may increase the risk for infarction (12). In males, larger brain volumes (34) may be associated with greater need for

cerebral blood flow increasing risk of infarction (12) and white matter microstructural damage associated with reduced PSI (32) (see Figure 3).

No studies have looked at the association between subcortical volumes and working memory in children with SCA, making our contribution to the literature important. Previous studies have found an association between subcortical volumes and working memory in adults with SCA aged 19–55 years (9). Our results provide only weak evidence, which was not statistically significant, to support this finding. However, there was only one older control, and our study was underpowered to exclude the association over the wide age range. We included pediatric patients with SCA alongside adult patients which may nullify the effect of subtle neurodevelopmental differences present in adult patients with SCA. In addition, working memory is a complex phenomenon using brain regions including the frontal lobe, parietal cortex, subcortical regions, and the cerebellum (35). Subcortical regions have smaller volumes in pediatric populations with SCA (16). Working memory in the SCA population may be affected by compensatory function in other brain regions. In this study, working memory showed decline with age in patients with SCA only, likely due to the effect of anemia on brain structure and function (36–38).

Our study compared regional brain volume trajectories across a wide age range and found similar trajectories in the controls to previously published data (31) for WMV and total subcortical gray matter volume. We compared WMV in ages 8–30 years and subcortical volumes in patients with SCA and controls aged 8–64 years. WMV did not differ between patients and controls at 8 years nor did the rate of development significantly differ between patients and controls. However, age significantly predicted WMV growth in both groups. Similar findings have been reported by Steen et al. (19). We suspect that lower white matter density (39) and microstructural abnormalities (13) alongside cerebral hemodynamic mechanisms (4) are a better predictor of cognition in SCA patients as compared to WMV. Total subcortical volumes were significantly negatively predicted by age, suggesting reducing volumes with age. Moreover, at onset, SCA patients tend to have larger volumes compared to controls. However, this difference is not significant in our participant group. Previous studies indicate that children with SCA tend to display subtle neurodevelopmental delay in total gray matter volume growth related to SCA pathology (9, 34).

4.1. Limitations

We did not examine the effect of SCI in the patient or control group. Although literature suggests that cognition and brain growth is affected in the SCA population regardless of the presence of SCI, patients with SCI may be more likely to have severe disease (2). Evaluating the effect of SCI on developmental trajectories is an important next step. Additionally, our study did not plot separate developmental trajectories for males and females. A previous study on brain volume growth has demonstrated that there is no effect of sex on brain volume trajectories after controlling for total brain volume (34). However, this effect is yet to be evaluated for cognitive variables. Our study is also limited by our use of cross-sectional data. Although it is possible to make inferences about development using cross-sectional data, this will always be inferior to interrogating longitudinal data. Developmental trajectories may also be non-linear, while we only considered linear trajectories. Looking at curve estimations should be considered in future research. We were underpowered to exclude the possibility that subcortical volumes predict working memory. Further research in larger populations should investigate other brain regions implicated in working memory which may be involved in compensatory functions in children with SCA. Additionally, we used MNI template space to process our images. MNI template is mainly based on non-Hispanic white population which may affect image processing results. Further research may consider creating and using template space adapted for non-white populations. We were not able to establish a link between cognitive end points and use of hydroxyurea in patients. We believe this is due to inconsistent use of hydroxyurea in our cohort. Hence, adding measures of compliance in future studies should be considered. In addition, randomized controlled trials of new treatments for SCA should consider including cognitive and MRI endpoints to investigate their effects on the brain.

5. Conclusions

Through our study, we have contributed to the current literature on SCA pathology. Our findings suggest an association between white matter volume and processing speed in male patients with SCA. They also suggest that developmental trajectories may differ between patients with SCA and controls. These findings contribute to current understanding of disease severity in SCA populations. Interventions targeting early life cerebrovascular and hemodynamic mechanisms might preserve cognitive function and contribute to improved quality of life in this population. Another important finding is that females appear to have protective mechanisms against disease severity. Appropriate timely intervention for males, including monitoring hemoglobin levels, should be considered as a target for intervention. The observation that anemia severity affects brain volumetric growth in the SCA population supports existing literature (12). Future research should explore the effect of SCI and sex on disease severity in the patients with SCA.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by West London and South Yorkshire Research Ethics Committees. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

SH conceptualized the paper, completed the review, analyzed the data, and drafted the manuscript. MK and HS were involved in data collection and processing. SH and MK analyzed the data supervised by FK, AH, RM, and JC. RM, JC, HS, and

AH contributed to the review and provided feedback on the manuscript. All authors provided feedback on the manuscript and read and approved the final version for submission.

Funding

MK and HS were funded by Action Medical Research (GN2509). RM was supported by the EPSRC-funded UCL Center for Doctoral Training in Medical Imaging (EP/L016478/1). The work was supported by the National Institute for Health Research and Biomedical Research Center at Great Ormond Street Hospital for Children NHS Foundation Trust and the Institute of Child Health (IS-BRC-1215-20012).

Conflict of interest

FK is a consultant for Global Blood Therapeutics.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Wastnedge E, Waters D, Patel S, Morrison K, Goh MY, Adeloye D, et al. The global burden of sickle cell disease in children under 5 years of age: a systematic review and meta-analysis. *J Glob Health.* (2018) 8:021103. doi: 10.7189/jogh.08.021103

2. Kawadler JM, Clayden JD, Clark CA, Kirkham FJ. Intelligence quotient in paediatric sickle cell disease: a systematic review and meta-analysis. *Dev Med Child Neurol.* (2016) 58:672–9. doi: 10.1111/dmcn.13113

3. Smith KE, Schatz J. Working memory in children with neurocognitive effects from sickle cell disease: contributions of the central executive and processing speed. *Dev Neuropsychol.* (2016) 41:231–44. doi: 10.1080/87565641.2016.1238474

4. Prussien KV, Compas BE, Siciliano RE, Ciriegio AE, Lee CA, Kassim AA, et al. Cerebral hemodynamics and executive function in sickle cell anemia. *Stroke.* (2021) 52:1830–4. doi: 10.1161/STROKEAHA.120.032741

5. Connolly ME, Bills SE, Hardy SJ. Cognitive functioning and educational support plans in youth with sickle cell disease. *J Ped Hematol Oncol.* (2021) 43:e666–e76. doi: 10.1097/MPH.0000000000002092

6. Downes M, Kirkham FJ, Telfer PT, de Haan M. Assessment of executive functions in preschool children with sickle cell anemia. *J Int Neuropsychol Soc.* (2018) 24:949–54. doi: 10.1017/S1355617718000255

7. Hood AM, Kölbel M, Stotesbury H, Kawadler J, Slee A, Inusa B, et al. Biopsychosocial predictors of quality of life in paediatric patients with sickle cell disease. *Front Psychol.* (2021) 12:681137. doi: 10.3389/fpsyg.2021.681137

8. Steen RG, Langston JW, Ogg RJ, Xiong X, Ye Z, Wang WC. Diffuse T1 reduction in gray matter of sickle cell disease patients: evidence of selective vulnerability to damage? *Mag Res Imag.* (1999) 17:503–15. doi: 10.1016/S0730-725X(98)00204-5

9. Mackin RS, Insel P, Truran D, Vichinsky EP, Neumayr LD, Armstrong FD, et al. Neuroimaging abnormalities in adults with sickle cell anemia: associations with cognition. *Neurology.* (2014) 82:835–41. doi: 10.1212/WNL.0000000000000188

10. Hamdule S, Kirkham FJ. *Brain Volumes and IQ in sickle cell anaemia: A systematic review*, F1000. (2023).

11. Brouwer RM, Hedman AM, van Haren NE, Schnack HG, Brans RG, Smit DJ, et al. Heritability of brain volume change and its relation to intelligence. *Neuroimage.* (2014) 100:676–83. doi: 10.1016/j.neuroimage.2014.04.072

12. Choi S, O'Neil SH, Joshi AA Li J, Bush AM, Coates TD, et al. Anemia predicts lower white matter volume and cognitive performance in sickle and non-sickle cell anemia syndrome. *Am J Hematol.* (2019) 94:1055–65. doi: 10.1002/ajh.25570

13. Stotesbury H, Kirkham FJ, Kölbel M, Balfour P, Clayden JD, Sahota S, et al. White matter integrity and processing speed in sickle cell anemia. *Neurology.* (2018) 90:e2042–e50. doi: 10.1212/WNL.0000000000005644

14. Spencer APC, Lee-Kelland R, Brooks JCW, Jary S, Tonks J, Cowan FM, et al. Brain volumes and functional outcomes in children without cerebral palsy after therapeutic hypothermia for neonatal hypoxic-ischaemic encephalopathy. *Dev Med Child Neurol.* 3:15369 (2022). doi: 10.1111/dmcn.15369

15. Total and regional brain volumes in a population-based normative sample from 4 to 18 years: the NIH MRI study of normal brain development. *Cereb Cortex.* (2012) 22:1–12. doi: 10.1093/cercor/bhr018

16. Kawadler JM, Clayden JD, Kirkham FJ, Cox TC, Saunders DE, Clark CA. Subcortical and cerebellar volumetric deficits in paediatric sickle cell anaemia. *Br J Haematol.* (2013) 163:373–6. doi: 10.1111/bjh.12496

17. Santini T, Koo M, Farhat N, Campos VP, Alkhateeb S, Vieira MAC, et al. Analysis of hippocampal subfields in sickle cell disease using ultrahigh field MRI. *Neuroimage: Clinical.* (2021) 30:102655. doi: 10.1016/j.nicl.2021.102655

18. Wang W, Enos L, Gallagher D, Thompson R, Guarini L, Vichinsky E, et al. Neuropsychologic performance in school-aged children with sickle cell disease: a report from the cooperative study of sickle cell disease. *J Pediatr.* (2001) 139:391–7. doi: 10.1067/mpd.2001.116935

19. Kawadler JM, Clark CA, McKinstry RC, Kirkham FJ. Brain atrophy in paediatric sickle cell anaemia: findings from the silent infarct transfusion (SIT) trial. *Br J Haematol.* (2017) 177:151–3. doi: 10.1111/bjh.14039

20. Darbari DS, Eigbire-Molen O, Ponisio MR, Milchenko MV, Rodeghier MJ, Casella JF, et al. Progressive loss of brain volume in children with sickle cell anemia and silent cerebral infarct: a report from the silent cerebral infarct transfusion trial. *Am J Hematol.* (2018) 93:E406–e8. doi: 10.1002/ajh.25297

21. Chen R, Krejza J, Arkuszewski M, Zimmerman RA, Herskovits EH, Melhem ER. Brain morphometric analysis predicts decline of intelligence quotient in children with sickle cell disease: A preliminary study. *Adv Med Sci.* (2017) 62:151–7. doi: 10.1016/j.advms.2016.09.002

22. Chen R, Arkuszewski M, Krejza J, Zimmerman RA, Herskovits EH, Melhem ER, et al. prospective longitudinal brain morphometry study of children with sickle cell disease. *AJNR Am J Neuroradiol.* (2015) 36:403–10. doi: 10.3174/ajnr.A4101

23. Clayden JD, Stotesbury H, Kawadler JM, Slee A, Koelbel M, Saunders DE, et al. Structural connectivity mediates the relationship between blood oxygenation and cognitive function in sickle cell anemia. *Blood Advances.* (2022) 3:6751. doi: 10.1182/bloodadvances.2021006751

24. McCrimmon AW, Smith AD. Review of the wechsler abbreviated scale of intelligence, second edition (WASI-II). *J Psychoeduc Assess.* (2012) 31:337–41. doi: 10.1177/0734282912467756

25. Ministry of Housing CLG. *English Indices of Deprivation 2010. Neighbourhoods Statistical Release*. (24 March 2011 ed: GOV.UK.) Department for Communities and Local Government (2011). p. 1-20. Available online at: www.gov.uk/government/collections/english-indices-of-deprivation

26. Castro OL, Haddy TB, Rana SR. Age and sex-related blood cell values in healthy black Americans. *Public Health Rep.* (1987) 102:232–7.

27. Heitzer AM, Longoria J, Okhomina V, Wang WC, Raches D, Potter B, et al. Hydroxyurea treatment and neurocognitive functioning in sickle cell disease from school age to young adulthood. *Br J Haematol.* (2021) 195:256–66. doi: 10.1111/bjh.17687

28. Fischl B, Salat DH, Busa E, Albert M, Dieterich M, Haselgrove C, et al. Whole brain segmentation: automated labeling of neuroanatomical structures in the human brain. *Neuron.* (2002) 33:341–55. doi: 10.1016/S0896-6273(02)00569-X

29. Fischl B, Salat DH, van der Kouwe AJ, Makris N, Ségonne F, Quinn BT, et al. Sequence-independent segmentation of magnetic resonance images. *Neuroimage.* (2004) 23(Supplement 1):S69–84. doi: 10.1016/j.neuroimage.2004.07.016

30. Thomas MS, Annaz D, Ansari D, Scerif G, Jarrold C, Karmiloff-Smith A. Using developmental trajectories to understand developmental disorders. *J Speech Lang Hear Res.* (2009) 52:336–58. doi: 10.1044/1092-4388(2009/07-0144)

31. Bethlehem RAI, Seidlitz J, White SR, Vogel JW, Anderson KM, Adamson C, et al. Brain charts for the human lifespan. *Nature.* (2022) 604:525–33. doi: 10.1038/s41586-022-04554-y

32. Hogan AM, Kirkham FJ, Prengler M, Telfer P, Lane R, Vargha-Khadem F, et al. An exploratory study of physiological correlates of neurodevelopmental delay in infants with sickle cell anaemia. *Br J Haematol.* (2006) 132:99–107. doi: 10.1111/j.1365-2141.2005.05828.x

33. Stotesbury H, Hales PW, Hood AM, Koelbel M, Kawadler JM, Saunders DE, et al. Individual watershed areas in sickle cell anemia: an arterial spin labeling study. *Front Physiol.* (2022) 13:865391. doi: 10.3389/fphys.2022.865391

34. Steen RG, Emudianughe T, Hunte M, Glass J, Wu S, Xiong X, et al. Brain volume in pediatric patients with sickle cell disease: evidence of volumetric growth delay? *Am J Neuroradiol.* (2005) 26:455–62.

35. Eriksson J, Vogel Edward K, Lansner A, Bergström F, Nyberg L. Neurocognitive architecture of working memory. *Neuron.* (2015) 88:33–46. doi: 10.1016/j.neuron.2015.09.020

36. Vichinsky EP, Neumayr LD, Gold JI, Weiner MW, Rule RR, Truran D, et al. Neuropsychological dysfunction and neuroimaging abnormalities in neurologically intact adults with sickle cell anemia. *JAMA.* (2010) 303:1823–31. doi: 10.1001/jama.2010.562

37. Jacob M, Stotesbury H, Kija E, Saunders D, Mtei RJ, Tutuba H, et al. Effect of age, cerebral infarcts, vasculopathy and haemoglobin on cognitive function, in Tanzanian children with sickle cell anaemia. *Eur J Paed Neurol.* (2022) 37:105–13. doi: 10.1016/j.ejpn.2022.01.010

38. Hijmans CT, Grootenhuis MA, Oosterlaan J, Heijboer H, Peters M, Fijnvandraat K. Neurocognitive deficits in children with sickle cell disease are associated with the severity of anemia. *Pediatr Blood Cancer.* (2011) 57:297–302. doi: 10.1002/pbc.22892

39. Baldeweg T, Hogan AM, Saunders DE, Telfer P, Gadian DG, Vargha-Khadem F, et al. Detecting white matter injury in sickle cell disease using voxel-based morphometry. *Ann Neurol.* (2006) 59:662–72. doi: 10.1002/ana.20790

frontiers | Frontiers in Neurology

TYPE Original Research
PUBLISHED 31 March 2023
DOI 10.3389/fneur.2023.1112865

OPEN ACCESS

EDITED BY
Fenella Jane Kirkham,
University College London,
United Kingdom

REVIEWED BY
Xiaodong Zhang,
Emory University,
United States
Adam Bush,
Stanford University,
United States
Shifa Hamdule,
University College London,
United Kingdom

*CORRESPONDENCE
Lori C. Jordan
✉ lori.jordan@vumc.org

SPECIALTY SECTION
This article was submitted to Stroke, a section of the journal Frontiers in Neurology

RECEIVED 30 November 2022
ACCEPTED 10 March 2023
PUBLISHED 31 March 2023

CITATION
Jones RS, Donahue MJ, Davis LT, Pruthi S, Waddle SL, Custer C, Patel NJ, DeBaun MR, Kassim AA, Rodeghier M and Jordan LC (2023) Silent infarction in sickle cell disease is associated with brain volume loss in excess of infarct volume.
Front. Neurol. 14:1112865.
doi: 10.3389/fneur.2023.1112865

Silent infarction in sickle cell disease is associated with brain volume loss in excess of infarct volume

R. Sky Jones[1], Manus J. Donahue[2,3,4], L. Taylor Davis[2], Sumit Pruthi[2], Spencer L. Waddle[2], Chelsea Custer[1,2], Niral J. Patel[1,2], Michael R. DeBaun[5], Adetola A. Kassim[6], Mark Rodeghier[7] and Lori C. Jordan[1,2,3]*

[1]Division of Pediatric Neurology, Department of Pediatrics, Vanderbilt University Medical Center, Nashville, TN, United States, [2]Department of Radiology, Vanderbilt University Medical Center, Nashville, TN, United States, [3]Department of Neurology, Vanderbilt University Medical Center, Nashville, TN, United States, [4]Department of Psychiatry, Vanderbilt University Medical Center, Nashville, TN, United States, [5]Vanderbilt-Meharry Center of Excellence in Sickle Cell Disease, Nashville, TN, United States, [6]Division of Hematology and Oncology, Department of Medicine, Vanderbilt University Medical Center, Nashville, TN, United States, [7]Rodeghier Consulting, Chicago, IL, United States

Introduction: Sickle cell disease (SCD) increases cerebral infarct risk, but reported effects on brain volume have varied. More detailed information using larger cohorts and contemporary methods could motivate the use of longitudinal brain volume assessment in SCD as an automated marker of disease stability or future progression. The purpose of this study was to rigorously evaluate whether children and young adults with SCD have reduced gray matter volume (GMV) and white matter volume (WMV) compared to healthy controls using high-resolution MRI. We tested the hypotheses that (i) elevated CBF, a marker of cerebral hemodynamic compensation in SCD, is associated with global and regional brain atrophy, and (ii) silent cerebral infarct burden is associated with brain atrophy in excess of infarct volume.

Methods: Healthy controls (n =49) and SCD participants without overt stroke (n =88) aged 7–32years completed 3T brain MRI; pseudocontinuous arterial spin labeling measured CBF. Multivariable linear regressions assessed associations of independent variables with GMV, WMV, and volumes of cortical/subcortical regions.

Results: Reduced hemoglobin was associated with reductions in both GMV (p =0.032) and WMV (p =0.005); reduced arterial oxygen content (CaO_2) was also associated with reductions in GMV (p =0.035) and WMV (p =0.006). Elevated gray matter CBF was associated with reduced WMV (p =0.018). Infarct burden was associated with reductions in WMV 30-fold greater than the infarct volume itself (p =0.005). Increased GM CBF correlated with volumetric reductions of the insula and left and right caudate nuclei (p =0.017, 0.017, 0.036, respectively). Infarct burden was associated with reduced left and right nucleus accumbens, right thalamus, and anterior corpus callosum volumes (p =0.002, 0.002, 0.009, 0.002, respectively).

Discussion: We demonstrate that anemia and decreased CaO2 are associated with reductions in GMV and WMV in SCD. Increased CBF and infarct burden were also associated with reduced volume in subcortical structures. Global WMV deficits associated with infarct burden far exceed infarct volume itself. Hemodynamic

compensation via increased cerebral blood flow in SCD seems inadequate to prevent brain volume loss. Our work highlights that silent cerebral infarcts are just a portion of the brain injury that occurs in SCD; brain volume is another potential biomarker of brain injury in SCD.

KEYWORDS

sickle cell, infarction, brain volume, cerebral blood flow, MRI, silent cerebral infarct

Introduction

Sickle cell disease (SCD) is a chronic hemolytic anemia (1) Silent cerebral infarcts (SCIs) are increasingly present with age in individuals with SCD (2) and are defined as infarcts visible on brain MRI though focal neurological deficits are not apparent (3) SCIs in children with SCD are associated with risk of overt stroke, academic deficits, and further infarction (4, 5), most often occurring in cerebral blood flow (CBF) borderzone regions (6). Thus, 2020 American Society of Hematology guidelines recommend MRI of the brain to screen for SCI at least once in childhood and adulthood (7). SCD also results in cerebral hemodynamic and metabolic aberrations (8). Anemia and altered oxygen affinity of hemoglobin S contribute to low arterial oxygen content (CaO_2), leading to compensatory increases in CBF (9–11). However, cerebral hemodynamic and metabolic compensation may insufficiently compensate for the extent of anemia (12), thereby leading to subtle brain injury and brain volume loss.

The advent of automated brain segmentation has led to the use of software for the clinical characterization of disorders involving cerebral atrophy such as Alzheimer's disease (13). Reductions in brain volume may serve as a clinical marker for other diseases such as SCD, however exemplar datasets from generalizable cohorts are not readily available. Furthermore, the relevance of brain volume in SCD has not been conclusively established in the context of tissue infarction and hemodynamic impairment. For instance, prior investigations into SCD and brain volume have reached various conclusions with studies finding reduced gray matter volume (GMV) but not white matter volume (WMV) (14), reduced WMV but not GMV (15) and a longitudinal study that identified decreases in total brain volume over time (16). Studies often were limited by small and heterogenous samples, lower spatial resolution MRI, and variation in field strength and contrast parameters which precludes comparison among data sets. More detailed information on these trends using larger cohorts and contemporary methods could motivate the use of longitudinal brain volume assessment in SCD as an automated marker of disease stability or future progression.

To address this limitation, the purpose of this study was to rigorously evaluate whether children and young adults with SCD, using a larger cohort with more homogenous imaging parameters and standardized inclusion criteria, have reduced GMV and WMV compared to healthy controls using high resolution MRI. We tested the hypotheses that (i) elevated CBF, a marker of cerebral hemodynamic compensation in SCD, is associated with global and regional brain atrophy, and (ii) silent cerebral infarct burden is associated with brain atrophy in excess of infarct volume.

Materials and methods

This study was approved by the Vanderbilt University Medical Center Institutional Review Board and written informed consent was obtained from all adult participants. For those less than 18 years of age, parents or guardians provided written informed consent and children assented to participate.

Participants with SCD defined as hemoglobin (Hb) SS or HbSβ0 thalassemia were recruited from SCD clinics at an academic medical center and a community clinic. Race-matched healthy controls (HbAA) were recruited via community outreach; attempts were made to age-match participants within 3 years. SCD inclusion criteria: age = 7–32 years. Exclusion criteria: contraindication to 3 Tesla MRI, prior overt stroke, receiving regular blood transfusions, intracranial stenosis >70%, major neurological or psychiatric condition besides SCD, major structural brain abnormality. Neurological history and exam to confirm that any infarcts were silent, hemoglobin (Hb, g/dL), hematocrit and pulse oximetry (SaO_2, %) readings were obtained at time of MRI. Arterial oxygen content (CaO_2, ml O_2/100g blood) was calculated as $SaO_2 \times Hgb \times 1.37$. Any SCI in a healthy control participant was an exclusion criterion. Five healthy controls found to have SCIs were excluded from analysis: these five individuals represented a subpopulation too small to adequately analyze but did not otherwise differ from the remaining healthy controls. White matter alterations are expected in a small portion of healthy children and young adults (17, 18). Full exclusion logic is shown in Figure 1.

Sequences

Each participant was scanned at 3 Tesla (Philips Healthcare, Best, Netherlands). A 3D T_1-weighted magnetization-prepared rapid gradient echo (MPRAGE) sequence (3D turbo-gradient-echo; spatial resolution = 1.0 mm isotropic, TR/TE = 8.20/3.76 ms) was acquired for tissue volume analysis. Axial and coronal 2D fluid-attenuated inversion-recovery (FLAIR) sequences (axial spatial resolution = 0.9 × 1.1 × 3.0 mm; coronal spatial resolution = 0.9 × 3.0 × 1.1 mm; TR/TI/TE = 11,000/2800/120 ms) were acquired for lesion quantification. 2D pseudocontinuous arterial spin labeling (pCASL) was used to evaluate CBF. Imaging parameters varied slightly between adults and children given expected variations in blood arrival time with age. The labeling volumes were planned so that they were perpendicular to the carotid and vertebrobasilar arteries to reduce the likelihood of the labeling plane traversing the carotid and vertebral arteries obliquely. A common spatial resolution of 3 × 3 × 7 mm and dual-pulse background suppression was used. Adults utilized a pCASL scan with

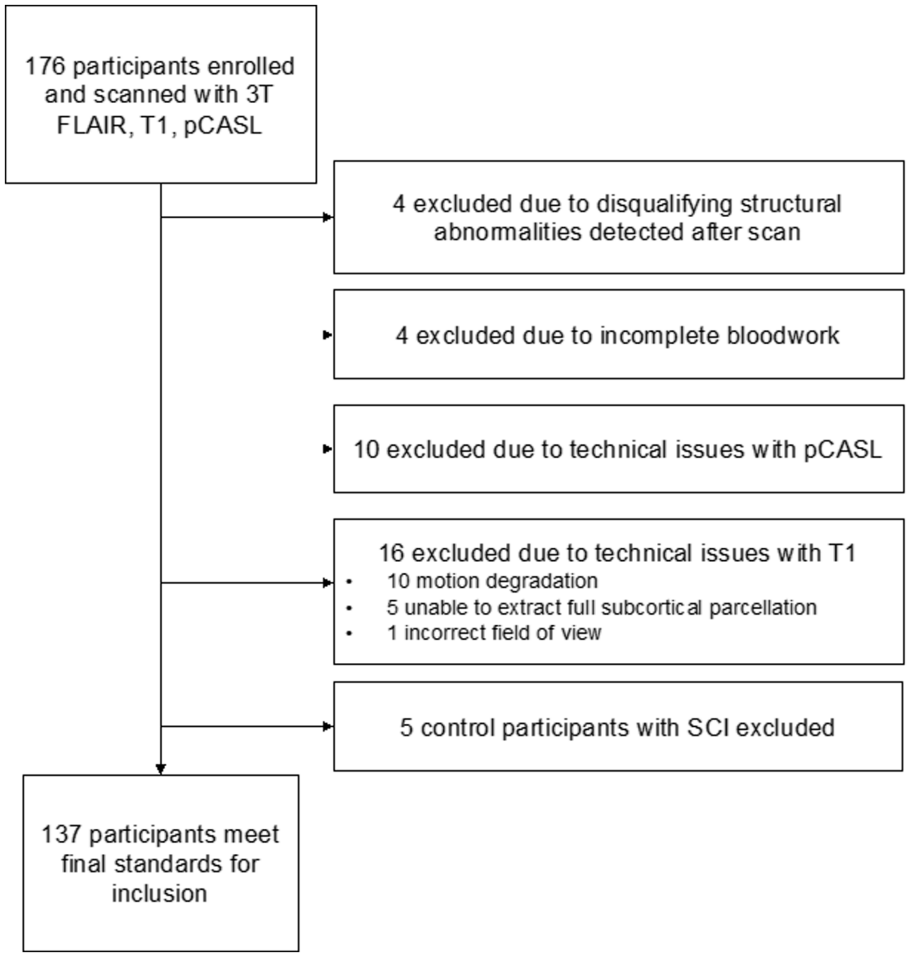


FIGURE 1
Study exclusion flowchart.

post-labeling delay = 1900 ms (label duration = 1,000 ms), averages = 20, scan duration = 168 s, whereas children utilized an ASL scan with labeling parameters of post-labeling delay = 1,650 ms (label duration = 1,650 ms), averages = 20, scan duration = 168 s. The difference in label delays was due to known differences in blood arrival between adults and children and a desire to titrate each sequence to the population. The shorter label duration was used in the adult protocol as this scan was part of a multi-delay pCASL protocol for transit time determination, and the shorter delay was selected to increase sensitivity to blood arrival time while maintaining adequate signal-to-noise ratio. Here, only the single delay of 1,900 ms was used to increase consistency with the single-delay pediatric scan, and in a direct comparison we have reported that both of these single-delay scans provide CBF values that are reproducible and not significantly different in healthy adults (19). Other scan parameters: TR = 4,200 ms, TE = 13 ms, spatial pre-saturation, bandwidth = 2,665 Hz, slice time = 23 ms, in-plane field-of-view = 240 × 240 mm, slice thickness = 7 mm, slice gap 0.5 mm, slices = 17, SENSE-factor = 2.3, echo planar imaging (EPI) factor = 35, *k*-space trajectory = cartesian. The M_0 scan was acquired with identical geometry but with the pCASL preparation removed, TR = 20s, and scanner gain, shimming, and scaling unchanged from the pCASL acquisition.

Image analysis

Tissue segmentations were created using FreeSurfer (version 7.1.1[1]). The standard automated FreeSurfer cortical reconstruction routine (recon-all) was applied to T_1-weighted images; participants with motion corrupted T_1 images were excluded. All resulting segmentations were manually inspected. GNU Parallel was used to reduce computation time (20).

Cerebral blood flow was calculated in each voxel from pCASL acquisitions using the single-PLD kinetic model corrected for the hematocrit, as described previously (21). pCASL data were corrected for motion and baseline drift, normalized by the TR = 20s equilibrium magnetization M_0 scan, and the solution to the flow-modified Bloch equation, using the measured hematocrit for blood T_1 determination and SCD labeling efficiency ($\alpha = 0.72$), was applied (21). The M_0 image itself was used for normalization, rather than a single measurement in pure blood, as this is now recommended (22), pure arterial blood is not straightforward to isolate, and the image-based approach reduces

1 http://surfer.nmr.mgh.harvard.edu

spatial artifacts from coil sensitivity. Of note, the ISMRM Perfusion Study Group white paper simplified kinetic model (22) was not used; rather, we used an expanded model that accounts for differences in blood and tissue T_1, as well as blood arrival time, which all change in the presence of anemia (23). The primary CBF parameter of interest was the gray matter CBF. For gray matter CBF determination, we utilized the whole brain blood–brain partition coefficient (λ) = 0.9 ml/g, $T_{1,tiss}$ = 1.2 s (24, 25), and bolus arrival time (BAT) = 1.10 s for controls and 1.02 s for SCD as measured previously (21). We calculated the participant-specific blood T_1 using the measured hematocrit and previously published relationship between arterial (oxygenation = 92 ± 7%) blood water T_1 and hematocrit: $1/T_1 = 0.52 \times Hct + 0.38$ (26). An alternative method has been proposed whereby individual T_1 measurements are made in venous blood water *in vivo* (27). We chose not to measure venous T_1 as the flow-modified Bloch equation requires arterial blood water T_1, and the venous and arterial T_1 differ by approximately 200 ms, depending on oxygenation status. However, it should be noted that both approaches are commonly used in the literature (27). Finally, for completeness, we also estimated the white matter CBF as a more exploratory measure. Here, we used the same whole brain blood–brain partition coefficient (λ) = 0.9 ml/g, but estimated BAT at 2.0 s for controls and 1.9 s for SCD under the assumption that transit delays in gray matter and white matter are similar between SCD and healthy participants (28, 29). The resulting CBF maps were registered to the T_1-weighted image using the M_0 image as the template, and the mean CBF within the gray and white matter was separately recorded using the white matter CBF parameters in the white matter mask and gray matter CBF parameters in the gray matter mask.

FreeSurfer estimates whole brain volume and total GMV (30); WMV was calculated as the difference between the total brain volume (excluding ventricles) and GMV. For exploratory analyses, subcortical values were recorded, as well as tissue volumes in all bilateral brain lobes and cerebellum.

Infarcts were traced on axial FLAIR sequences by a staff scientist (RSJ) and independently confirmed by an experienced board-certified neuroradiologist (LTD). For the purposes of this study, only lesions meeting the minimum dimension of a silent cerebral infarct as defined in the Silent Infarct Transfusion Trial (4) (at least 3 mm in one plane and visible in a second imaging plane) were traced. Intracranial volume (ICV) estimates were obtained for each participant; FreeSurfer estimates ICV by dividing a reference atlas ICV by the subject-to-atlas scaling factor calculated during cortical registration.

Statistical testing

Demographic statistics included mean and standard deviation (normal data) or median and interquartile range (non-normal data) for continuous variables and count and percent for categorical variables. Tests for differences in continuous variables were done with independent samples *t*-tests, or Mann–Whitney *U* tests for non-normally distributed data. Categorial variables were evaluated using a $\chi 2$ test or Fisher's exact test for small cell counts. All tests were two-sided.

Multivariate linear regression was used to evaluate the relationship between the presence of SCD as well as physiologic parameters known to be altered by SCD (hemoglobin concentration, CBF, CaO_2 and lesion burden) and measures of tissue volume. Separate multivariate regressions were created for disease state and each SCD-associated physiologic parameter; these parameters are highly correlated with one another. Separate regressions allows the magnitude of parameter effect on and suitability as a standalone biomarker for changes in brain volume to be quantified and avoids model instability introduced by collinearity.

Models were generated using *statsmodels* (31). All models use age, sex and ICV as covariates in addition to those explicitly stated. Significance was defined as two-sided $p < 0.05$ after Benjamini–Hochberg correction, with each set of regressions using the same independent variable of interest corrected together. Our primary analysis utilized total gray and total white matter volume as dependent variables; a secondary analysis of 66 subcortical and cortical structures was performed as well. Supplementary material includes coefficients for all variables in each regression model are available online.

The infarct burden in our participant population was exponentially distributed; thus, infarct burden was $\log_{10}$ transformed before regression. This transformation necessarily removes participants with no lesion burden infarcts from the regression as the logarithm of 0 is undefined.

Results

A total of 137 participants were included: 88 had SCD (median age = 18.1 years, IQR 15.4; 48.9% male) and 49 were healthy controls (median age = 23.75 years, IQR 12.8; 42.9% male). SCIs were present in 31 participants (35%) with SCD. Participant demographic information is summarized in Table 1.

Representative comparisons of anatomical images of individuals with and without SCD, along with their FreeSurfer-derived GM and WM segmentations can be seen in Figure 2, demonstrating the quality of the segmentation as well as the minimal differences apparent on standard anatomic neuroimaging in participants with SCD with and without infarcts and healthy controls. Regression plots of total brain volume against factors hypothesized to impact brain size (SCD presence, hemoglobin and infarct burden) are presented in Figure 3.

In the primary multivariable analysis (Table 2) including all study participants, SCD was associated with reduced WMV of 16 mm^3 after controlling for age, sex and ICV ($p = 0.0075$), compared to healthy controls, Table 2; GMV was not reduced. Lower hemoglobin was associated with decreases in both GMV ($p = 0.032$) and WMV ($p = 0.005$). Reductions in CaO_2 were associated with reductions in both GMV ($p = 0.035$) and WMV ($p = 0.006$). Increased GM CBF was associated with decreased WMV ($p = 0.018$) but not GMV. In participants with SCD, an increase in infarct burden was associated with diminished WMV ($p = 0.005$). Because infarct burden was log transformed before regressing, the coefficient of this regression (−30.2) translates to approximately 30.2 mm^3 of WMV loss for each 10-fold increase in infarct burden. Thus, an increase of infarct burden from 0.1 to 1 mm^3 (the range within approximately two-thirds of infarct burdens for study participants fall) results in an expected WMV deficit that is over 30 times larger than the increase in infarct volume itself. Infarct burden was not associated with GMV.

A secondary and exploratory analysis of regional brain volumes is presented in Table 3, which were corrected for multiple volume comparisons as stated in the Methods. Increased GM CBF was associated with decreased volume of the insular lobe ($p = 0.017$), the

TABLE 1 Study demographics.

	Control (n =49)	SCD (n =88)	p[a]
Black race, n (percent)	49 (100.0%)	86 (97.73%)	0.54[c]
Male sex, n (percent)	21 (42.86%)	43 (48.86%)	0.25
Hemoglobin genotype	49 HbAA 100%)	80 HbSS (91%) 8 HbSB0 (9%)	n/a
Age at MRI, years, median (IQR)	23.75 (12.83)	18.06 (15.42)	0.26[d]
SCI present, n (percent)	n/a	31 (35.23%)	n/a
SCI count[e], median (IQR)	n/a	4 (5)	n/a
Total SCI burden[e], mL, median (IQR)	n/a	0.14 (0.33)	n/a
Gray matter volume, mean (SD)	619.40 (74.16)	626.21 (67.99)	0.588
White matter volume, mean (SD)	441.31 (55.17)	435.62 (62.52)	0.595
GM CBF, ml/100 g/min, mean (SD)	50.64 (8.9)	82.47 (17.92)	<0.001
WM CBF, ml/100 g/min, mean (SD)	24.99 (4.16)	40.49 (10.81)	<0.001
Regular blood transfusions, n (percent)	n/a	0 (0.0%)	n/a
CaO_2, mL/dL, mean (SD)	17.38 (2.12)	11.63 (1.85)	<0.001
SaO_2, percent, median (IQR)	98.0 (2.0)	96.0 (3.26)	<0.001[d]
Hemoglobin, g/dL, mean (SD)	13.28 (1.57)	9.04 (1.37)	<0.001
Hemoglobin S fraction, percent, median (IQR)	n/a	0.77 (0.16)	n/a
Intracranial stenosis > 70%, n, (percent)	0 (0.0%)	0 (0.0%)	n/a
Hydroxyurea therapy, n (percent)	n/a	79 (89.77%)	n/a
Type 1 diabetes mellitus[b], n (percent)	3 (6.12%)	2 (2.27%)	0.35[c]
Hypercholesterolemia[b], n (percent)	0 (0.0%)	0 (0.0%)	n/a
Coronary artery disease[b], n (percent)	0 (0.0%)	0 (0.0%)	n/a
Smoking currently, n (percent)	2 (4.08%)	8 (9.09%)	0.49[c]
Body mass index, kg/m^2, mean (SD)	23.29 (8.23)	21.14 (4.54)	0.054

[a]Chi-square test for categorical variables and t-test for continuous variables, unless otherwise noted. [b]Documented in medical records and/or self-reported by study participant. [c]Fisher exact test used if one cell < 5. [d]Mann–Whitney U test used for non-normally distributed data: interquartile range (IQR) reported instead of SD and median reported instead of mean. [e]Statistics for lesion count and volume calculated using only participants with 1 or more SCI. CaO_2, arterial oxygen content; SaO_2, blood oxygen saturation; SCD, sickle cell disease; SCI, silent cerebral infarct; HbSB0, hemoglobin S β0 thalassemia.

left caudate nucleus (p =0.017) and the right caudate nucleus (p = 0.036). Greater lesion burden was associated with reduced volume of the left nucleus accumbens (p = 0.002), the right nucleus accumbens (p = 0.002), the right thalamus (p = 0.009), and the anterior corpus callosum (p = 0.002). Figure 4 shows these structures with reduced brain volume highlighted with a 3-dimesional lobe parcellation. Hemoglobin, CaO_2 and presence of SCD alone were not associated with any focal deficits in brain volume. Structures with significant associations with CBF or lesion burden are shown in Figure 4.

Discussion

In a relatively well-characterized cohort of children and young adults with SCD treated at a single institution and excluding potential confounding comorbidities (e.g., overt stroke, regular blood transfusion therapy, vasculopathy), we found SCD to be associated with decreased WMV compared to race and sex matched controls (total N = 137). The measured brain volume deficits, however, were subtle: SCD was associated with a 16 mm^3 loss of WMV after controlling for age, sex, and ICV. The small magnitude of the volume (32) difference hints at the complexity of volumetric brain analysis of individuals with SCD and may explain the variable findings in prior studies of volumetric deficits related to SCD.

Interestingly, WMV losses associated with silent infarction in our study outpaced the measured infarct volume by a factor of 30; the WMV deficits seen are too large to be explained by loss in tissue volume at the site of infarction alone. This volume loss suggests that global white matter injury cannot be easily visualized with typical clinical imaging modalities may co-occur with silent infarction. One possible explanation supported by prior work is that white matter microstructural injury occurs prior to and possibly concurrently with the development of cerebral infarcts in SCD, which has been visualized with diffusion tensor imaging (33); our cohort does not have diffusion tensor data for evaluation. Furthermore, increased infarct burden is associated with focal volume loss in several subcortical structures including bilateral volume loss in the nucleus accumbens, a structure partly involved in learning, executive function, and impulse control (34). Increases in infarct burden were also associated with decreased thalamic volume, though the effect was only significant in the right thalamus. Given that there is no biological evidence otherwise to suggest why volumetric losses would be unilateral, we expect that the true effect is bilateral. Though SCI has been defined as infarction without focal neurological deficits, subcortical volume deficits

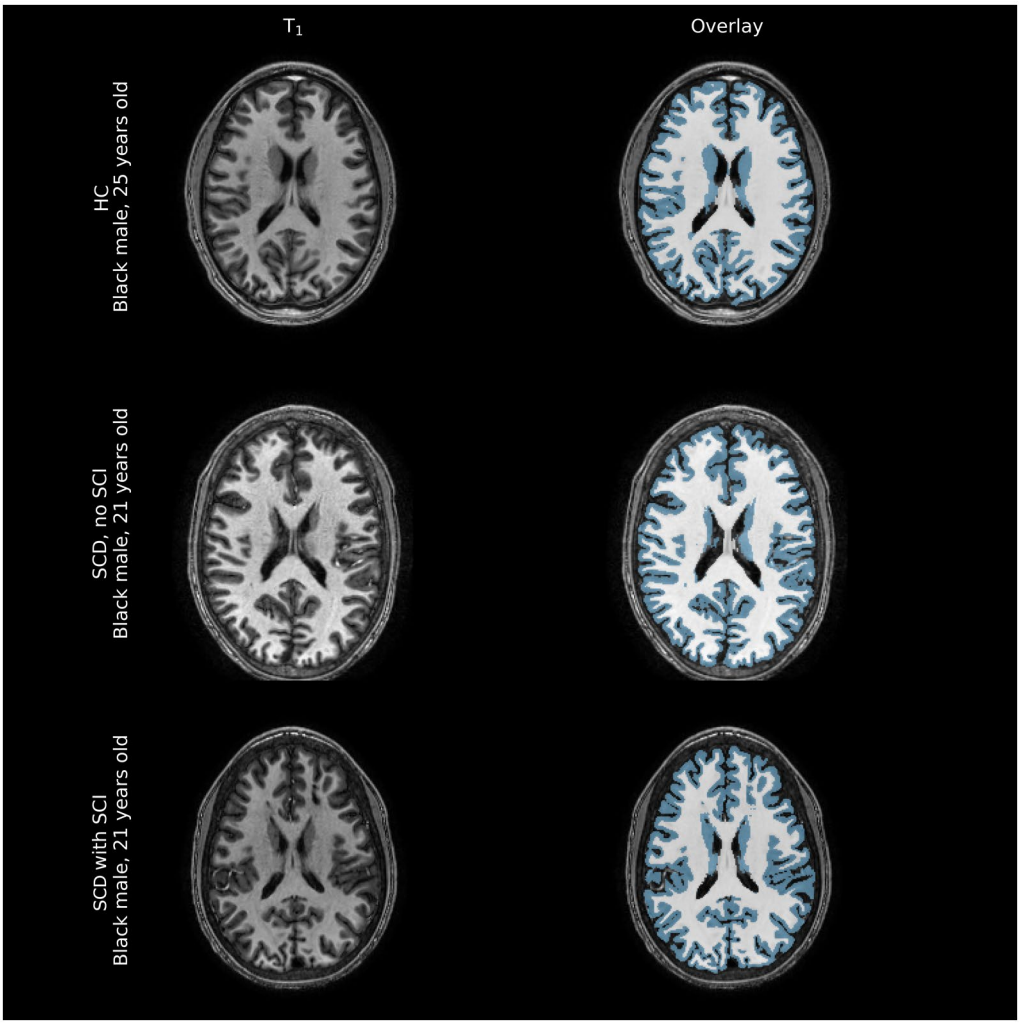


FIGURE 2
Representative comparison of structural images of participant brains along with FreeSurfer-derived gray and white matter segmentations (blue overlay and white overlay, respectively). Tissue volume differences between healthy controls (HC) and individuals with sickle cell disease (SCD) with and without silent cerebral infarcts (SCI) are often not immediately apparent on structural imaging modalities, barring large overt ischemic strokes that were excluded from this sample.

associated with SCI may partly explain why individuals with SCD and SCI have a high risk of executive and cognitive dysfunction (5, 15).

Reductions in hemoglobin and CaO_2 were both associated with deficits in GMV and WMV. These findings extend the work of a previous study that showed reductions in hemoglobin were associated with WMV deficits (15). However, neither hemoglobin nor CaO_2 were associated with focal changes in brain volume whereas GM CBF was associated with bilateral losses of volume in the caudate nuclei, structures important for stimulus control learning (35), as well as the insular cortex. Overall, brain volume losses were associated with elevated CBF, consistent with impaired cerebral hemodynamic compensation in SCD (10).

This work adds to the SCD literature, where prior studies have come to various conclusions about brain volumes in people with SCD. One previous study, using a semi-automated neural network to classify brain tissue, reported gray matter development was delayed in 83 children with SCD compared to 43 healthy controls (14), while another found decreased hemoglobin was associated with reduced WMV in a cohort of 52 adults and children with SCD, 26 with non-sickle cell anemia and 40 controls (15) when using BrainSuite's tensor-based morphometry.[2] Using FSL-FAST, a decrease in total brain volume in 34 adults with SCD compared to 11 controls was found (11); these participants were included in the cohort presented in this study. A clinical trial investigating monthly blood transfusions for the prevention of new silent infarcts in 196 children with SCD and SCI which utilized SIENA and paired MRIs at study entry and exit suggested that SCD results in whole-brain volume loss over time (16). These studies excluded patients with overt stroke but are complicated by variable MRI resolution (1.5 versus 3 T), heterogeneous, intermediate-sized cohorts and the complexities of SCI, vasculopathy, age, and different software, possibly accounting for the variability in conclusions regarding the relationship between SCD and brain volume. The breadth of methods and consistent

2 http://brainsuite.org/

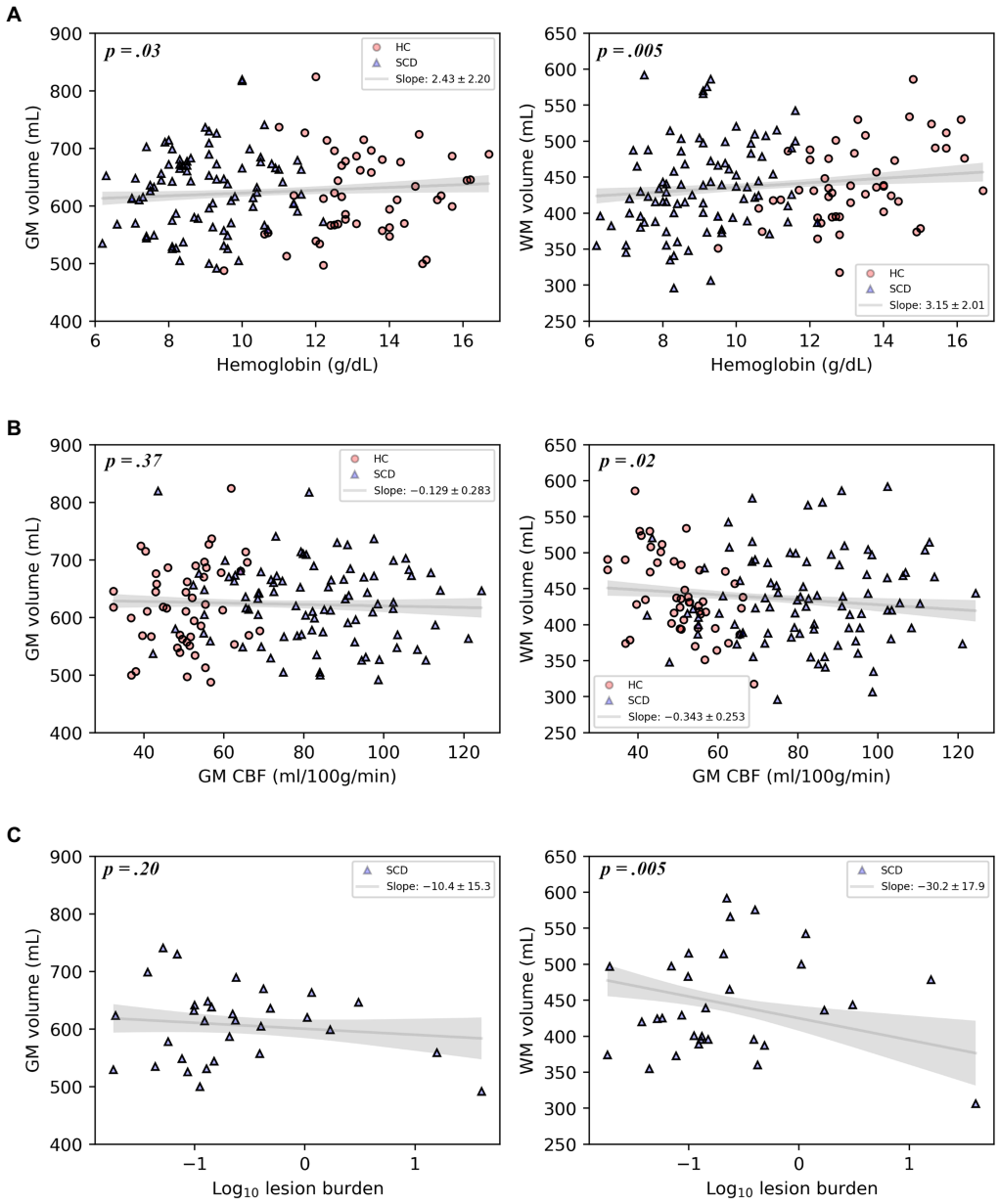


FIGURE 3
Plots of gray matter volume and white matter volume with hemoglobin, gray matter CBF, or log-transformed infarct (lesion) burden, displaying the multivariable regression model-predicted association between each covariate and volume, controlling for age, sex, and intracranial volume, with 95% confidence intervals (gray line and shaded area). Models include healthy controls and participants with SCD **(A,B)** or participants with SCD and silent cerebral infarcts only **(C)**. Overlaid on the model predictions are the raw data.

prior findings of reductions in some aspect of brain volume suggests a real effect, but the subtlety of the deficits highlights the relevance of functional indicators of hemodynamic compensation in SCD (6, 11, 36) and longitudinal follow up.

Our study differs from previous cross-sectional investigations of brain volume in SCD with reasonably large cohort that includes both children and adults and includes assessment of CBF in the analysis of brain volume. We excluded SCD participants undergoing regular blood transfusions because blood assays were collected at one time point and may not be representative of physiological steady state; hemoglobin and oxygen saturation levels peak after transfusion and then steadily decrease. Individuals receiving transfusions represent a more clinically symptomatic subset of individuals with SCD compared to those without, so we expect this group would exhibit the greatest brain volume deficits. Thus, our cohort is a healthier group of participants with SCD. Our findings may not be representative of deficits in cohorts with overt stroke, severe cerebral vasculopathy, and those receiving aggressive therapies, for whom we would expect even more severe brain volumetric deficits.

Study strengths include a larger sample size and race-matched healthy controls than evaluated in previous cross-sectional studies of brain volume in SCD with a larger range in ages, along with 3 Tesla high resolution MRI for all participants. This is also the first study to examine either CBF or a continuous measure of infarct burden volume

TABLE 2 Components of multivariable linear regression models for predicting gray and white matter volume (primary analysis), organized by independent variables of interest.

Independent variable	Coefficient	*p*	Dependent variable	Participant group
SCD	−10.4	0.10	GMV	HC + SCD ($n = 137$)
SCD	−15.6	0.008	WMV	HC + SCD ($n = 137$)
Hemoglobin	2.43	0.032	GMV	HC + SCD ($n = 137$)
Hemoglobin	3.15	0.005	WMV	HC + SCD ($n = 137$)
GM CBF	−0.129	0.37	GMV	HC + SCD ($n = 137$)
GM CBF	−0.343	0.018	WMV	HC + SCD ($n = 137$)
CaO_2	1.76	0.035	GMV	HC + SCD ($n = 137$)
CaO_2	2.27	0.006	WMV	HC + SCD ($n = 137$)
Infarct burden*	−10.4	0.19	GMV	SCD w/ SCI ($n = 31$)
Infarct burden*	−30.2	0.005	WMV	SCD w/ SCI ($n = 31$)

Each row represents a separate regression model. Corrections for multiple comparisons made using the Benjamini–Hochberg method, treating *p*-values from each independent variable as its own set for correction purposes. All regressions include age, sex and ICV as additional covariates. GM, gray matter; WM, white matter; CBF, cerebral blood flow, HC, healthy control. *Log_{10} of infarct burden.

TABLE 3 Components of multivariable linear regression models for predicting cortical and subcortical structure volumes, organized by independent variables of interest.

Independent variable	Coefficient	*p*	Dependent variable	Participant group
GM CBF	−0.010	0.017	Insula volume	HC + SCD ($n = 137$)
GM CBF	−0.0053	0.017	Left caudate volume	HC + SCD ($n = 137$)
GM CBF	−0.0053	0.036	Right caudate volume	HC + SCD ($n = 137$)
Infarct burden*	−0.11	0.002	Left nucleus accumbens volume	SCD w/ SCI ($n = 31$)
Infarct burden*	−0.085	0.002	Right nucleus accumbens volume	SCD w/ SCI ($n = 31$)
Infarct burden*	−0.44	0.009	Right thalamus volume	SCD w/ SCI ($n = 31$)
Infarct burden*	−0.098	0.002	Anterior corpus callosum volume	SCD w/ SCI ($n = 31$)

Each row represents a separate regression model. Due to space constraints, only significant models are shown. Corrections for multiple comparisons made using the Benjamini–Hochberg method, treating the *p*-values from each independent variable as its own set for correction purposes. All regressions include age, sex and ICV as additional covariates. GM, gray matter; WM, white matter; CBF, cerebral blood flow. *Log_{10} of infarct burden.

as a marker of brain volume in SCD. Limitations of our study include cross-sectional design, which poses issues for detecting subtle effects or the ordering of associations, but our cohort of 137 participants provides good sensitivity for a study of this design and our limited selection of covariates used in our models limits overfitting.

Brain volumes have been measured in SCD clinical trials and have been used clinically in other conditions such as Alzheimer's disease. The findings presented in this work suggest that common physiological consequences of SCD (reduced hemoglobin, reduced CaO_2, increased GM CBF, increased risk of silent infarction) and SCD itself result in both diffuse and focal brain tissue volume deficits. Still, these deficits are subtle, and so brain volume measurements at a single timepoint should be treated cautiously. Hemodynamic and metabolic changes, approximately 1.5-fold different in SCD vs. healthy race-matched controls, are stronger biomarkers of disease severity though they require more advanced neuroimaging techniques (9, 37). Additionally, the white matter volume deficits associated with silent infarction are more than 30 times larger than the infarct volume itself. A possible explanation is that a common mechanism controls infarction risk and volume loss, and that the presence of infarcts in SCD could be a visible marker of subtle diffuse brain injury. The consistent reduction in WMV is logical because despite elevated CBF, silent infarcts primarily occur in borderzone of cerebral blood flow which is predominantly subcortical white matter. Vaclavu et al. (37) also found CBF was higher in patients with SCD versus controls; after an acetazolamide challenge causing cerebral vasodilation and further elevating CBF, cerebral oxygen utilization seemed to worsen in SCD patients. Juttukonda et al. (38) and others (39) have proposed that elevated CBF may lead to capillary level shunting. This work suggests that elevated CBF may not be nourishing to the brain, indicating a mechanism other than cerebral infarction that may lead to brain volume loss.

While the current study is cross-sectional, further longitudinal investigation of the relationships between SCI, brain volume losses and cognitive impairment in SCD may enable the use of infarct burden and brain volume assessments as easily accessible and potentially automated prognostic markers of impairment that could then guide clinical care decisions. This is currently clinically relevant because in many settings, MRI of the brain will be completed as part of standard care for SCD at least once in childhood and once in early adulthood. To allow longitudinal assessment of brain volume from clinically available neuroimaging, we recommend that MRI of the brain be acquired at the same field strength, ideally 3 T, and with a 3D-T1 imaging sequence that will allow brain volume assessment over time. The associations of infarct burden and CBF with volumetric deficits in specific structures suggest potential as biomarkers of specific structure-related impairment, which future studies may elucidate.

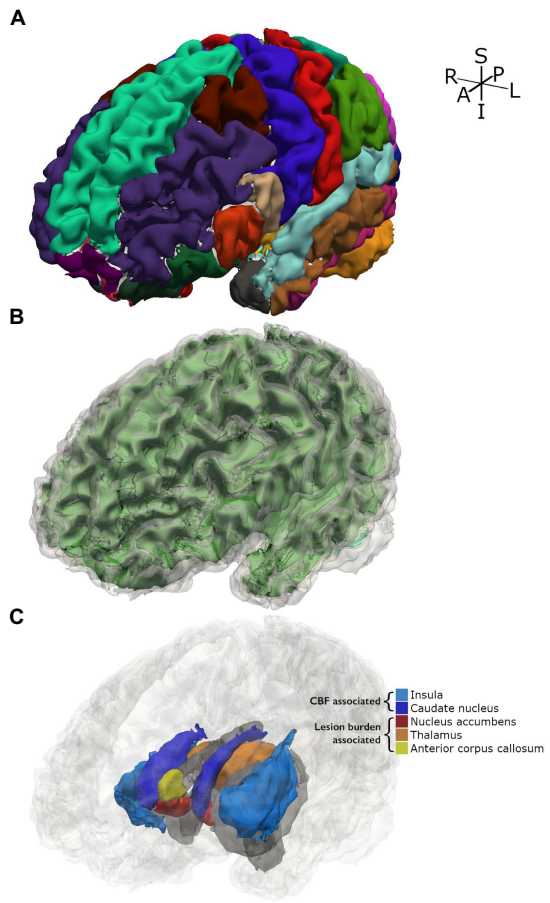


FIGURE 4
3D parcellation of a representative participant with SCD. **(A)** Shows FreeSurfer cortical parcellation. **(B)** Shows gray matter segmentation (translucent gray) over white matter segmentation (green). **(C)** Shows subcortical and cortical structures for which significant associations with tested independent variables were found. Other structures are displayed in gray as spatial references.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Vanderbilt University Medical Center Institutional Review Board. Written informed consent to participate in this study was provided by the participants' or for those less than 18 years of age, by legal guardian. Children also provided assent.

Author contributions

RJ: study conception and design, data acquisition, analysis and interpretation of data, and manuscript drafting and revision. SW: data acquisition, analysis and interpretation of data, and manuscript revision. LD, AK, SP, and MRD: interpretation of data and manuscript revision. MR: analysis and interpretation of data and manuscript revision. CC and NP: data acquisition and manuscript revision. MJD and LJ: study conception and design, analysis and interpretation of data, and manuscript revision. All authors contributed to the article and approved the submitted version.

Funding

The authors acknowledge receipt of the following financial support for the research, authorship, and/or publication of this article: the NIH (K24-HL147017, R01 NS096127, R01 NS097763, and UL1 TR000445) and the American Heart Association (AHA #14CSA20380466).

Conflict of interest

MJD is a paid consultant for Global Blood Therapeutics, receives advisory board receives research-related support from Philips North America, and is the CEO of Biosight LLC, which provides healthcare technology consulting services. These agreements have been approved by Vanderbilt University Medical Center in accordance with its conflict-of-interest policy. MR is an independent statistical consultant. He owns Rodeghier Consulting, Chicago, IL, United States. He was paid for his work on this manuscript via NIH grant funding. MRD and his institution are the sponsor of two externally funded research investigator-initiated projects. Global Blood Therapeutics will provide funding for the cost of these clinical studies but will not be a cosponsor of either study. MRD is not receiving any compensation for the conduct of these two-investigator initiated observational studies. MRD is a member of the Global Blood Therapeutics advisory board for a proposed randomized controlled trial for which he receives compensation. MRD is the steering committee for a Novartis-sponsored phase 2 trial to prevent priapism in men. MRD was a medical advisor for the development of the CTX001 Early Economic Model. MRD provided medical input on the economic model as part of an expert reference group for Vertex/CRISPR CTX001 Early Economic Model in 2020. MRD provided a onetime consultation to the Forma Pharmaceutical company about sickle cell disease in 2021.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2023.1112865/full#supplementary-material

References

1. Piel FB, Steinberg MH, Rees DC. Sickle cell disease. *N Engl J Med*. (2017) 376:1561–73. doi: 10.1056/NEJMra1510865

2. Kassim AA, Pruthi S, Day M, Rodeghier M, Gindville MC, Brodsky MA, et al. Silent cerebral infarcts and cerebral aneurysms are prevalent in adults with sickle cell anemia. *Blood*. (2016) 127:2038–40. doi: 10.1182/blood-2016-01-694562

3. Sacco RL, Kasner SE, Broderick JP, Caplan LR, Connors JJ, Culebras A, et al. An updated definition of stroke for the 21st century: a statement for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke*. (2013) 44:2064–89. doi: 10.1161/STR.0b013e318296aeca

4. DeBaun MR, Gordon M, McKinstry RC, Noetzel MJ, White DA, Sarnaik SA, et al. Controlled trial of transfusions for silent cerebral infarcts in sickle cell anemia. *N Engl J Med*. (2014) 371:699–710. doi: 10.1056/NEJMoa1401731

5. Prussien KV, Jordan LC, DeBaun MR, Compas BE. Cognitive function in sickle cell disease across domains, cerebral infarct status, and the lifespan: a meta-analysis. *J Pediatr Psychol*. (2019) 44:948–58. doi: 10.1093/jpepsy/jsz031

6. Ford AL, Ragan DK, Fellah S, Binkley MM, Fields ME, Guilliams KP, et al. Silent infarcts in sickle cell anemia occur in the borderzone region and are associated with low cerebral blood flow. *Blood*. (2018) 132:1714–23. doi: 10.1182/blood-2018-04-841247

7. DeBaun MR, Jordan LC, King AA, Schatz J, Vichinsky E, Fox CK, et al. American Society of Hematology 2020 guidelines for sickle cell disease: prevention, diagnosis, and treatment of cerebrovascular disease in children and adults. *Blood Adv*. (2020) 4:1554–88. doi: 10.1182/bloodadvances.2019001142

8. Fields ME, Guilliams KP, Ragan D, Binkley MM, Mirro A, Fellah S, et al. Hydroxyurea reduces cerebral metabolic stress in patients with sickle cell anemia. *Blood*. (2019) 133:2436–44. doi: 10.1182/blood-2018-09-876318

9. Fields ME, Guilliams KP, Ragan DK, Binkley MM, Eldeniz C, Chen Y, et al. Regional oxygen extraction predicts border zone vulnerability to stroke in sickle cell disease. *Neurology*. (2018) 90:e1134–42. doi: 10.1212/WNL.0000000000005194

10. Afzali-Hashemi L, Baas KPA, Schrantee A, Coolen BF, van Osch MJP, Spann SM, et al. Impairment of cerebrovascular hemodynamics in patients with severe and milder forms of sickle cell disease. *Front Physiol*. (2021) 12:645205. doi: 10.3389/fphys.2021.645205

11. Jordan LC, Gindville MC, Scott AO, Juttukonda MR, Strother MK, Kassim AA, et al. Non-invasive imaging of oxygen extraction fraction in adults with sickle cell anaemia. *Brain*. (2016) 139:738–50. doi: 10.1093/brain/awv397

12. Vaclavu L, Meynart BN, Mutsaerts H, Petersen ET, Majoie C, VanBavel ET, et al. Hemodynamic provocation with acetazolamide shows impaired cerebrovascular reserve in adults with sickle cell disease. *Haematologica*. (2019) 104:690–9. doi: 10.3324/haematol.2018.206094

13. Brewer JB, Magda S, Airriess C, Smith ME. Fully-automated quantification of regional brain volumes for improved detection of focal atrophy in Alzheimer disease. *AJNR Am J Neuroradiol*. (2009) 30:578–80. doi: 10.3174/ajnr.A1402

14. Steen RG, Emudianughe T, Hunte M, Glass J, Wu S, Xiong X, et al. Brain volume in pediatric patients with sickle cell disease: evidence of volumetric growth delay? *AJNR Am J Neuroradiol*. (2005) 26:455–62.

15. Choi S, O'Neil SH, Joshi AA, Li J, Bush AM, Coates TD, et al. Anemia predicts lower white matter volume and cognitive performance in sickle and non-sickle cell anemia syndrome. *Am J Hematol*. (2019) 94:1055–65. doi: 10.1002/ajh.25570

16. Darbari DS, Eigbire-Molen O, Ponisio MR, Milchenko MV, Rodeghier MJ, Casella JF, et al. Progressive loss of brain volume in children with sickle cell anemia and silent cerebral infarct: a report from the silent cerebral infarct transfusion trial. *Am J Hematol*. (2018) 93:E406–8. doi: 10.1002/ajh.25297

17. Strauss LD, Cavanaugh BA, Yun ES, Evans RW. Incidental findings and Normal anatomical variants on brain MRI in children for primary headaches. *Headache*. (2017) 57:1601–9. doi: 10.1111/head.13221

18. Mehrhof SZ, Popel N, Mio M, Lu W, Heyn CC, Fiksenbaum LM, et al. Prevalence of white matter hyperintensities is not elevated in a large sample of adolescents and young adults with bipolar disorder. *Braz J Psychiatry*. (2021) 43:147–52. doi: 10.1590/1516-4446-2020-0886

19. Johnson SE, McKnight CD, Jordan LC, Claassen DO, Waddle S, Lee C, et al. Choroid plexus perfusion in sickle cell disease and moyamoya vasculopathy: implications for glymphatic flow. *J Cereb Blood Flow Metab*. (2021) 41:2699–711. doi: 10.1177/0271678x211010731

20. Tange O. GNU parallel 20200722 ('privacy shield') (2020). Available at: https://org/10.5281

21. Juttukonda MR, Jordan LC, Gindville MC, Davis LT, Watchmaker JM, Pruthi S, et al. Cerebral hemodynamics and pseudo-continuous arterial spin labeling considerations in adults with sickle cell anemia. *NMR Biomed*. (2017) 30:e3681. doi: 10.1002/nbm.3681

22. Alsop DC, Detre JA, Golay X, Gunther M, Hendrikse J, Hernandez-Garcia L, et al. Recommended implementation of arterial spin-labeled perfusion MRI for clinical applications: a consensus of the ISMRM perfusion study group and the European consortium for ASL in dementia. *Magn Reson Med*. (2015) 73:102–16. doi: 10.1002/mrm.25197

23. Wang J, Alsop DC, Li L, Listerud J, Gonzalez-At JB, Schnall MD, et al. Comparison of quantitative perfusion imaging using arterial spin labeling at 1.5 and 4.0 tesla. *Magn Reson Med*. (2002) 48:242–54. doi: 10.1002/mrm.10211

24. Lu H, Nagae-Poetscher LM, Golay X, Lin D, Pomper M, van Zijl PC. Routine clinical brain MRI sequences for use at 3.0 tesla. *J Magn Reson Imaging*. (2005) 22:13–22. doi: 10.1002/jmri.20356

25. Donahue MJ, Lu H, Jones CK, Edden RA, Pekar JJ, van Zijl PC. Theoretical and experimental investigation of the VASO contrast mechanism. *Magn Reson Med*. (2006) 56:1261–73. doi: 10.1002/mrm.21072

26. Lu H, Clingman C, Golay X, van Zijl PC. Determining the longitudinal relaxation time (T1) of blood at 3.0 tesla. *Magn Reson Med*. (2004) 52:679–82. doi: 10.1002/mrm.20178

27. Václavů L, van der Land V, Heijtel DF, van Osch MJ, Cnossen MH, Majoie CB, et al. In vivo T1 of blood measurements in children with sickle cell disease improve cerebral blood flow quantification from arterial spin-labeling MRI. *AJNR Am J Neuroradiol*. (2016) 37:1727–32. doi: 10.3174/ajnr.A4793

28. Stotesbury H, Hales PW, Hood AM, Koelbel M, Kawadler JM, Saunders DE, et al. Individual watershed areas in sickle cell anemia: An arterial spin labeling study. *Front Physiol*. (2022) 13:865391. doi: 10.3389/fphys.2022.865391

29. Donahue MJ, Faraco CC, Strother MK, Chappell MA, Rane S, Dethrage LM, et al. Bolus arrival time and cerebral blood flow responses to hypercarbia. *J Cereb Blood Flow Metab*. (2014) 34:1243–52. doi: 10.1038/jcbfm.2014.81

30. Reuter M, Schmansky NJ, Rosas HD, Fischl B. Within-subject template estimation for unbiased longitudinal image analysis. *NeuroImage*. (2012) 61:1402–18. doi: 10.1016/j.neuroimage.2012.02.084

31. Seabold S., Perktold J., editors. Statsmodels: Econometric and statistical modeling with python. In Proceedings of the 9th python in science conference. (2010). Austin, TX.

32. Jacob M, Stotesbury H, Kawadler JM, Lapadaire W, Saunders DE, Sangeda RZ, et al. White matter integrity in Tanzanian children with sickle cell anemia: a diffusion tensor imaging study. *Stroke*. (2020) 51:1166–73. doi: 10.1161/STROKEAHA.119.027097

33. Wang Y, Fellah S, Fields ME, Guilliams KP, Binkley MM, Eldeniz C, et al. Cerebral oxygen metabolic stress, microstructural injury, and infarction in adults with sickle cell disease. *Neurology*. (2021) 97:e902–12. doi: 10.1212/wnl.0000000000012404

34. Basar K, Sesia T, Groenewegen H, Steinbusch HW, Visser-Vandewalle V, Temel Y. Nucleus accumbens and impulsivity. *Prog Neurobiol*. (2010) 92:533–57. doi: 10.1016/j.pneurobio.2010.08.007

35. Chiu YC, Jiang J, Egner T. The caudate nucleus mediates learning of stimulus-control state associations. *J Neurosci*. (2017) 37:1028–38. doi: 10.1523/jneurosci.0778-16.2016

36. Li W, Xu X, Liu P, Strouse JJ, Casella JF, Lu H, et al. Quantification of whole-brain oxygenation extraction fraction and cerebral metabolic rate of oxygen consumption in adults with sickle cell anemia using individual T (2) -based oxygenation calibrations. *Magn Reson Med*. (2020) 83:1066–80. doi: 10.1002/mrm.27972

37. Václavů L, Petr J, Petersen ET, Mutsaerts H, Majoie CBL, Wood JC, et al. Cerebral oxygen metabolism in adults with sickle cell disease. *Am J Hematol*. (2020) 95:401–12. doi: 10.1002/ajh.25727

38. Juttukonda MR, Donahue MJ, Waddle SL, Davis LT, Lee CA, Patel NJ, et al. Reduced oxygen extraction efficiency in sickle cell anemia patients with evidence of cerebral capillary shunting. *J Cereb Blood Flow Metab*. (2020) 41:546–60. doi: 10.1177/0271678X20913123

39. Bush AM, Coates TD, Wood JC. Diminished cerebral oxygen extraction and metabolic rate in sickle cell disease using T2 relaxation under spin tagging MRI. *Magn Reson Med*. (2018) 80:294–303. doi: 10.1002/mrm.27015

frontiers | Frontiers in Neurology

TYPE Opinion
PUBLISHED 24 May 2023
DOI 10.3389/fneur.2023.1104101

OPEN ACCESS

EDITED BY
Andria Ford,
Washington University in St. Louis, United States

REVIEWED BY
Fenella Jane Kirkham,
University College London, United Kingdom

*CORRESPONDENCE
Bruno Bonnechère
✉ bruno.bonnechere@uhasselt.be

RECEIVED 21 November 2022
ACCEPTED 02 May 2023
PUBLISHED 24 May 2023

CITATION
Boma PM, Panda J, Ngoy Mande JP and Bonnechère B (2023) Rehabilitation: a key service, yet highly underused, in the management of young patients with sickle cell disease after stroke in DR of Congo. *Front. Neurol.* 14:1104101. doi: 10.3389/fneur.2023.1104101

Rehabilitation: a key service, yet highly underused, in the management of young patients with sickle cell disease after stroke in DR of Congo

Paul Muteb Boma[1], Jules Panda[1,2], Jean Paul Ngoy Mande[3] and Bruno Bonnechère[4,5]*

[1]Reference Centre for Sickle Cell Disease of Lubumbashi, Institut de Recherche en Science de la Santé, Lubumbashi, Democratic Republic of Congo, [2]Department of Surgery, Faculty of Medicine, University of Lubumbashi, Lubumbashi, Democratic Republic of Congo, [3]Department of Neurology and Psychiatry, Faculty of Medicine, University of Lubumbashi, Lubumbashi, Democratic Republic of Congo, [4]REVAL Rehabilitation Research Center, Faculty of Rehabilitation Sciences, University of Hasselt, Hasselt, Belgium, [5]Technology-Supported and Data-Driven Rehabilitation, Data Science Institute, University of Hasselt, Hasselt, Belgium

KEYWORDS

sickle cell disease, stroke, rehabilitation, management, prevention stroke rehabilitation in sickle cell disease

Context

Sickle cell disease (SCD) is an inherited disorder that affects red blood cells, causing them to take on a sickle-like shape. This condition can lead to cerebrovascular accidents (CVA) in children, the incidence of which is an estimated 220 to 300 times higher in affected children than in healthy children of the same age. Studies have estimated the prevalence of stroke in this condition to be between 3.7 and 7% (1–3).

Recurrent cerebral infarction can occur in two thirds of SCD patients, usually within 2–3 years after the first stroke (2). Microvascular damage and impaired vasoreactivity can lead to silent infarcts, which are associated with impaired cognitive development (4). A focal neurological deficit that lasts more than 24 h in an SCD child or adolescent is defined as stroke.

It is recommended to screen with transcranial Doppler (TCD) all SCD children from the age of 12-18 months, with further yearly evaluations to identify cerebral vasculopathy before the onset of cerebrovascular accidents (5). Without screening and prophylactic treatment, between 5 and 17% of SCD patients develop a first stroke during childhood or adolescence (6). In 98% of cases, children with SCD survive their first episode of stroke with complete motor recovery, although cognitive sequelae are common. Inadequate management exposes to recurrence of stroke after 12 to 24 months in 67% of patients (3). Chronic transfusions, ideally in partial exchange transfusion mode, reduces this risk to 10% and it has been recently demonstrated that even low-dose hydroxyurea (10 mg/kg/day), cuts the risk by two, demonstrating that these interventions are efficacious for secondary stroke prevention in low-resource settings (7). However, due to its cost and the relatively low acceptance of chronic blood transfusions, these treatments are only used by a minority of patients. A study conducted in Nigeria found that only 10% of caregivers of children who had an indication for chronic blood transfusion for stroke prevention consented to the treatment (8), to the authors' best knowledge there is not such data in the Democratic Republic of Congo (DRC), but according to authors' experience the most important limiting factor is financial.

Current situation

With an estimated 39,700 SCD births per year, the DRC is the third-most affected country in the world after Nigeria and India (9). The clinical manifestations of SCD are highly variable, as they are influenced by a combination of environmental and genetic factors such as the coexistence of α-triplication and α-homozygous deletion, which leads to less severe forms of SCD (10).

Currently, the only statistics available suggest a prevalence of stroke of 2.7% observed in SCD patients in the south of the country (including Lubumbashi) (11). Despite this, the overall management of SCD remains inadequate, and access to necessary treatments such as hydroxyurea and appropriate blood transfusions is limited due to the precarious socioeconomic status, the very limited availability of hydroxyurea in pharmacies (12), and the scarcity of appropriate blood products (13). Patients with sickle cell disease who have a history of childhood stroke are at an increased risk of global cognitive deficits. Despite the high prevalence of these cognitive disorders documented by several studies, there is no standardized approach to their screening and there is still, currently, a lack of data on rehabilitation for stroke and cognitive difficulties in people with sickle cell disease across the world, including even in high income countries (14, 15).

Therefore, it is crucial to develop effective rehabilitation strategies to minimize the negative impact of stroke on the functions and quality of life of SCD patients. Such strategies should be combined with primary and secondary stroke prevention measures to reduce the overall burden of neurological complications of sickle cell disease, especially in high-prevalence countries such as India and African countries, including DRC (16).

A lack of resources

Stroke is a major cause of disability that can have a significant impact on people's lives (17, 18). Effective rehabilitation strategies are critical for stroke survivors, as they help to reduce disability, improve quality of life, and support recovery (19). The World Health Organization (WHO) emphasizes the importance of putting health promotion, prevention, and rehabilitation methods in place, as well as strengthening health information systems, evidence, and research (20). Access to high-quality health care, including rehabilitation facilities, has thus been designated as one of the Sustainable Development Goals' pillars (SDGs goal 3) (21). Rehabilitation can be provided in a variety of settings, including inpatient or outpatient hospital settings, private clinics, and community settings such as a person's home. Rehabilitation professionals include physiotherapists, occupational therapists, speech and language therapists and audiologists, orthotists and prosthetists, clinical psychologists, physical medicine and rehabilitation physicians, and rehabilitation nurses, among others (22).

Unfortunately, access to high-quality healthcare and rehabilitation facilities is not always available, particularly in certain parts of the world. For example, in the DRC, there is a ratio of only 1.3 physiotherapists per 100,000 population (23). This means that stroke rehabilitation is often delayed, inadequate, and irregular, resulting in poorer outcomes for stroke survivors. Although it is challenging to create evidence-based guidelines for managing SCD (24), a panel of experts made strong recommendations for chronic complications including use of analgesics and physical therapy for treatment of avascular necrosis (25). Evidence currently suggests the need and positive impact of both motor and cognitive rehabilitation for SCD patients after CVA (26–29). Improving access to healthcare and rehabilitation facilities is therefore essential for ensuring stroke survivors receive the best possible treatment and outcomes. Additionally, healthcare personnel should be educated on the latest (neuro)rehabilitation techniques, so that patients receive the most suitable and effective treatments (30).

The modified Rankin Scale for neurologic disability (mRS) is a tool that has been developed to measures the degree of disability or dependence in the daily activities of people who have suffered a stroke or other causes of neurological disability (31). The mRS is a generic ordinal clinician-rated tool that categorizes the severity and the overall level of disability. This scale rates individuals on 7 levels, ranging from 0 ("no symptom at all") to 6 ("death") (32). It is commonly used to describe all dimensions of recovery and disability after acute stroke (33).

Observations

We conducted a retrospective analysis of the 893 children and adolescents' patients followed at the Sickle Cell Reference Center of Lubumbashi, a medical unit of the Institute of Research in Health Sciences. We collected from the medical records of patients who presented with stroke, the age at the onset of the accident, the sex, the side of hemiplegia, secondary prevention strategy, the delay in rehabilitation after the stroke, the regularity and duration of rehabilitation as well as the mRS assessed 1 year after the stroke during the annual check-up. We performed a linear multivariate regression to try to explain the mRS with the time from stroke to rehabilitation, the duration of the rehabilitation and its frequency adjusted for age.

A prevalence of 3.6% of stroke (32 out of 890 patients) was observed in our series, with a predominance of male patients (n = 21, 66%). These clinical manifestations are illustrated in Table and Figures 1A, B.

Out of the 32 patients who developed a stroke, 25 (78%) have had at least one session of rehabilitation. However, there are two issues concerning the access to these services: most of the patients (14 out of the 25) did not have regularly scheduled sessions, and most of the patients only obtained access to the rehabilitation services long after the stroke (median = 16 days [IQR = 32.5], see Figure 1C). The median duration of rehabilitation was 10.5 months [IQR = 15]. After the initial stroke, 21 out of 32 patients (66%) were regularly treated with hydroxyurea as a secondary preventive measure, which proved to be beneficial. Additionally, among those receiving regular hydroxyurea treatment, only 9 patients (28%) were able to access a long-term transfusion exchange program. The results of the regression show a significant effect of the time from stroke to rehabilitation ($\beta = 0.028$, SE = 0.006, $p < 0.001$) and the frequency ($\beta = -1.24$, SE = 0.36, $p = 0.002$) on the mRS, with a trend for an effect of the duration of the intervention ($\beta = 0.031$, SE = 0.016, $p = 0.073$). These three components explained

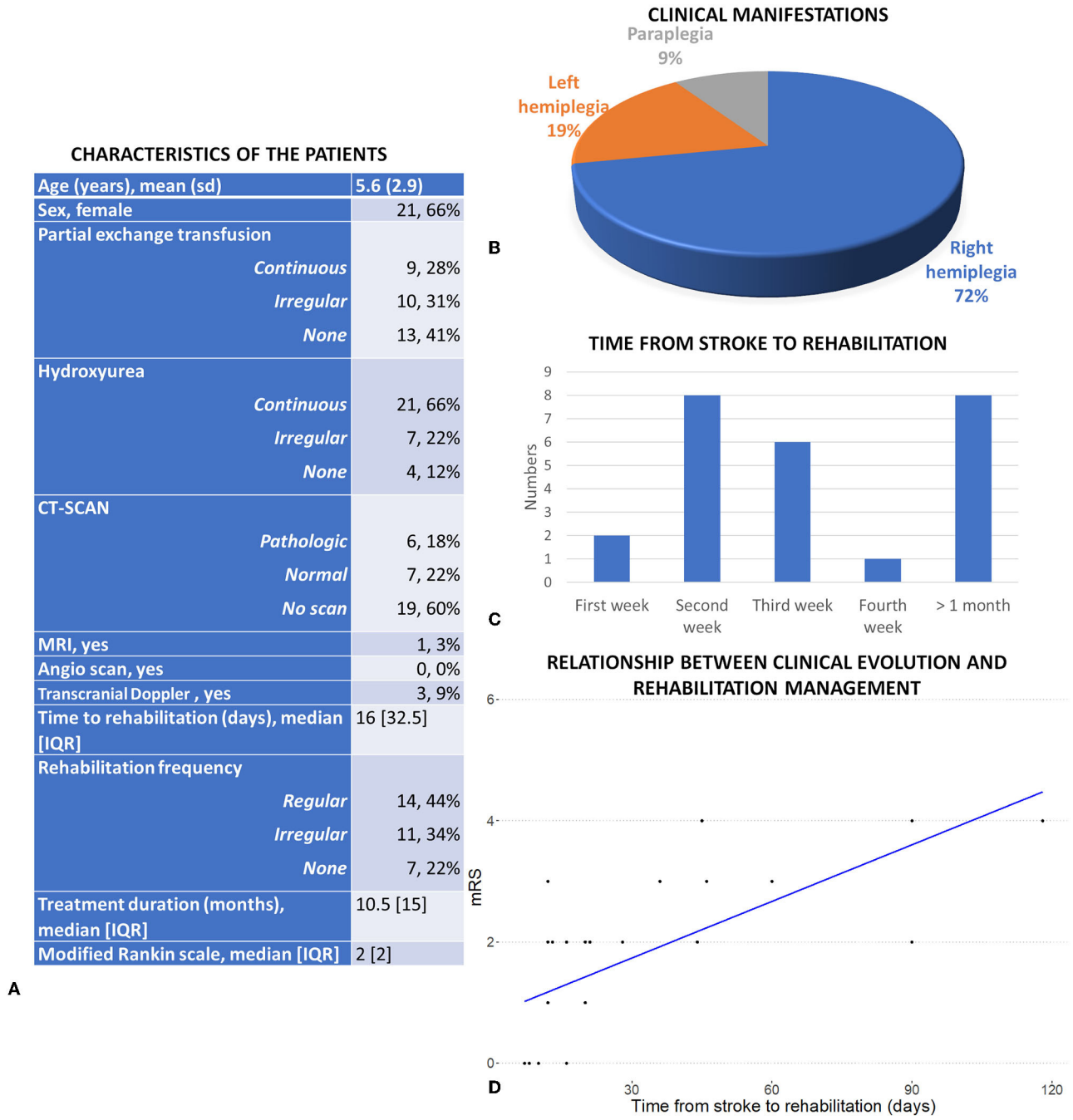


FIGURE 1
(A) Clinical characteristics of the patients and complementary examination. **(B)** Clinical manifestation of the 32 included patients. **(C)** Repartition of the average time from stroke to treatment. **(D)** Relationship between the clinical evolution and the time from stroke to rehabilitation services (after adjustment for frequency, duration of the rehabilitation and age).

73% of the variance in the mRS, highlighting the importance of the rehabilitation service to predict mRS and of an early intervention (Figure 1D).

Problems to solve

Two major problems stand in the way of improving the rehabilitation of children with SCD suffering from stroke in Lubumbashi. The first point, according to the WHO, relates to the lack of access to rehabilitation services, such as healthcare professionals, infrastructures, and funding (22). The second issue is determining the best rehabilitation strategies adapted to the sequelae present, as the current research in stroke rehabilitation are mainly done with adult populations in high-income countries (34). Addressing these challenges should be an essential part of stroke prevention, which should be established alongside neurorehabilitation programs.

Potential solutions

Current modalities of rehabilitation include both supervised and unsupervised exercises, but advances in technology are opening new horizons in this field (35, 36). Potential solutions to increase both the quantity and the quality of the rehabilitation include the use of mobile technologies and virtual reality (VR) (37). These solutions broaden the potential of the healthcare sector, at least in high-income nations (38), and can be a low-cost alternative to current rehabilitation modalities, as they do not require immediate access to healthcare clinics or personnel (39). To develop and implement this type of innovative solution, two important points must be addressed: the education and training of healthcare professionals, and the development of local research to develop guidelines adapted to the regional situation (40).

At the WHO level, a rehabilitation competency framework has been developed to provide foundations for curricula for rehabilitation specialists (19). It is important to include new technology tools related to rehabilitation in this framework, as part of the current and future training programs.

In terms of research, it is necessary to look into the acceptance of new technologies as part of medical diagnosis or intervention, as a large portion of the population in low- and middle-income countries still rely on traditional medicine (41). Additionally, raising health literacy levels of patients and the population is important before testing these solutions on a large scale. It is also key to involve the family and community in the management to increase the chances of success, as demonstrated by the community-based rehabilitation approach (42). Regarding the essential preventive method, TCD has emerged as the most effective screening tool for identifying stroke risk in children. This has enabled primary prevention measures, such as blood transfusion and hydroxyurea treatment, to be implemented with success. We believe that hydroxyurea is the most feasible solution in LMICs, especially since its efficacy in other indications shows promising results in improving sickle cell disease morbidity (43). Prescribing it in early childhood can therefore be beneficial for these patients to reduce the risk of stroke.

Conclusion and call to action

Incorporating VR solutions into rehabilitation services in LMICs is a highly innovative and ambitious project, fraught with potential dangers (44). The challenge is not only to implement VR in rehabilitation, but to raise awareness of the benefits of rehabilitation among healthcare professionals and the population (45). It is clear that technology can play an important role in providing improved healthcare to LMICs. To ensure the successful implementation of (technology-supported) rehabilitation in daily practice, strong local infrastructures need to be developed using North-South consortiums to facilitate inter and multidisciplinary collaborations. Considering the high prevalence of cognitive impairments in both silent cerebral infarcts-associated and non-associated children with SCD, it is reasonable to explore the potential benefits of cognitive rehabilitation interventions for all children with SCD. With the extensive use of mobile phones in the DRC (37), it is possible to conduct high-quality research in this field that could prove cost-effective in the long run without the need for MRI screening. Additionally, this could be a valuable contribution to the field, as a recent systematic review has highlighted the scarcity of research on this topic in developing countries (46). It is interesting to note that randomized controlled trials have conducted on cognitive rehabilitation in children exposed to cerebral malaria (47) and HIV (48), but there is limited data available on SCD. Therefore, it is worth exploring the evidence base for cognitive rehabilitation interventions in children with SCD, with and without cerebral infarction, to potentially benefit children with this condition (49).

In the particular context of SCD, it is important to develop local scientific research capacity and generate evidence supporting the use of rehabilitation, as the vast majority of the patients are in LMICs and therefore there is not enough research performed in HIC due to the lack of patients (and better prevention and control) (50).

Additionally, the development of new affordable and portable technology and solutions can enable continuous patient monitoring and follow-up (51, 52), leading to more individualized rehabilitation (53) and could allow the validation of new interventions or technologies (54). People-centered care is essential for this, as it involves actively involving the patient in the care, providing them with the knowledge and support they need to make decisions and engage in their own care, and ensuring that caregivers are performing optimally within a supportive working environment (55).

To ensure successful integration of these interventions into the healthcare system, a multi-level approach must be taken, with attention to the legal, regulatory, and economic aspects at the macro-level, local health service and community at the meso-level, and the needs of the patient at the micro-level (56). Challenges include providing access to hardware, mobile connection, and education for healthcare practitioners and patients (57), but also the rapid pace of technological change, where hardware and software may quickly become obsolete (58). Additionally, it is essential to consider the financial burden on families who may not be able to afford extended stays in rehabilitation centers. In light of these challenges, it may be worth considering the feasibility of offering rehabilitation services in the home environment. For instance, in the DRC, where mobile phone and tablet use is widespread, utilizing the family's existing technology for cognitive rehabilitation could be a viable option. Such an approach could potentially provide more cost-effective and accessible care to those who require rehabilitation services. However, it is crucial to acknowledge that this approach has its limitations and should be carefully evaluated to ensure that it aligns with the specific needs and context of the population being served.

In regions with a high prevalence of sickle cell disease and stroke, access to rehabilitation for children remains extremely limited. Although the figures presented here are from the southern part of DR Congo, the conclusions can be generalized to other endemic countries like Nigeria and India. It is crucial to prioritize the development of rehabilitation services in these countries for both individual and public health. Furthermore, early detection of cerebral vasculopathy using TCD and MRI allows for the

identification of higher-risk patients and implementation of preventive management, such as establishing a chronic transfusion program or prescribing hydroxyurea treatment to minimize the risk of stroke.

VR offers the possibility of functional rehabilitation and stimulation of cognition, while promoting the performance of exercises at home with a better compliance rate. The DRC faces a significant digital divide that exacerbates disparities within and between urban and rural areas, as well as across different provinces (59). Despite this, there has been a noticeable surge in the adoption of affordable mobile phones and digital touch pads connected to the internet in larger cities like Lubumbashi. This trend offers a promising solution for families who cannot afford long-term care in rehabilitation centers, as these devices provide a viable alternative for at-home exercise programs. To improve global health, reduce the burden of CVA, and address the shortage of rehabilitation professionals, new technology must be used. As effective and accessible rehabilitation therapies are lacking in many LMICs, these VR applications could prove to be extremely beneficial in the context of improving the supply of health care, also taking into account access to the necessary disease-modifying treatments, in particular hydroxyurea, which appears to be the easiest to implement.

Author contributions

PB, JP, and JN carried out the data collection. PB, JP, JN, and BB contributed to the analysis of the results and to the writing of the manuscript. All authors read and approved the final manuscript.

Funding

This work was supported by the VLIR-UOS through the Short Initiative Program (328-SI2022 - 77969).

Acknowledgments

The authors would like to thank Dr. Patience Muhau Pfutila for his precious help in selecting patient files and collecting data.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. Corvest V, Blais S, Dahmani B, De Tersant M, Etienney A-C, Maroni A, et al. [Cerebral vasculopathy in children with sickle cell disease: Key issues and the latest data]. *Arch Pediatr Organe Off Soc Francaise Pediatr.* (2018) 25:63–71. doi: 10.1016/j.arcped.2017.11.015

2. Ohene-Frempong K, Weiner SJ, Sleeper LA, Miller ST, Embury S, Moohr JW, et al. Cerebrovascular accidents in sickle cell disease: rates and risk factors. *Blood.* (1998) 91:288–94.

3. Njamnshi AK, Mbong EN, Wonkam A, Ongolo-Zogo P, Djientcheu V. P, et al. The epidemiology of stroke in sickle cell patients in Yaounde, Cameroon. *J Neurol Sci.* (2006) 250:79–84. doi: 10.1016/j.jns.2006.07.003

4. Kossorotoff M, Grevent D, de Montalembert M. Drépanocytose et atteinte vasculaire cérébrale chez l'enfant. *Arch Pédiatrie.* (2014) 21:404–14. doi: 10.1016/j.arcped.2014.01.005

5. Verlhac S, Bernaudin F, Brugières P. Transcranial color Doppler in children with sickle cell disease. *J Radiol.* (2003) 84:131–8.

6. Kirkham FJ, Lagunju IA. Epidemiology of stroke in sickle cell disease. *J Clin Med.* (2021) 10:4232. doi: 10.3390/jcm10184232

7. Abdullahi S, Sunusi SM, Abba MS, Sani S, Inuwa HA, Gambo S, et al. Hydroxyurea for secondary stroke prevention in children with sickle cell anemia in nigeria: a randomized controlled trial. *Blood.* (2022) 141:825–34. doi: 10.1182/blood.2022016620

8. Lagunju IA, Brown BJ, Sodeinde OO. Chronic blood transfusion for primary and secondary stroke prevention in Nigerian children with sickle cell disease: A 5-year appraisal: Chronic Blood Transfusion in Sickle Cell Disease. *Pediatr Blood Cancer.* (2013) 60:1940–5. doi: 10.1002/pbc.24698

9. Piel FB, Hay SI, Gupta S, Weatherall DJ, Williams TN. Global burden of sickle cell anaemia in children under Five, 2010–2050: modelling based on demographics, excess mortality, and interventions. *PLoS Med.* (2013) 10:e1001484. doi: 10.1371/journal.pmed.1001484

10. Mikobi TM, Lukusa Tshilobo P, Aloni MN, Akilimali PZ, Mvumbi-Lelo G, Mbuyi-Muamba JM. Clinical phenotypes and the biological parameters of Congolese patients suffering from sickle cell anemia: a first report from Central Africa. *J Clin Lab Anal.* (2017) 31:e22140. doi: 10.1002/jcla.22140

11. Tshilolo L, Mukendi R, Girot R. La drépanocytose dans le sud du Zaïre. Étude de deux séries de 251 et 340 malades suivis entre 1988 et 1992. *Arch Pédiatrie.* (1996) 3:104–111. doi: 10.1016/0929-693X(96)85059-7

12. Mukinayi BM, Cibeyibeyi GK, Tumba GD, Gulbis B. Drépanocytose en République Démocratique du Congo: quels sont les obstacles à un traitement par hydroxyurée? *Pan Afr Med J.* (2021) 38:41. doi: 10.11604/pamj.2021.38.41.18718

13. Boma Muteb P, Kaluila Mamba JFJ, Muhau Pfutila P, Bilo V, Panda Mulefu JD, Diallo DA. Effectiveness, safety, and cost of partial exchange transfusions in patients with sickle-cell anemia at a sickle cell disease center in sub-Saharan Africa. *Med Sante Trop.* (2017) 27:387–91. doi: 10.1684/mst.2017.0719

14. Couette M, Forté S, Oudin Doglioni D, Mekontso-Dessap A, Calvet D, Kuo KHM, et al. Early strokes are associated with more global cognitive deficits in adults with sickle cell disease. *J Clin Med.* (2023) 12:1615. doi: 10.3390/jcm12041615

15. Forté S, Blais F, Castonguay M, Fadiga N, Fortier-St-Pierre M, Couette M, et al. Screening for cognitive dysfunction using the rowland universal dementia assessment scale in adults with sickle cell disease. *JAMA Netw Open.* (2021) 4:e217039. doi: 10.1001/jamanetworkopen.2021.7039

16. Davies L, Delcourt C. Current approach to acute stroke management. *Intern Med J.* (2021) 51:481–7. doi: 10.1111/imj.15273

17. Coleman ER, Moudgal R, Lang K, Hyacinth HI, Awosika OO, Kissela BM, et al. Early rehabilitation after stroke: a narrative review. *Curr Atheroscler Rep.* (2017) 19:59. doi: 10.1007/s11883-017-0686-6

18. Stinear CM, Lang CE, Zeiler S, Byblow WD. Advances and challenges in stroke rehabilitation. *Lancet Neurol.* (2020) 19:348–60. doi: 10.1016/S1474-4422(19)30415-6

19. Mills J-A, Cieza A, Short SD, Middleton JW. Development and validation of the WHO rehabilitation competency framework: a mixed methods study. *Arch Phys Med Rehabil.* (2021) 102:1113–23. doi: 10.1016/j.apmr.2020.10.129

20. Stucki G, Bickenbach J, Gutenbrunner C, Melvin J. Rehabilitation: the health strategy of the 21st century. *J Rehabil Med.* (2018) 50:309–16. doi: 10.2340/16501977-2200

21. United Nation. *Take Action for the Sustainable Development Goals*. Available online at: https://www.un.org/sustainabledevelopment/sustainable-development-goals/ (accessed February 4, 2023).

22. WHO. *Fact sheets: Rehabilitation*. (2020). Available online at: https://www.who.int/news-room/fact-sheets/detail/rehabilitation (accessed April 2, 2023).

23. World Physiotherapy. *Congo Physical Therapists Union*. Available online at: https://world.physio/membership/congo (accessed July 11, 2022).

24. DeBaun MR. The challenge of creating an evidence-based guideline for sickle cell disease. *JAMA.* (2014) 312:1004–5. doi: 10.1001/jama.2014.11103

25. Yawn BP, Buchanan GR, Afenyi-Annan AN, Ballas SK, Hassell KL, James AH, et al. Management of sickle cell disease: summary of the 2014 evidence-based report by expert panel members. *JAMA.* (2014) 312:1033–48. doi: 10.1001/jama.2014.10517

26. King AA, DeBaun MR, White DA. Need for cognitive rehabilitation for children with sickle cell disease and strokes. *Expert Rev Neurother.* (2008) 8:291–6. doi: 10.1586/14737175.8.2.291

27. Seidman C, Kirkham F, Pavlakis S. Pediatric stroke: current developments. *Curr Opin Pediatr.* (2007) 19:657–62. doi: 10.1097/MOP.0b013e3282f1883b

28. King AA, White DA, McKinstry RC, Noetzel M, Debaun MR, A. pilot randomized education rehabilitation trial is feasible in sickle cell and strokes. *Neurology.* (2007) 68:2008–11. doi: 10.1212/01.wnl.0000264421.24415.16

29. Yerys BE, White DA, Salorio CF, McKinstry R, Moinuddin A, DeBaun M. Memory strategy training in children with cerebral infarcts related to sickle cell disease. *J Pediatr Hematol Oncol.* (2003) 25:495–8. doi: 10.1097/00043426-200306000-00014

30. Ontario Health (Quality). Continual long-term physiotherapy after stroke: a health technology assessment. *Ont Health Technol Assess Ser.* (2020) 20:1–70.

31. Banks JL, Marotta CA. Outcomes validity and reliability of the modified Rankin scale: implications for stroke clinical trials: a literature review and synthesis. *Stroke.* (2007) 38:1091–6. doi: 10.1161/01.STR.0000258355.23810.c6

32. Broderick JP, Adeoye O, Elm J. Evolution of the modified rankin scale and its use in future stroke trials. *Stroke.* (2017) 48:2007–12. doi: 10.1161/STROKEAHA.117.017866

33. Kasner SE. Clinical interpretation and use of stroke scales. *Lancet Neurol.* (2006) 5:603–12. doi: 10.1016/S1474-4422(06)70495-1

34. Jolliffe L, Lannin NA, Cadilhac DA, Hoffmann T. Systematic review of clinical practice guidelines to identify recommendations for rehabilitation after stroke and other acquired brain injuries. *BMJ Open.* (2018) 8:e018791. doi: 10.1136/bmjopen-2017-018791

35. Berton A, Longo UG, Candela V, Fioravanti S, Giannone L, Arcangeli V, et al. Virtual reality, augmented reality, gamification, and telerehabilitation: psychological impact on orthopedic patients' rehabilitation. *J Clin Med.* (2020) 9:E2567. doi: 10.3390/jcm9082567

36. Bonnechère B, Jansen B, Van Sint Jan S. Cost-effective (gaming) motion and balance devices for functional assessment: Need or hype? *J Biomech.* (2016) 49:2561–5. doi: 10.1016/j.jbiomech.2016.07.011

37. Bonnechère B, Sahakian BJ. Can mobile technology help prevent the burden of dementia in low- and mid-income countries? *Front Public Health.* (2020) 8:554938. doi: 10.3389/fpubh.2020.554938

38. Rutkowski S, Kiper P, Cacciante L, Cieślik B, Mazurek J, Turolla A, Szczepańska-Gieracha J. Use of virtual reality-based training in different fields of rehabilitation: a systematic review and meta-analysis. *J Rehabil Med.* (2020) 52:jrm00121. doi: 10.2340/16501977-2755

39. Krug E, Cieza A. Strengthening health systems to provide rehabilitation services. *Bull World Health Organ.* (2017) 95:167–167. doi: 10.2471/BLT.17.191809

40. Bonnechère B, Kossi O, Adoukonou T, Coninx K, Spooren A, Feys P. Improving public health through the development of local scientific capacity and training in rehabilitation in LMICs: A proof-of-concept of collaborative efforts in Parakou, Benin. *Front Public Health.* (2022) 10:952727. doi: 10.3389/fpubh.2022.952727

41. Asuzu CC, Akin-Odanye EO, Asuzu MC, Holland J. A socio-cultural study of traditional healers role in African health care. *Infect Agent Cancer.* (2019) 14:15. doi: 10.1186/s13027-019-0232-y

42. Zeng X, Balikuddembe JK, Liang P. Impact of community-based rehabilitation on the physical functioning and activity of daily living of stroke patients: a systematic review and meta-analysis. *Disabil Rehabil.* (2022) 45:403–414. doi: 10.1080/09638288.2022.2037755

43. Abdullahi SU, Jibir BW, Bello-Manga H, Gambo S, Inuwa H, Tijjani AG, et al. Hydroxyurea for primary stroke prevention in children with sickle cell anaemia in Nigeria (SPRING): a double-blind, multicentre, randomized, phase 3 trial. *Lancet Haematol.* (2022) 9:e26–37. doi: 10.1016/S2352-3026(21)00368-9

44. Bonnechère B, Kossi O, Mapinduzi J, Panda J, Rintala A, Guidetti S, et al. Mobile health solutions: An opportunity for rehabilitation in low- and middle income countries? *Front Public Health.* (2022) 10:1072322. doi: 10.3389/fpubh.2022.1072322

45. Bernhardt J, Urimubenshi G, Gandhi DBC, Eng JJ. Stroke rehabilitation in low-income and middle-income countries: a call to action. *Lancet Lond Engl.* (2020) 396:1452–62. doi: 10.1016/S0140-6736(20)31313-1

46. Hoyt CR, Hurwitz S, Varughese TE, Yaeger LH, King AA. Individual-level behavioral interventions to support optimal development of children with sickle cell disease: a systematic review. *Pediatr Blood Cancer.* (2023) 70:e30178. doi: 10.1002/pbc.30178

47. Boivin MJ, Nakasujja N, Sikorskii A, Ruiseñor-Escudero H, Familiar-Lopez I, Walhof K, et al. Neuropsychological benefits of computerized cognitive rehabilitation training in Ugandan children surviving severe malaria: a randomized controlled trial. *Brain Res Bull.* (2019) 145:117–28. doi: 10.1016/j.brainresbull.2018.03.002

48. Boivin MJ, Nakasujja N, Sikorskii A, Opoka RO, Giordani B. A randomized controlled trial to evaluate if computerized cognitive rehabilitation improves neurocognition in ugandan children with HIV. *AIDS Res Hum Retroviruses.* (2016) 32:743–55. doi: 10.1089/aid.2016.0026

49. Boivin MJ, Giordani B. Neuropsychological assessment of African children: evidence for a universal brain/behavior omnibus within a coconstructivist paradigm. *Prog Brain Res.* (2009) 178:113–35. doi: 10.1016/S0079-6123(09)17808-1

50. Idris IM, Botchwey EA, Hyacinth HI. Sickle cell disease as an accelerated aging syndrome. *Exp Biol Med Maywood NJ.* (2022) 247:368–74. doi: 10.1177/15353702211068522

51. Bonnechère B. Evaluation of processing speed of different cognitive functions across the life span using cognitive mobile games. *Games Health J.* (2022) 11:132–40. doi: 10.1089/g4h.2021.0144

52. Bonnechère B, Sholukha V, Omelina L, Van Vooren M, Jansen B, Van Sint Jan S. Suitability of functional evaluation embedded in serious game rehabilitation exercises to assess motor development across lifespan. *Gait Posture.* (2017) 57:35–9. doi: 10.1016/j.gaitpost.2017.05.025

53. Termoz A, Delvallée M, Damiolini E, Marchal M, Preau M, Huchon L, et al. Co-design and evaluation of a patient-centred transition programme for stroke patients, combining case management and access to an internet information platform: study protocol for a randomized controlled trial - NAVISTROKE. *BMC Health Serv Res.* (2022) 22:537. doi: 10.1186/s12913-022-07907-5

54. Bonnechère B, Timmermans A, Michiels S. Current technology developments can improve the quality of research and level of evidence for rehabilitation interventions: a narrative review. *Sensors.* (2023) 23:875. doi: 10.3390/s23020875

55. WHO. *Framework on Integrated, People-Centred Health Services*. (2016). Available online at: https://apps.who.int/gb/ebwha/pdf_files/WHA69/A69_39-en.pdf (accessed May 3, 2023).

56. Sawatzky R, Kwon J-Y, Barclay R, Chauhan C, Frank L, van den Hout WB, et al. the Response Shift – in Sync Working Group. Implications of response shift for micro-, meso-, and macro-level healthcare decision-making using results of patient-reported outcome measures. *Qual Life Res.* (2021) 30:3343–57. doi: 10.1007/s11136-021-02766-9

57. Smith T, McNeil K, Mitchell R, Boyle B, Ries N, A. study of macro-, meso- and micro-barriers and enablers affecting extended scopes of practice: the case of rural nurse practitioners in Australia. *BMC Nurs.* (2019) 18:14. doi: 10.1186/s12912-019-0337-z

58. Bonnechère B, Omelina L, Kostkova K, Van Sint Jan S, Jansen B. The end of active video games and the consequences for rehabilitation. *Physiother Res Int J Res Clin Phys Ther.* (2018) 23:e1752. doi: 10.1002/pri.1752

59. Madjidi A, Bayubasire Ishingwa A. COVID-19 pandemic: the need for digital in the DRC. *Rev Int Cherch.* (2020) 1:255–78.

frontiers | Frontiers in Neurology

TYPE Original Research
PUBLISHED 25 July 2023
DOI 10.3389/fneur.2023.1087054

OPEN ACCESS

EDITED BY
Jean-Claude Baron,
University of Cambridge, United Kingdom

REVIEWED BY
Clio González-Zacarías,
University of Southern California, United States
Panagiotis G. Simos,
University of Crete, Greece

*CORRESPONDENCE
Melanie Koelbel
melanie.koelbel.15@ucl.ac.uk
Fenella J. Kirkham
fenella.kirkham@ucl.ac.uk

RECEIVED 01 November 2022
ACCEPTED 26 June 2023
PUBLISHED 25 July 2023

CITATION
Koelbel M, Hamdule S, Kirkham FJ, Stotesbury H, Hood AM and Dimitriou D (2023) Mind the gap: trajectory of cognitive development in young individuals with sickle cell disease: a cross-sectional study. *Front. Neurol.* 14:1087054. doi: 10.3389/fneur.2023.1087054

Mind the gap: trajectory of cognitive development in young individuals with sickle cell disease: a cross-sectional study

Melanie Koelbel[1,2]*, Shifa Hamdule[1], Fenella J. Kirkham[1,3]*, Hanne Stotesbury[1], Anna Marie Hood[1,4] and Dagmara Dimitriou[2]

[1]Developmental Neurosciences Section, UCL Great Ormond Street Institute of Child Health, London, United Kingdom, [2]Sleep Education and Research Laboratory, UCL Institute of Education, London, United Kingdom, [3]Clinical and Experimental Sciences, University of Southampton, Southampton, United Kingdom, [4]Division of Psychology and Mental Health, Manchester Centre for Health Psychology, University of Manchester, Manchester, United Kingdom

Study objectives: Compared to typically developing children and young adults (CYA-TD), those living with Sickle Cell Disease (CYA-SCD) experience more cognitive difficulties, particularly with executive function. Few studies have examined the relative importance of silent cerebral infarction (SCI), haemoglobin and arterial oxygen content on age-related cognitive changes using cross-sectional or longitudinal (developmental trajectory) data. This study presents cohort data from a single timepoint to inform studies with multiple timepoints.

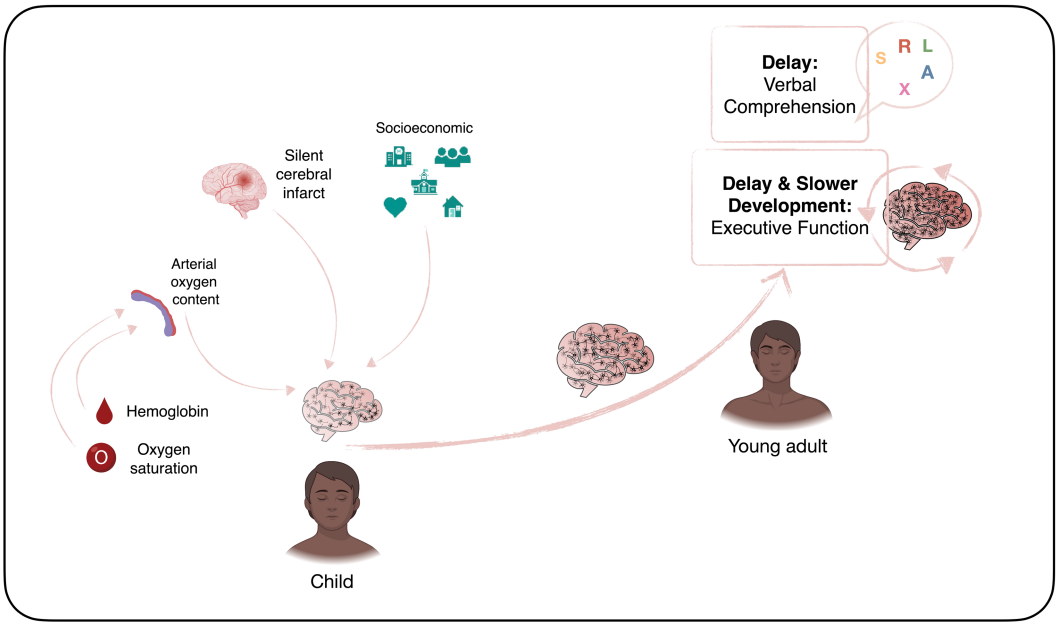
Methods: We compared cross-sectional raw and scaled scores as age-related changes in cognition (trajectories) in CYA-SCD and age-and ethnicity-matched CYA-TD. We also compared cross-sectional age-related changes in cognition (trajectories) in CYA-SCD with and without SCI to CYA-TD. General cognitive abilities were assessed using Wechsler Intelligence Scales, including the Verbal Comprehension Index (VCI) and Perceptual Reasoning Index (PRI) underpinning IQ. Executive function was evaluated using the Delis-Kaplan Executive Function System (D-KEFS) Tower subtest and the Behaviour Rating Inventory of Executive Function (BRIEF) questionnaire. SCI were identified from contemporaneous 3T MRI; participants with overt stroke were excluded. Recent haemoglobin was available and oxygen saturation (SpO_2) was measured on the day of the MRI.

Results: Data were available for 120 CYA-SCD [62 male; age=16.78±4.79 years; 42 (35%) with SCI] and 53 CYA-TD (23 male; age=17.36±5.16). Compared with CYA-TD, CYA-SCD experienced a delayed onset in VCI and slower rate of development for BRIEF Global Executive Composite, Metacognition Index (MI), and Behaviour Regulation Index. The rate of executive function development for the BRIEF MI differed significantly between CYA-TD and CYA-SCD, with those with SCI showing a 26% delay compared with CYA-TD. For CYA-SCD with SCI, arterial oxygen content explained 22% of the variance in VCI and 37% in PRI, while haemoglobin explained 29% of the variance in PRI.

Conclusion: Age-related cognitive trajectories of CYA-SCD may not be impaired but may progress more slowly. Longitudinal studies are required, using tests unaffected by practice. In addition to initiation of medical treatment, including measures to improve arterial oxygen content, early cognitive intervention, educational support, and delivery of extracurricular activities could support cognitive development for CYA-SCD.

KEYWORDS

sickle cell disease, cognition, development, executive function, age-related changes, cross-sectional study, verbal comprehension, silent cerebral infarction



GRAPHICAL ABSTRACT

1. Introduction

Sickle cell disease (SCD) is the most common inherited blood disorder, with about 275,000 babies born annually worldwide (1). In the homozygous form [sickle cell anaemia (SCA); HbSS], a combination of low haemoglobin and low oxygen saturation means that children are at risk of central nervous system complications, including stroke and seizures (2, 3). Accumulation of silent cerebral infarcts (SCI) on Magnetic Resonance Imaging (MRI) from an early age may result in cognitive difficulties that impact not only on academic attainment and life achievements, but also quality of life (4). Schatz and McClellan (4) identified SCD as a neurodevelopmental disorder because of the multifaceted impact of genes, vascular health, and social and environmental factors on early brain development (Figure 1). However, it is still unclear if individuals with SCD experience developmental delay, loss of skills related to brain injury after normal development, or atypical development due to a combination of both.

Previous research suggests that stroke and SCI contribute to the cognitive difficulties frequently seen in this population (5, 6). Systematic literature reviews have demonstrated that full-scale intelligence quotient (FSIQ) is lower than controls in individuals living with SCD, even those with no brain pathology (i.e., "normal MRI": no SCI or stroke) (5, 7). However, cross-sectional data from studies using higher MRI field strengths have not found a robust association of SCI with cognition (8). As genetically determined and potentially modifiable environmental exposures may alter the development of cognition (9), there is a case for investigating cognitive profiles and trajectories in conditions where both are at play, such as SCD (10). To inform early interventions which might positively change the course of development, it is important to know when and why any difference(s) in development occur.

Full-Scale IQ (FSIQ) of adults or children may be measured with the Wechsler Intelligence Scales (11, 12), which include tests of different cognitive skills including the Verbal Comprehension Index (VCI), as well as Perceptual Reasoning (PRI), Working Memory (WMI) and Processing Speed Indices (PSI). The VCI subtests measure problem solving skills (e.g., subtest Similarities) (13, 14) and acquired general knowledge (e.g., Vocabulary) (14, 15). Executive Function (EF), including working memory, response selection (i.e., inhibition) and meta-tasking (16–18), is important for academic achievement and well-being in children. These cognitive domains develop at different stages throughout childhood from infancy to young adulthood (19), with multiple sensitive periods for each cognitive sub-domain (20). EF is typically measured using standardised tests, from batteries such as the Delis-Kaplan Executive Function System, or validated questionnaires such as the Behaviour Rating Inventory of Executive Function (BRIEF). Various socioeconomic factors (e.g., income-to-needs and maternal education) appear to be predictors of a child's performance on EF tasks over time (21). Research has also shown that compromised health, such as is experienced in an inherited disease like SCD, might play an important role in the development of cognitive difficulties (22). Critically, children with SCD show early signs of reduced performance on measures of EF as well as general IQ (23, 24).

Previous research suggests that cognitive abilities are *impaired* in children and adults living with SCD and that difficulties are present at an early age (7). Most previous studies have only looked at cognitive abilities in specific age groups cross-sectionally. However, an approach which takes into account age-related changes is needed when studying neurodevelopmental disorders (25). Specific cognitive abilities (e.g., processing speed, EF) might impact on the early development of other cognitive abilities (26).

Wang et al. (27) reported cognitive data from 1 to 4 (mean 2.9 ± 1.0) timepoints in 467 children living with SCD (255 with HbSS) aged 6–18 years. At baseline those with HbSS and SCI had lower scores for FSIQ, Verbal IQ (VIQ) and Performance IQ (PIQ), as well as Digit Span, mathematics and reading achievement, at all ages. The hypothesis that there would be a decrease in psychometric

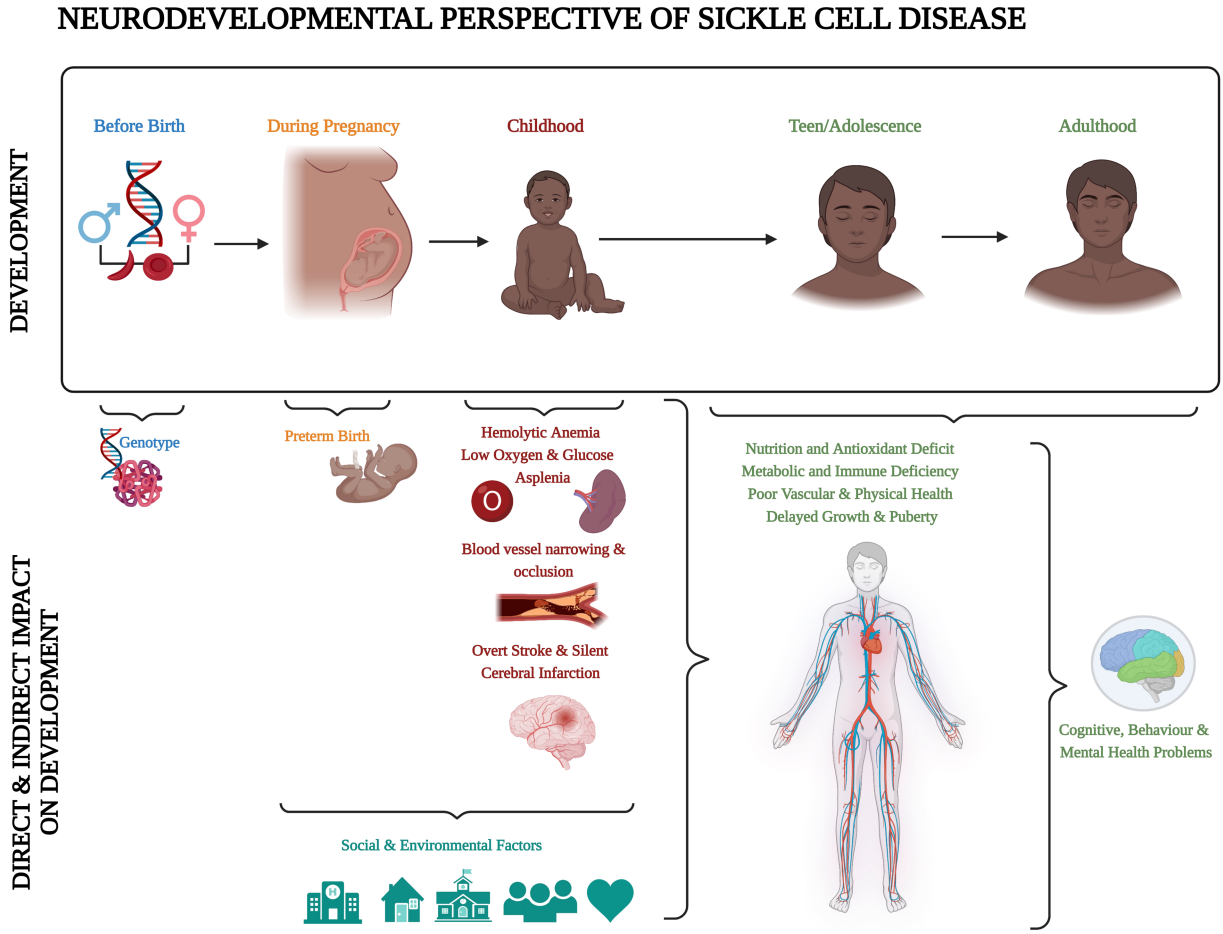


FIGURE 1
Neurodevelopmental perspective of sickle cell disease. Created with BioRender.com according to the information given in the review by Schatz and McClellan (4).

performance with increasing age was addressed with follow-up data in between 122 and 173 children with HbSS and normal MRI only. It is important to note that during the study period, two versions of the Wechsler IQ scales were used with different scaling, so that age-related change, rather than longitudinal data, were reported for IQ. There was a significant decline in VIQ but not in PIQ or FSIQ with increasing age. On average VIQ declined with increasing age by 0.5 points/year ($p < 0.04$). After rescoring of the effectively equivalent subtests, longitudinal data could be reported for Working Memory (assessed using the Digit Span subtest) for which there was no evidence of decline, and Processing Speed (assessed using the Coding subtest) which declined 0.2 points per year ($p < 0.01$) (27). From the Woodcock-Johnson Tests of Achievement, there was a decline in performance for Mathematics ($p < 0.01$) but not for Reading. This study was conducted prior to the introduction of disease-modifying therapies, e.g., hydroxyurea. A Ghanaian study in adults aged 18–50 years with follow-up data after 7 years found a significant decline in verbal, non-verbal and global PIQ on the Revised Quick Cognitive Screening Test, as well as significant decreases in visuospatial abilities, processing speed, and EF (28). The rarity of these types of longitudinal evaluations in high and low resource countries is in part due to the logistical difficulties and expense.

An alternative to longitudinal evaluation is to examine existing cross-sectional databases to compare cognitive scores against age between typically developing children and young adults (CYA-TD) and those living with a long-term condition that may affect cognitive development. Although evaluating age-related changes in cross-sectional data is not truly a measure of developmental trajectory, it can help gain valuable insight in understanding developmental change over time to inform longitudinal studies. The assessment of age-related changes in cognitive development has been used in the previous studies of other developmental disorders (e.g., autism, Williams Syndrome and Down Syndrome) (29, 30), and was originally termed the developmental trajectory approach but has received little attention so far in children living with SCD (10, 31).

The approach described by Thomas et al. (32) helps to identify cognitive change with age and provides a more in-depth analysis of scores compared with simply reporting means across all ages. A significant linear relationship between age and the cognitive variable of interest in typically developing children is required to perform the developmental trajectory analysis in those with a long-term condition. Further analysis compares the two groups to identify if there might be a delay or slower rate of development in the non-typically developing population. The latter may be because there is a plateau or

because there is no relationship at all with age, which can be distinguished by a rotational analysis (32).

Scaling to age-appropriate scores allows comparison with normative data, to see whether the groups develop differently as compared to their CYA-TD peers but might confound an analysis of age-related changes. Evaluation of raw scores is encouraged in longitudinal data as it provides a robust measure of individual change in the outcome measure, hence allowing evaluation of progress or decline for a particular individual across different time points. Exploring trajectories for raw as well as scaled scores might yield important insights when considering using cognitive endpoints in randomised clinical trials (33). Following this paradigm, we explored associations with both raw as well as scaled scores.

In this study, we investigated the cognitive development of children and young adults living with SCD (CYA-SCD) by adopting a cross-sectional age-related change (developmental trajectory) approach used in several previous studies (30, 32). The key aim was to investigate if there was a delay in the development of cognitive function, or if the rate of development differed, by comparing ethnicity and age-matched controls with patients living with SCD. Since previous literature has also showed an association between cognition and blood oxygenation measures [haemoglobin, peripheral oxygen saturation (SpO_2) and arterial oxygen content (CaO_2)] as well as SCI, we also aimed to investigate the effects of SCI, haemoglobin, SpO_2 and CaO_2 on cognitive development. Fox example, previous research found lower VIQ in adolescents with SCD and lower SpO_2 (34), while lower processing speed was associated with lower CaO_2 in CYA-SCD (35).

We hypothesised that:

1. CYA-SCD would perform worse on tests of intelligence and EF compared to age-and ethnicity-matched CYA-TD.
2. Developmental trajectories would be delayed, and the rate of development would be slower for CYA-SCD, with and without SCI, as compared to CYA-TD.
3. Factors explaining the cognitive differences between CYA-SCD and CYA-TD would include haemoglobin, SpO_2 and CaO_2.

2. Methods

2.1. Participants

Cross-sectional data were collected from Black CYA aged >8 years and ≤ 30 years with SCD and TD. A total of 173 Participants were enrolled in three different studies conducted at University College London: (1) The Sleep and Asthma Cohort (SAC) (36), (2) the Prevention of Morbidity in SCA 2b (POMS) (37), and (3) a community study in London which recruited through convenience and snowball sampling including advertisements on Twitter, Instagram, and in community centres and charities (38). For all three studies, inclusion criteria included the ability to speak English fluently and homozygosity for sickle cell haemoglobin (i.e., HbSS) or compound heterozygosity for sickle β thalassaemia zero (HbSβ0) for CYA-SCD. CYA-SCD were ineligible for SAC and POMS study participation if they were receiving nocturnal respiratory support at the time of enrolment, participating in a clinical trial evaluating blood transfusion or oxygen therapy, had chronic lung disease (other than asthma), or existing respiratory failure.

Additional exclusion criteria for the POMS study were hospital admissions within 1 month of enrolment or more than six hospital admissions within 12 months of enrolment (both for acute sickle-related complications), overnight oximetry showing mean overnight saturation of less than 90% for more than 30% of total sleep time, severe sleep apnoea defined by 4% oxygen desaturation index >15/h, and chronic blood transfusion or transfusion within 3 months of enrolment. The use of the disease-modifying therapy, hydroxyurea, was not an exclusion criterion. For the SAC study, patients were enrolled without regard to past sickle-or sleep-related morbidity or transfusion status. Black-British siblings, family members, peers of CYA-SCD, and people from the community in London, with no known neurological and psychological difficulties, were recruited as CYA-TD, whether they had sickle cell trait or SCI on MRI. Participants with or without SCD who had experienced an overt stroke were excluded. Each participant had a study protocol MRI on a Siemens 3 T PRISMA scanner (8). An independent neuroradiologist classified SCI according to the Silent Infarction Transfusion trial (39) (i.e., hyperintensity on FLAIR: >3 mm in diameter, present on two planes).

Detailed inclusion and exclusion criteria for individuals with SCD are explained elsewhere (36, 37, 40). West London NHS (SAC; 05/Q0408/42, 11/EM/0084, 15/LO/0347), Yorkshire NHS (POMS; 15/YH/0213), and University College London (14475/001) ethics committees provided ethical approval. Written informed consent was obtained from all participants and for children from their parent/guardian; the children also gave written assent.

2.2. Measures

2.2.1. Questionnaires

Basic demographic information was obtained at the start of each study (i.e., age, sex, and postcode). The postcode-based Index of Multiple Deprivation was used as an indication of socioeconomic status (SES), as previously described (41). It consists of seven domains: income, employment, education, health, crime, barriers to housing and service, and living environment (42). The data are open-source, and downloadable from the UK Ministry of Housing, Communities & Local Government.[1] The index ranges from rank 1st (most deprived) to 32,844th (least deprived).

2.2.2. Cognitive assessment

General cognitive abilities were assessed using the Wechsler Intelligence Scale for Children (WISC-IV; SAC patients and CYA-TD < 16 years) (11), the Wechsler Adult Intelligence Scale (WAIS-IV; SAC patients and CYA-TD > 16 years) (12) or were estimated using the Wechsler Abbreviated Scale of Intelligence (WASI-II Vocabulary and Matrix Reasoning; POMS patients) (43). Scaled and raw scores for all indices and subtests were calculated to compare age-related changes. Scaled scores were derived from raw scores for each subtest, enabling comparison to the normative group based on the age range. Scaled scores from various subtests were combined together, resulting in indices for various domains of cognitive development: VCI (Verbal Comprehension:

1 http://imd-by-postcode.opendatacommunities.org/imd/2019

Similarities, Vocabulary, Information/Comprehension), PRI (Perceptual Reasoning: Block Design, Matrix Reasoning/Picture Concepts, Visual Puzzles), WMI (Working Memory: Digit Span, Arithmetic/Letter-Number-Sequencing), PSI (Processing Speed: Symbol Search, Coding/Cancellation) and FSIQ (Full-Scale IQ: sum of all raw scores). Raw scores have a mean of 10 and a standard deviation of 3. Scaled scores have a mean of 100 and a standard deviation of 15.

EF was assessed using the examiner-administered Tower subtest from the D-KEFS (44). Tower time was the outcome investigated, which has a mean of 10 and a standard deviation of 3. In addition, for a patient-centric measure, the caregiver-reported Behaviour Rating Inventory of Executive Function (BRIEF) (45) or adult self-reported BRIEF (BRIEF-A) were administered. The Global Executive Composite (GEC), Behaviour Regulation Index (BRI) and Metacognition Index (MI) and all subscale scores were investigated (46). The GEC is a summary index score of the BRI and MI. The BRI contains four different subscales: Inhibit, Shift, Emotional Control and Self-Monitor. The MI contains five subscales: Initiate, Working Memory, Plan/Organize, Task Monitor, and Organization of Materials. The caregiver-reported BRIEF does not contain the subscales of the BRI Self-Monitor and subscale for the MI Task Monitor. It also has an additional subscale the MI Monitor. T scores were used to interpret the BRIEF indices (normal < 60; mildly elevated: 60–64; potentially clinically elevated 65–69; clinically elevated above 70). Scaled and raw scores for all indices and subtests were calculated to compare developmental trajectories.

2.2.3. Procedure

Participants completed their cognitive assessment on a single day in a quiet and comfortable room to minimise distractions. On the day of cognitive assessment, a fingertip pulse oximeter was used to measure daytime SpO_2 (%). Haemoglobin (Hb in g/L) and Haematocrit (%) were obtained from the most recent laboratory test, partial pressure of oxygen (PaO_2) was assumed to be 100 mmHg and arterial oxygen content (CaO_2 in mL/dL) was calculated as

$$\left[\mathrm{SpO2} \times \mathrm{Hb}\left(\mathrm{g}/\mathrm{L}\right) \times 1.37\right] + \left[\mathrm{PaO2}\left(\mathrm{mmHg}\right) \times 0.003\right]$$

2.3. Statistical analysis

Statistical analysis was performed using SPSS® version 26 (IBM Corporation, Armonk, NY, United States) for Mac®. Mean ± standard deviation (SD) was reported unless otherwise stated. For each variable, normality and homogeneity of variance were assessed using the Shapiro–Wilk test. Cook's distance was used to identify potential outliers. Appropriate parametric (e.g., *t*-test, ANCOVA) or non-parametric (e.g., Mann–Whitney-U, Kruskal-Wallis) tests were then chosen to compare demographic and cognitive variables between CYA-SCD and CYA-TD, while controlling for age, sex, and SES. We investigated the relationship of haemoglobin, CaO_2, SpO_2 and SES in CYA-SCD on cognitive scores using simple linear regression models. We also inspected the scatter-and residual plots to check for linearity and outliers before assessing linear relationships.

2.4. Age-related change (developmental trajectory) analysis

To calculate and compare cross-sectional age-related changes (developmental trajectories), we used the method outlined by Thomas et al. (32), as summarised in the sections below. Detailed descriptions of this statistical approach have been published previously (30, 32) and used in cross-sectional studies of neurodevelopmental disorders (29, 30, 47). Further information is available at: http://www7.bbk.ac.uk/psychology/dnl/old_site/stats/Thomas_trajectories.html#Section2.

2.4.1. Pre-analysis: simple linear regression

Trajectories can only be compared if there is a significant linear relationship between mental age and the cognitive variable in the CYA-TD group (32). To explore the importance of any differences in socioeconomic status across different cohorts, all models were corrected for SES. However, SES did not significantly influence the scores on cognitive tests. Hence, it was excluded from the ANCOVA analyses. For the analysis, mental age was calculated as (FSIQ score/100) * chronological age. Hence, the first step of our analysis was to generate simple linear regression models with each *scaled* cognitive variable as the outcome and mental age as the predictor for the CYA-TD group and the CYA-SCD group separately. Additionally, for *raw* scores, separate regression lines were calculated for WISC and WAIS data as the total raw scores for each subtest differ (See Supplementary Tables).

2.4.2. Age-related changes (developmental trajectories) analysis

For our next step, we adjusted the age such that zero years represented the lowest age at which we collected data, i.e., 8 years. To do this, we deducted the lowest age from each participant's mental age (30). Following this adjustment for age, we generated ANCOVA models that compared the developmental trajectory for cognitive scores only for those who showed a significant linear relationship with mental age in the CYA-TD group. The developmental trajectory analyses using ANCOVA resulted in two metrics: (1) if there was a *delay at onset*, i.e., if the two groups differed in performance at the youngest age at which the measurement is taken (also known as overall effect of the group), and (2) if the *rate of development* differed between the two groups which is the interaction between group performance and mental age (group × MA). It was also possible to calculate the difference between the two scores at onset by subtracting the intercept of the CYA-SCD group from the intercept of the CYA-TD group (*delay at onset* = intercept of CYA-TD group − intercept of CYA-SCD group). Similarly, difference in the rate of development is calculated by dividing the gradient of CYA-SCD group by that of the CYA-TD group (*rate of development* = gradient of CYA-SCD group/gradient of CYA-TD group).

2.4.3. Rotational analysis (null trajectory analysis)

For cognitive domains where the CYA-TD group showed a significant relationship between scores and age, but the CYA-SCD group did not, we conducted a *rotation analysis* (X-Y coordinates) to distinguish between a *zero trajectory* and *no systematic relationship* in the CYA-SCD group, since results can be influenced by other factors such as cross-sectional design artifacts, missing disease severity data, and variability and static development in a particular cognitive domain

[see detailed discussion in Thomas et al. (32)]. This allows distinction in the CYA-SCD group between plateauing (zero trajectory) or whether, alternatively, there is no systematic relationship, suggesting that there might be other variables that predict the relationship better.

Using the same approach, we also examined if developmental trajectories differed between CYA-SCD with and without SCI.

3. Results

3.1. Participant characteristics

Our study included 120 CYA-SCD participants [Genotypes: HbSS: 99%, $N = 119$; HbSB0-thal: 1%, $N = 1$, 62 males; mean age = 16.78 ± 4.79; age range 8–30 years; $N = 53$ (44%) ≤ 16 years]. Fifty-three CYA-TD [34% HbAS; 23 males; mean age = 17.36 ± 5.16; age range 8.2–30 years; $N = 23$ (43%) ≤ 16 years] were eligible for inclusion in the developmental trajectory analysis (Table 1). Just over one third of CYA-SCD had SCI (Table 1). No significant differences for age, sex, or SES ($p > 0.05$) were observed between CYA-SCD and CYA-TD, or for CYA-SCD with ($N = 49$) or without hydroxyurea prescription ($N = 71$). CYA-SCD had significantly lower SpO_2, CaO_2, haemoglobin and haematocrit compared to CYA-TD (Table 1).

3.2. Cognitive profile

After controlling for age, sex and SES, there were significant mean group differences between CYA-SCD and CYA-TD for IQ, VCI Similarities and Vocabulary, PRI and PRI Block Design and Matrix Reasoning, WMI and WMI Digit Span and Arithmetic, PSI and PSI Coding and Symbol Search but no significant differences for variables measuring EF (Table 1).

After controlling for age, sex and SES, there were no significant mean group differences in demographics, for CYA-SCD with (CYA-SCD-SCI+) and without (CYA-SCD-SCI−) silent cerebral infarct (Supplementary Tables). Significant group differences for CYA-SCD-SCI+ and CYA-TD were found for PRI and PRI Block Design, WMI and WMI Digit Span, PSI and PSI Symbol Search and Cancellation. Significant group differences for CYA-SCD-SCI+, CYA-SCD-SCI− and CYA-TD were found for PRI, WMI and WMI Digit Span and Arithmetic, PSI and PSI Coding and Cancellation. No significant differences were found for variables measuring EF. All values were controlled for age, sex and SES and are available in Supplementary Table S1.

3.3. Developmental trajectories (age-related changes) for scaled cognitive scores

The results for scaled scores developmental trajectories are presented below for each step of the analysis, with the data on delay at onset and rate of development for CYA-SCD compared with CYA-TD presented in Table 2.

3.3.1. Pre-analysis: simple linear regression

Cross-sectional developmental trajectory analyses explored cognitive profiles in CYA-SCD in comparison to CYA-TD. SES did not significantly influence any of the cognitive scores and was excluded from further analyses. All simple linear regression results examining the relationships between mental age and performance on cognitive scores are shown in Supplementary Table S2. To compare the groups in the later steps of the analysis, the pre-analysis (simple linear regression) required a statistically significant result for the CYA-TD group (Supplementary Table S2) which was the case for VCI, the VCI Similarities and Vocabulary subtests, PRI, the PRI Block Design subtest and WMI as well as the PSI Coding subtest. For the measures of EF the BRIEF GEC was close to being significantly related to mental age. The BRIEF MI and its subscales Initiate, Working Memory, and Plan/Organize as well as the BRIEF BRI and its subscale Inhibit, all showed a significant relationship with mental age (see Supplementary Table S2).

3.3.2. Cross-sectional developmental trajectory analysis

As our second step, we examined developmental trajectories between cognition and mental age for CYA-SCD and CYA-TD. ANCOVA models were constructed to evaluate the overall effect of the group in terms of delay at onset and the Group × Mental Age (MA) interaction effect to determine the differences in the rate of cognitive development. Significant results from this analysis are summarised in Table 2 with regressions are plotted in Figures 2–7.

3.3.2.1. Wechsler scales for Intelligence

For Wechsler Indices, there was an overall effect of group for VCI only; thus, the intercepts of the two groups were reliably different at the youngest age of measurement (Table 2 and Figure 2). CYA-SCD appeared to experience a delayed onset in VCI cognitive development, with a lower VCI score of 9.26 points compared to the CYA-TD group at the youngest age. However, there was no significant Group × MA interaction, i.e., verbal comprehension for CYA-SCD did not appear to develop more slowly compared to CYA-TD peers, indicating a possible period for catch up.

3.3.2.2. BRIEF scores

There was an overall effect of group for BRIEF GEC (Figure 3A), BRIEF MI (Figure 3B) and BRIEF MI Initiate, BRIEF MI Working memory and BRIEF MI Plan/Organise subscales (Figures 4A–C), as well as BRIEF BRI (Figure 3C) and BRIEF BRI Inhibit subscale (Figure 5) scores, thus, the intercepts of the CYA-SCD and CYA-TD groups were reliably different at the youngest age of measurement (See Table 2), with CYA-SCD showing worse EF scores at the youngest age. These data indicate that CYA-SCD have a delayed onset for EF compared to CYA-TD, with BRIEF MI Working Memory showing the greatest delay at onset (−27.96) followed by MI Plan/Organise (−21.9) (Table 2 and Figure 4). Significant Group x MA interactions were found for all these measures mentioned, with CYA-SCD demonstrating a slower rate of development compared to their CYA-TD peers, with BRIEF BRI (43%), and MI subscales Working Memory (42%) and Plan/Organise (38%) showing the greatest differences in rate of development.

3.3.3. Rotational analysis (null trajectory analysis)

For cognitive domains where there was a significant relationship between mental age and cognitive scores only for CYA-TD (Supplementary Table S2), but not for the CYA-SCD, an

TABLE 1 Participant comparison.

	N	CYA-SCD	CI 95%	*N*	CYA-TD	CI 95%	p^1	Effect size	p^2	Effect size
Demographics										
Gender	120	62 Male		53	23 Male		> 0.05			
		58 Female			30 Female					
MRI (*N*), SCI (%)	106	42, 35%			46, 10%					
Genotype (*N*)		HbSS (119)			HbAA (35)					
		HbSB0-thal (1)			HbAS (18)					
Age in years	120	16.78 (4.79)	[15.91, 17.65]	53	17.36 (5.16)	[15.94, 18.78]	> 0.05*	0.12		
		8.02–29.40			8.24–30.67					
SES	115	9995.15 (6541.42)	[8786.76, 11205.54]	53	9322.94 (6537.98)	[7520.85, 11125.03]	> 0.05*	0.14		
		1.443–32.371			3.056–32.371					
SpO_2%	55	96.41 (3.41)	[95.50, 97.34]	39	98.56 (1.43)	[98.10, 99.03]	< 0.001*	0.75	0.001	0.11
		86.91–100			93–100					
CaO_2 mL/d	106	11.73 (1.93)	[11.36, 12.10]	47	18.13 (1.04)	[17.82, 18.43]	< 0.00*	2.59	0.001	0.73
		7.79–17.55			16.86–20.68					
Hemoglobin g/L	106	88.30 (14.23)	[85.30, 90.78]	47	134.89 (8.55)	[132.38, 137.41]	< 0.00*	2.51	< 0.001	0.73
		60–134			126–152					
Hematocrit %	106	26.34 (4.57)	[25.46, 27.22]	47	39.60 (5.91)	[37.86, 41.34]	< 0.00*	2.23	< 0.001	0.57
		16.10–40			3.4–45.3					
Wechsler cognitive scores										
FSIQ	120	93.16 (13.07)	[90.80, 95.52]	53	98.43 (12.10)	[95.10, 101.77]	0.01	0.41	0.006	0.05
		58–123			75–130					
VCI	69	95.48 (13.71)	[92.19, 98.77]	53	99.40 (11.72)	[96.17, 102.63]	0.09	0.30	0.07	0.03
		57–138			71–130					
Similarities	69	9 (2.72)	[8.35, 9.65]	53	10.17 (2.23)	[9.56, 10.78]	0.01	0.46	0.02	0.05
		3–17			6–16					
Vocabulary	120	9.2 (2.90)	[8.68, 9.72]	53	10.11 (2.73)	[9.36, 10.87]	0.10*	0.25	0.015	0.04
		2–17			5–17					
PRI	69	91.07 (10.79)	[88.48, 93.66]	53	96.94 (10.53)	[94.04, 99.85]	0.003	0.55	0.003	0.08
		61–111			73–117					
Block Design	69	7.54 (2.18)	[7.01, 8.06]	53	8.43 (2.21)	[7.83, 9.04]	0.02*	0.44	0.03	0.04
		3–14			4–13					
Matrix Reasoning	120	9.62 (2.82)	[9.11, 10.13]	53	10.60 (2.66)	[9.87, 11.34]	0.03*	0.33	0.01	0.04
		1–16			4–15					
WMI	120	92.71 (13.70)	[90.23, 95.19]	53	100.68 (13.18)	[97.05, 104.31]	0.001	0.59	< 0.001	0.07
		56–136			65–136					
Digit Span	120	8.84 (2.57)	[8.38, 9.31]	53	10.60 (2.73)	[9.85, 11.36]	< 0.001*	0.62	< 0.001	0.08
		3–17			5–19					
Arithmetic	114	8.54 (3.04)	[7.97, 9.10]	49	10.16 (2.73)	[9.38, 10.95]	0.001*	0.52	0.001	0.07

(Continued)

TABLE 1 (Continued)

	N	CYA-SCD	CI 95%	N	CYA-TD	CI 95%	p^1	Effect size	p^2	Effect size
		2–19			5–18					
PSI	120	89.25 (13.28)	[86.85, 91.65]	53	98.51 (14.29)	[94.57, 102.45]	< 0.001	0.68	< 0.001	0.11
		59–131			53–122					
Coding	120	7.77 (2.65)	[7.29, 8.25]	53	9.42 (3.04)	[8.58, 10.25]	< 0.001*	0.54	< 0.001	0.08
		2–16			2–15					
Symbol Search	120	8.43 (3.01)	[7.88, 8.97]	53	9.64 (2.92)	[8.84, 10.45]	0.01*	0.41	0.01	0.04
		1–15			1–15					
Cancellation	120	9.38 (3.60)	[8.72, 10.03]	52	10.29 (2.89)	[9.49, 11.09]	0.08	0.27	0.10	0.02
Executive function scores										
BRIEF GEC	95	52.44 (10.79)	[50.24, 54.64]	39	50.67 (11.08)	[47.07, 54.26]	0.40	−0.16	0.38	0.01
		34–80			33–78					
BRIEF BRI	95	52.04 (10.92)	[49.82, 54.27]	39	51.59 (11.13)	[47.98, 55.20]	0.85*	0.03	0.88	0.00
		34–84			34–75					
Inhibit	67	51.72 (11.54)	[48.90, 54.53]	39	51.82 (10.75)	[48.34, 55.31]	0.84*	0.04	0.72	0.001
		36–103			36–87					
Shift	67	52.75 (11.87)	[49.85, 55.64]	39	52.77 (10.75)	[49.29, 56.25]	0.87*	0.03	0.79	0.001
		38–81			38–73					
Emotional Control	66	50.76 (11.99)	[47.81, 53.70]	39	51.56 (12.97)	[47.36, 55.77]	0.83*	0.04	0.63	0.002
		37–80			37–83					
BRIEF MI	96	52.66 (10.81)	[50.47, 54.85]	39	50.41 (11.55)	[46.67, 54.15]	0.19*	0.23	0.27	0.01
		33–82			34–85					
Initiate	67	53.61 (11.36)	[50.84, 56.38]	39	49.36 (11.01)	[45.79, 52.93]	0.08*	0.34	0.10	0.03
		35–79			35–69					
Working Memory	67	56.31 (12.88)	[53.17, 59.45]	39	52.23 (9.96)	[49.00–55.46]	0.14*	0.29	0.16	0.02
		38–89			38–71					
Plan/Organize	67	54.07 (11.24)	[51.33, 56.82]	39	49.36 (10.20)	[46.05, 52.66]	0.04*	0.42	0.07	0.03
		38–86			37–74					
Organise Material	67	49.97 (9.97)	[47.54, 52.40]	39	47.74 (8.19)	[45.09, 50.40]	0.31*	0.20	0.48	0.01
		34–71			34–67					
Delis-Kaplan Tower Time	114	556.67 (145.05)	[529.75, 583.58]	50	563.74 (146.25)	[522.18, 605.39]	0.78	0.05	0.73	0.001

Results are given in mean (±SD) and range. *p value for Mann–Whitney U test. p^1, mean difference without confounding variables. p^2, mean difference with confounding variables (age, sex, and SES). CYA-SCD, children and young adults living with sickle cell disease; CYA-TD, children and young adults who are typically developing; MRI, Magnetic resonance imaging; SCI, silent cerebral infarcts; SES, Socioeconomic status; SpO_2, Oxygen saturation; CaO_2, arterial oxygen content; FSIQ, Full Scale IQ; VCI, Verbal Comprehension Index; PRI, Perceptual Reasoning Index; WMI, Working Memory Index; PSI, Processing Speed Index; BRIEF, Behaviour Rating Inventory of Executive Function; BRIEF GEC, Global Executive Composite; BRIEF BRI, Regulation Index; BRIEF MI, Metacognition Index.

additional rotation analysis or null trajectory analysis was conducted (Supplementary Table S3) to distinguish between Zero trajectory and no systematic relationship. While there were significant relationships between mental age and cognition for the CYA-TD group none were found for CYA-SCD for PRI Block Design and PSI Coding, BRIEF GEC, BRI and its subscale Inhibition, BRIEF MI and its subscales Initiate, Working Memory and Plan/Organize as well as Tower Time score (Supplementary Table S3).

3.3.3.1. No systematic relationship

We found no systematic relationships (i.e., other factors more important than age) for CYA-SCD between age and PSI subscale Coding, PRI subscale Block Design and BRIEF GEC, BRI, MI, and its

TABLE 2 Developmental trajectories for scaled scores.

Variable	Slope: cognitive variable=(intercept for group)+(age * gradient)		Delay at onset	*p*	CI 95%	Rate of development	*p*	CI 95%
	CYA-SCD	CYA-TD	CYA-SCD compared with CYA-TD					
General cognitive scores								
VCI	81.79 + age * 1.48	91.05 + age * 0.8	9.26	0.05	[0.219, 18.311]	1.85	0.11	[−1.491, 0.147]
Executive function scores								
BRIEF GEC	53.53 + age * −0.07	33.03 + age * 1.03	−20.5	0.003	[−21.48, −4.58]	−0.07	0.005	[0.028, 0.156]
BRIEF BRI	57.5 + age * −0.36	37.38 + age * 0.83	−20.12	0.004	[−20.66, −3.50]	−0.43	0.003	[0.034, 0.164]
BRI Inhibit	53.38 + age * −0.1	33.32 + age * 1.08	−20.06	0.01	[−21.56, −2.56]	−0.09	0.01	[0.029, 0.168]
BRIEF MI	50.73 + age * 0.13	30.59 + age * 1.16	−20.14	0.003	[−21.61, −4.65]	0.11	0.01	[0.022, 0.150]
MI Initiate	52.48 + age * 0.07	32.09 + age * 1.01	−20.39	0.01	[−23.55, −4.45]	0.07	0.03	[0.009, 0.149]
MI Working Memory	63.39 + age * −0.42	35.43 + age * 0.99	−27.96	<0.001	[−28.51, −8.35]	−0.42	0.002	[0.044, 0.191]
MI plan/organise	58.69 + age * −0.28	36.79 + age * 0.74	−21.9	0.01	[−24.42, −5.66]	−0.38	0.03	[0.016, 0.153]

Delay at onset = intercept of CYA-TD group − intercept of CYA-SCD group; rate of development = gradient of CYA-SCD group/gradient of CYA-TD group. CYA-SCD, children and young adults living with sickle cell disease; CYA-TD, children and young adults who are typically developing; VCI, Verbal Comprehension Index; BRIEF GEC, Global Executive Composite; BRIEF BRI, Regulation Index; BRIEF MI, Metacognition Index; BRIEF, Behaviour Rating Inventory of Executive Function.

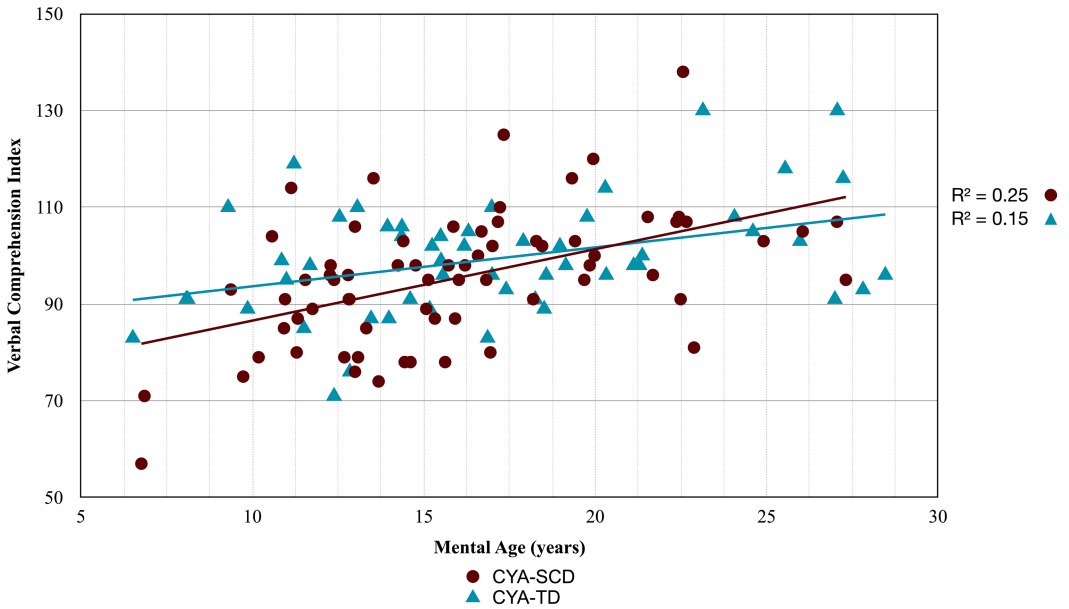


FIGURE 2
Developmental trajectory of Verbal Comprehension Index. CYA-SCD, children and young adults with sickle cell disease; CYA-TD, children and young adults who are typically developing.

subscales Working Memory and Plan/Organize, as well as Tower Time score (Supplementary Table S3). The results suggest that there are factors other than disease status contributing to the relationship between age and these cognitive domains. These potential relationships have been explored in the analyses described below (see Sections 3.4 and 3.5).

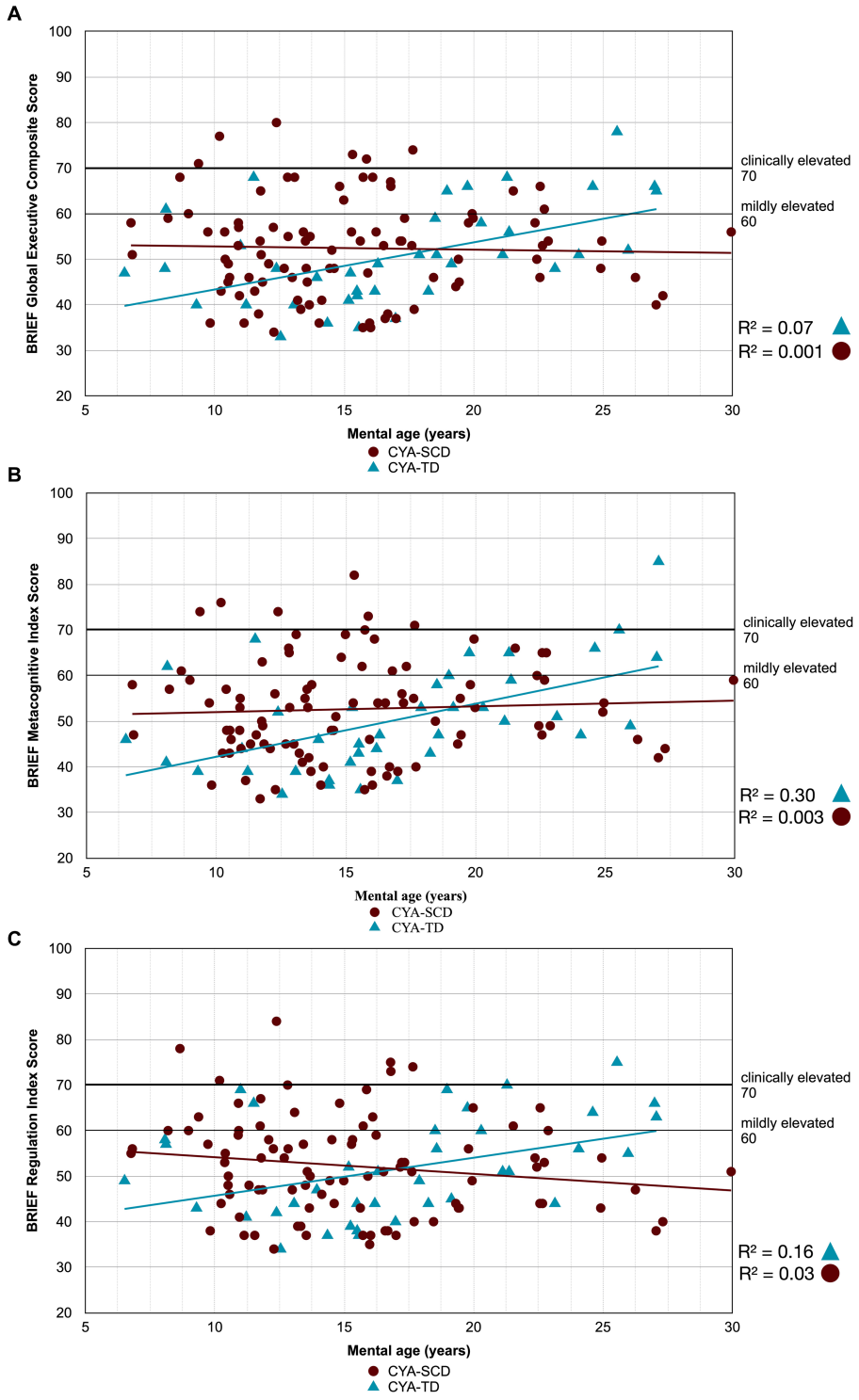


FIGURE 3
Developmental trajectory of BRIEF Composite Scores. CYA-SCD, children and young adults with sickle cell disease; CYA-TD, children and young adults who are typically developing; BRIEF, Behavior Rating Inventory of Executive Function. **(A)** BRIEF Global Executive Composite Score; **(B)** BRIEF Metacognitive Index Score; **(C)** BRIEF Regulation Index Score.

3.3.3.2. Zero trajectories

We found a significant zero trajectory only for BRIEF subscale Inhibition in relation to mental age in CYA-SCD. Non-significant zero trajectories were observed for the BRIEF GEC and the MI subscale Initiate (Supplementary Table S3). The results suggest that the development of behaviour regulation skills (e.g., inhibition) prematurely plateaued in CYA-SCD.

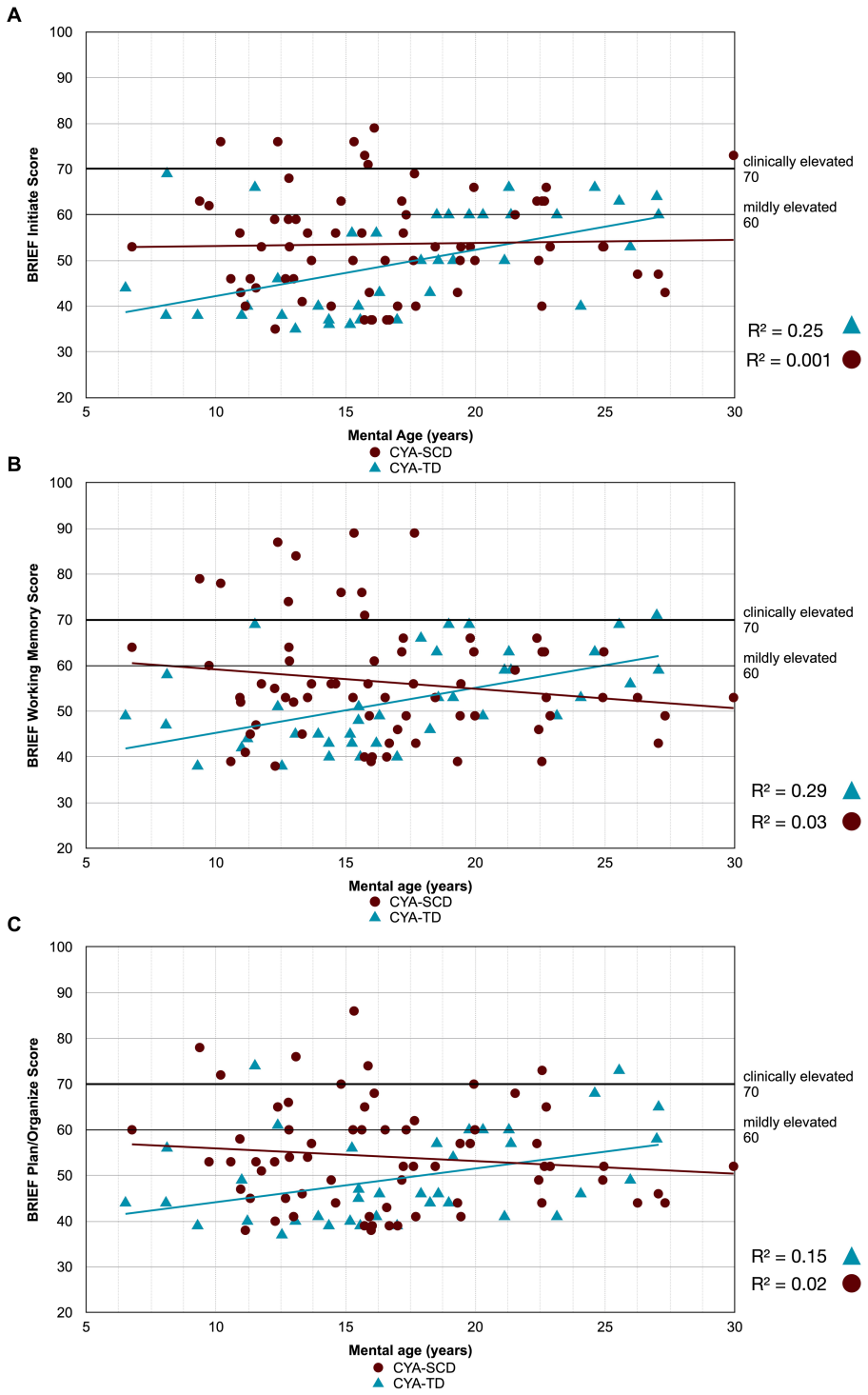


FIGURE 4
Developmental trajectory of BRIEF MI subscales. CYA-SCD, children and young adults with sickle cell disease; CYA-TD, children and young adults who are typically developing; BRIEF, Behavior Rating Inventory of Executive Function. **(A)** BRIEF Initiate Score; **(B)** BRIEF Working Memory Score; **(C)** BRIEF Plan/Organize Score.

3.4. Cross-sectional developmental trajectories based on SCI

3.4.1. Pre-analysis: simple linear regression

Similar analyses were conducted by dividing the participants in three groups: CYA-SCD and silent cerebral infarction (CYA-SCD-SCI+), CYA-SCD but no SCI (CYA-SCD-SCI−) and CYA-TD. Simple linear regression analyses examining the relationships between mental age and performance on cognition are shown in Supplementary Table S4. As the statistically significant results for the CYA-TD group were for the same domains, we conducted the analyses comparing the CYA-SCD-SCI+ with the CYA-TD group.

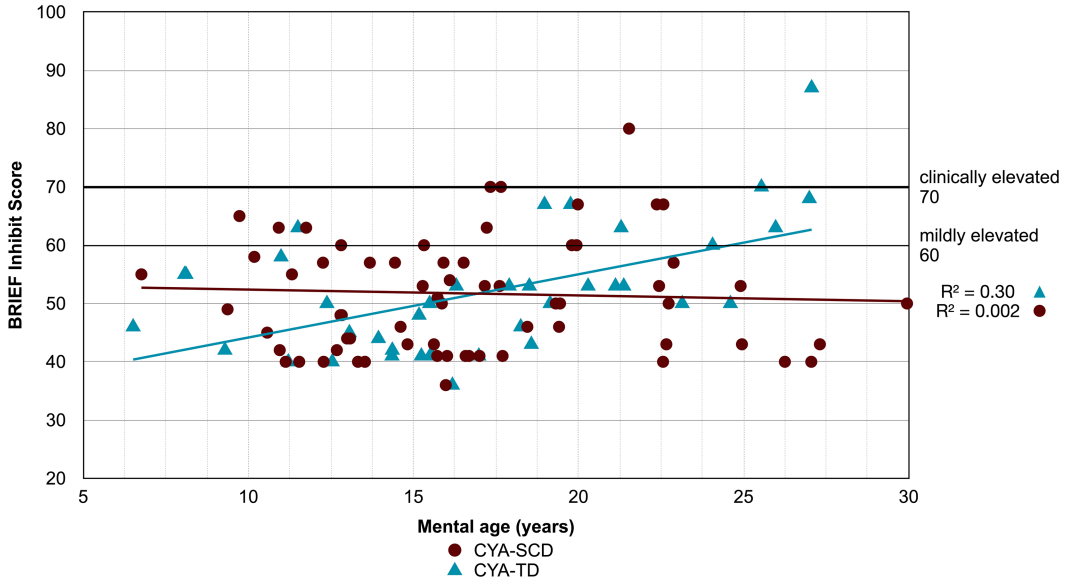


FIGURE 5
Developmental trajectory of BRIEF BRI Inhibit. CYA-SCD, children and young adults with sickle cell disease; CYA-TD, children and young adults who are typically developing; BRIEF, Behavior Rating Inventory of Executive Function.

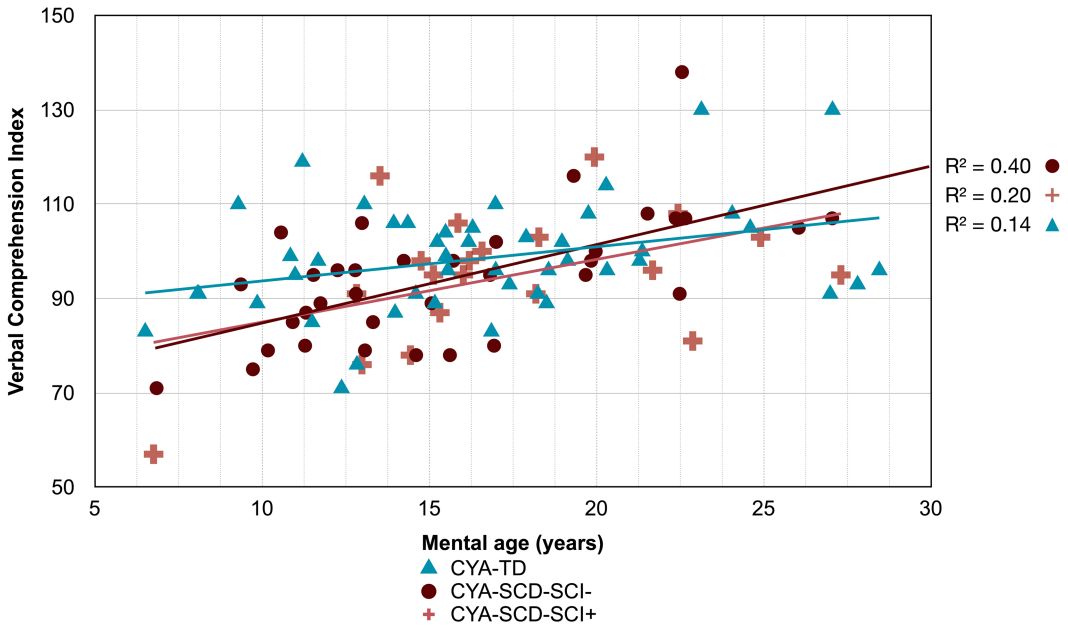


FIGURE 6
Developmental trajectory of Verbal Comprehension Index. CYA-SCD-SCI+, children and young adults with sickle cell disease with silent cerebral infarcts; CYA-SCD-SCI–, children and young adults with sickle cell disease without silent cerebral infarcts; CYA-TD, children and young adults who are typically developing.

3.4.2. Cross-sectional developmental trajectory analysis

3.4.2.1. Weschler scales for Intelligence

Although non-significant, only VCI showed a trend of delay at onset (8 years of age) in the CYA-SCD-SCI+ group with a lower score of 10 points compared to the CYA-TD group at the youngest age (Table 3 and Figure 6). The rate of development did not differ significantly between the three groups.

3.4.2.2. BRIEF scores

The developmental trajectory analysis was significantly different for BRIEF MI, with CYA-SCD showing worse EF scores at the youngest age. At onset, CYA-SCD-SCI+ scored 10 points above

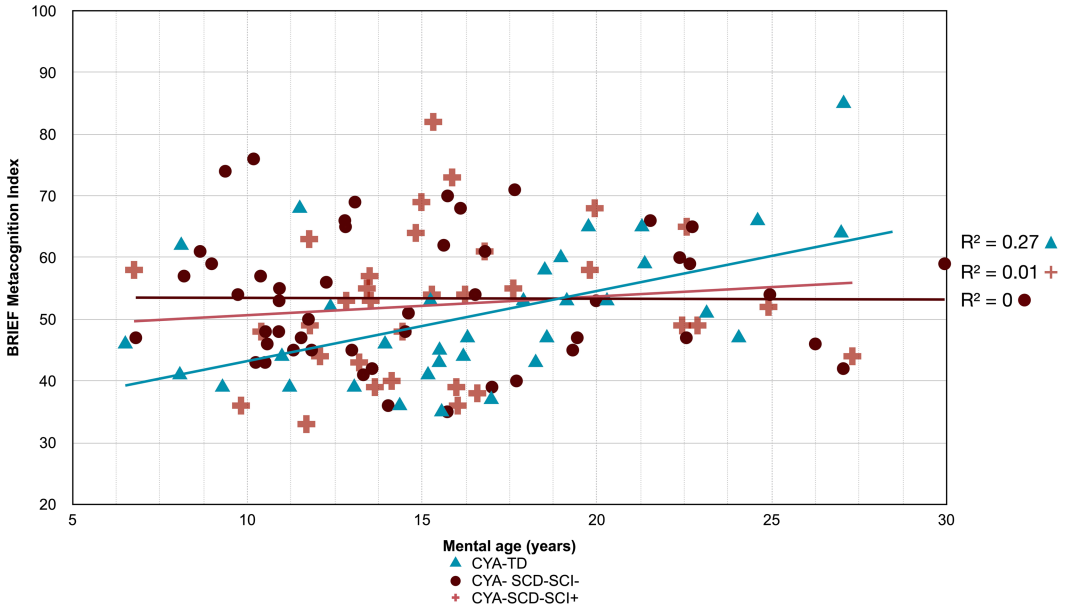


FIGURE 7
Developmental trajectory of BRIEF MI. CYA-SCD-SCI+, children and young adults with sickle cell disease with silent cerebral infarcts; CYA-SCD-SCI–, children and young adults with sickle cell disease without silent cerebral infarcts; CYA-TD, children and young adults who are typically developing.

CYA-TD, while CYA-SCD-SCI– scored 14 points above CYA-TD, indicative of early EF challenges pertaining to planning and organising, task initiation, self-monitoring, and working memory. The rate of development for BRIEF MI significantly differed between CYA-SCD-SCI+ and CYA-TD which was not the case for the CYA-SCD-SCI– group. CYA-SCD-SCI+ showed a 26% delay in the *rate* of development as compared to the CYA-TD group. This pattern of results suggests that presence of SCI has a significant effect on the development of EF in CYA-SCD (Table 3 and Figure 7).

3.5. Cross-sectional developmental trajectory analysis for raw scores

Results for raw scores were tabulated and can be found in the supplementary material for all analyses conducted (see Supplementary Table S5–S8). There was a statistically significant result for the CYA-TD group (Supplementary Table S5) for the Wechsler VCI Similarities and PRI Block design and for the BRIEF GEC, BRI and BRI subtest Inhibit as well as for BRIEF MI subtests Initiate and Organise Material and the Tower Total, while PSI Symbol Search was close to significance. There were no differences in developmental trajectory for the Wechsler raw scores but there were delays at onset and slower rates of development for BRIEF GEC, BRI, BRI Inhibit and MI Initiate raw scores (See Supplementary Table S6). On rotation analysis (see Supplementary Table S7), there was no systematic relationship between BRIEF GEC while there were zero trajectories for BRIEF BRI and BRIEF MI subtest Initiate with a significant delay at onset and a trend for a slower rate of development for CYA-SCD-SCI+ compared with CYA-TD (see Supplementary Table S8).

3.6. Exploratory analysis: effect of haemoglobin, CaO_2, SpO_2 and SES on cognition

To examine the effect of blood oxygenation measures (haemoglobin, SpO_2, and CaO_2) and socioeconomic status (SES) on cognition (scaled scores) in CYA-SCD, we generated simple linear regression models for each of the blood oxygenation measures predicting each cognitive variable separately for CYA-SCD only. All significant results are shown in Table 4 for CYA-SCD and Supplementary Table S9 for CYA-SCD-SCI+.

3.6.1. Haemoglobin

For CYA-SCD we found that haemoglobin significantly predicted the variance in VCI (10%, $p = 0.021$), VCI Similarities (8%, $p = 0.037$) and Vocabulary (4% $p = 0.041$), PSI (4%, $p = 0.04$) and BRIEF Working Memory subscale (8.2%, $p = 0.036$). Greater variance was explained by haemoglobin for PRI in the CYA-SCD-SCI+ (29%, $p = 0.013$).

3.6.2. Arterial oxygen content (CaO_2)

For CYA-SCD CaO_2 significantly predicted the variance in VCI (12%, $p = 0.01$) and its subscales Similarities (10%, $p = 0.022$) and Vocabulary (4%, $p = 0.038$), PSI (4%, $p = 0.033$) and its subscale Symbol Search (4%, $p = 0.032$) and BRIEF Working Memory (7.2%, $p = 0.049$). Greater variance was explained by CaO_2 for VCI (22%, $p = 0.004$) and PRI in the CYA-SCD-SCI+ (37%, $p = 0.005$).

3.6.3. Oxygen saturation (SpO_2)

For CYA-SCD SpO_2 only significantly predicted the variance in VCI (9%, $p = 0.027$).

TABLE 3 Developmental trajectories for CYA-SCD with and without SCI and CYA-TD.

Variable	Slope: cognitive variable=(intercept for group)+(age * gradient)			
	CYA-SCD		CYA-TD	
	SCI+	SCI−		
Wechsler cognitive score				
VCI	80.358 + age * 0.111	78.993 + age * 0.083	90.732 + age * 0.139	
BRIEF				
BRIEF MI	49.600 + age * 0.025	53.513 + age * −0.001	39.734 + age * 0.093	

Developmental trajectories for SCD with and without SCI and controls.												
Variable	Delay at onset SCD		*p*		CI 95%		Rate of development for SCD		*p*		CI 95%	
	CYA-SCD compared with CYA-TD											
	SCI+	SCI−	SCI+	SCI−	SCI+	SCI−	SCI+	SCI	SCI+	SCI−	SCI+	SCI−
Wechsler cognitive score												
VCI	10.37	11.73	0.07	0.85	[67.936, 92.779]	[−16.028, 13.297]	0.79	0.59	0.61	0.36	[−0.150, 0.055]	[−0.080, 0.135]
BRIEF												
BRIEF MI	−9.87	−13.77	0.03	0.11	[41.056, 58.144]	[−21.942, 2.211]	0.26	−0.01	0.05	0.53	[−0.044, 0.095]	[−0.110, 0.057]

Delay at onset = intercept of CYA-TD group − intercept of CYA-SCD group; rate of development = gradient of CYA-SCD group/gradient of CYA-TD group. CYA-SCD-SCI+, children and young adults with sickle cell disease with silent cerebral infarcts; CYA-SCD-SCI−, children and young adults with sickle cell disease without silent cerebral infarcts; CYA-TD, children and young adults who are typically developing; VCI, Verbal Comprehension Index; BRIEF, Behaviour Rating Inventory of Executive Function; BRIEF MI, Metacognition Index.

3.6.4. Effect of socioeconomic status on cognition

For CYA-SCD, SES significantly predicted the variance in BRIEF MI subscale Organize Material (9%, $p = 0.017$). Greater variance was explained by BRIEF BRI Inhibit in the CYA-SCD-SCI+ (22%, $p = 0.058$), although near-significant.

4. Discussion

Compared to intensively researched neurodevelopmental disorders such as Autism and Attention Deficit Hyperactivity Disorder, which are common in the general paediatric population, there are relatively few studies examining cognitive and behavioural profiles in children with underlying medical conditions such as those living with SCD. Developmental delay implies that children do not reach their typical developmental milestone/s in cognition or motor development (48), deviating months from the typically-developing population. A recent systematic review in infants living with SCD (0–48 months) found that 17.5%–50% experience developmental delay in cognition and language development, which had a significant impact by the second year of life (49). Given that developmental delay had received limited attention in CYA-SCD, the main objective of our study was to compare cross-sectional developmental trajectories between CYA-SCD and CYA-TD, who were matched on age and ethnicity. The cross-sectional developmental trajectory approach gives an initial understanding of how cognitive performance might develop with age and differ compared to CYA-TD.

We used common measures of intelligence (Weschler Scales of Intelligence) and EF (BRIEF, D-KEFS). Our findings indicate that CYA-SCD perform poorly on tests of cognitive ability as compared to the CYA-TD. These findings are consistent with previous literature (5, 35, 50). However, the differences are small after controlling for confounders. In our study, CYA-SCD showed developmental delay (difference at age of testing) in verbal comprehension, and developmental delay as well as slower development (rate) in EF (see Summary findings, Figure 8) compared to the CYA-TD. The same was observed for CYA-SCD-SCI+ compared to CYA-TD. However, greater delay and slower development was observed for CYA-SCD-SCI+ and EF (BRIEF MI: meta cognition), suggesting greater EF challenges in CYA-SCD-SCI+ (see Summary findings, Figure 8).

Our initial analyses prompted us to examine if blood oxygenation measures such as haemoglobin, SpO_2, and CaO_2 have a significant effect on cognitive development in CYA-SCD (see Summary findings, Figure 9). We found that all blood oxygenation measures (haemoglobin, SpO_2, and CaO_2) had a significant relationship with verbal comprehension, whereas haemoglobin and CaO_2 alone predicted processing speed, suggesting a dominant effect of haemoglobin in this analysis. Interestingly, haemoglobin and socioeconomic status (SES) alone predicted caregiver and self-reported EF. In CYA-SCD-SCI+, haemoglobin and CaO_2 predicted perceptual reasoning (problem solving), while SES alone predicted caregiver and self-reported EF. These data suggest that development of cognitive function, especially EF and problem solving skills, are impaired in CYA-SCD and may be associated with poor blood oxygenation, possibly leading to haemodynamic stress, during development (51, 52).

TABLE 4 Simple linear regression models for blood oxygenation measures and SES predicting cognitive variables in children and young adults with sickle cell disease.

Measure	Cognitive variables	CYA-SCD						
		N	*F*	*p*	*R2*	Unst. B	Std. Beta	CI 95%
Haemoglobin								
	VCI	55	5.67	0.02	0.10	0.28	0.31	[0.05, 0.53]
	Similarities	55	4.58	0.04	0.08	0.05	0.28	[0,0.10]
	Vocabulary	106	4.30	0.04	0.04	0.04	0.20	[0, 0.08]
	PSI	106	4.33	0.04	0.04	0.18	0.20	[0.01, 0.36]
	BRIEF Working Memory	54	4.63	0.04	0.08	−0.24	−0.29	[−0.50, −0.02]
CaO_2								
	VCI	55	7.07	0.01	0.12	2.35	0.34	[0.58, 4.11]
	Similarities	55	5.60	0.02	0.10	0.43	0.31	[0.07, 0.79]
	Vocabulary	106	4.43	0.04	0.04	0.30	0.20	[0.02, 0.59]
	PSI	106	4.65	0.03	0.04	1.38	0.21	[0.11, 2.66]
	Symbol Search	106	4.74	0.03	0.04	0.21	0.06	[0.03, 0.63]
	BRIEF Working Memory	54	4.05	0.05	0.07	−1.72	−0.27	[−3.4, −0.01]
SpO_2								
	VCI	55	5.18	0.03	0.09	1.47	0.30	[0.17, 2.76]
SES								
	BRIEF Organise Material	64	6.04	0.02	0.07	0	0.30	[0, 0]

CYA-SCD, children and young adults with sickle cell disease; Unst., unstandardised; Std., standardised; CaO_2, arterial oxygen content; SpO_2, Oxygen saturation; SES, Socioeconomic status; VCI, Verbal Comprehension Index; PSI, Processing Speed Index; BRIEF, Behaviour Rating Inventory of Executive Function; BRIEF MI, Metacognition Index.

4.1. Cross-sectional developmental trajectories

4.1.1. Wechsler scales

The main findings of our paper indicate that CYA-SCD tend to have a delay in verbal comprehension skills, but they also appear to have a catch-up period and are no longer delayed as young adults. However, this initial delay could impact on later successful reading skills (50) and successful academic performance in school-aged children (53). Additionally, research in healthy infants between the ages of 7–12 months has shown a significant correlation between language development (Mullen Scales of Early Learning) and grey and white matter volume (e.g., subcortical region hippocampus) (54). Specifically, for CYA-SCD, Steen et al. (55) demonstrated that grey matter volume growth is delayed in children living with SCD aged 3.9–18.5 years. Another factor to consider is that grey matter volume reduces between 2 to 9 years of age in parallel with white matter myelination, after which grey matter volumes stabilise until late adulthood (55). Therefore, the observed developmental delay in verbal comprehension could be related to delayed grey matter volume development in CYA-SCD. Neuroimaging studies have shown associations between frontal lobe volumes and verbal comprehension tasks in CYA-TD (56). While this association remains unexplored in CYA-SCD, it is possible that reduced volumes in the frontal lobe could be contributing factors to the delay observed in verbal comprehension development.

Additional research in CYA-SCD aged 7–17 years found lower verbal comprehension performance (Woodcock-Johnson-III) in those with greater frontal–parietal cortical lesions (57), which was also related to lower cognitive control (Examiner Battery: measuring inhibition and dysregulation of EF). Interestingly, cognitive control is important for successful language comprehension (58). We know that children living with SCD experience EF difficulties (59) affecting the central executive and working memory performance. It is possible that there is a relationship between EF and verbal comprehension in CYA living with SCD as well, as previously shown in a healthy adult population (60). However, these hypotheses need further investigation.

It is important to mention that there may be other factors contributing to the delay observed in verbal comprehension, such as prematurity (61), SES (62), low school attendance and missed schooling due to hospital visits and health related problems. For example, painful crisis is common and is also associated with reduced grey matter volumes in CYA-SCD (63). This evidence suggests that there could be multiple factors affecting verbal comprehension development in CYA-SCD which need to be explored in future research.

4.1.2. Executive function

Several studies have documented impaired EF skills in CYA-SCD. Our results not only agree with the previous literature (64, 65), but demonstrate that the development of EF skills in CYA-SCD are not only delayed, but show a slower rate of development. Our

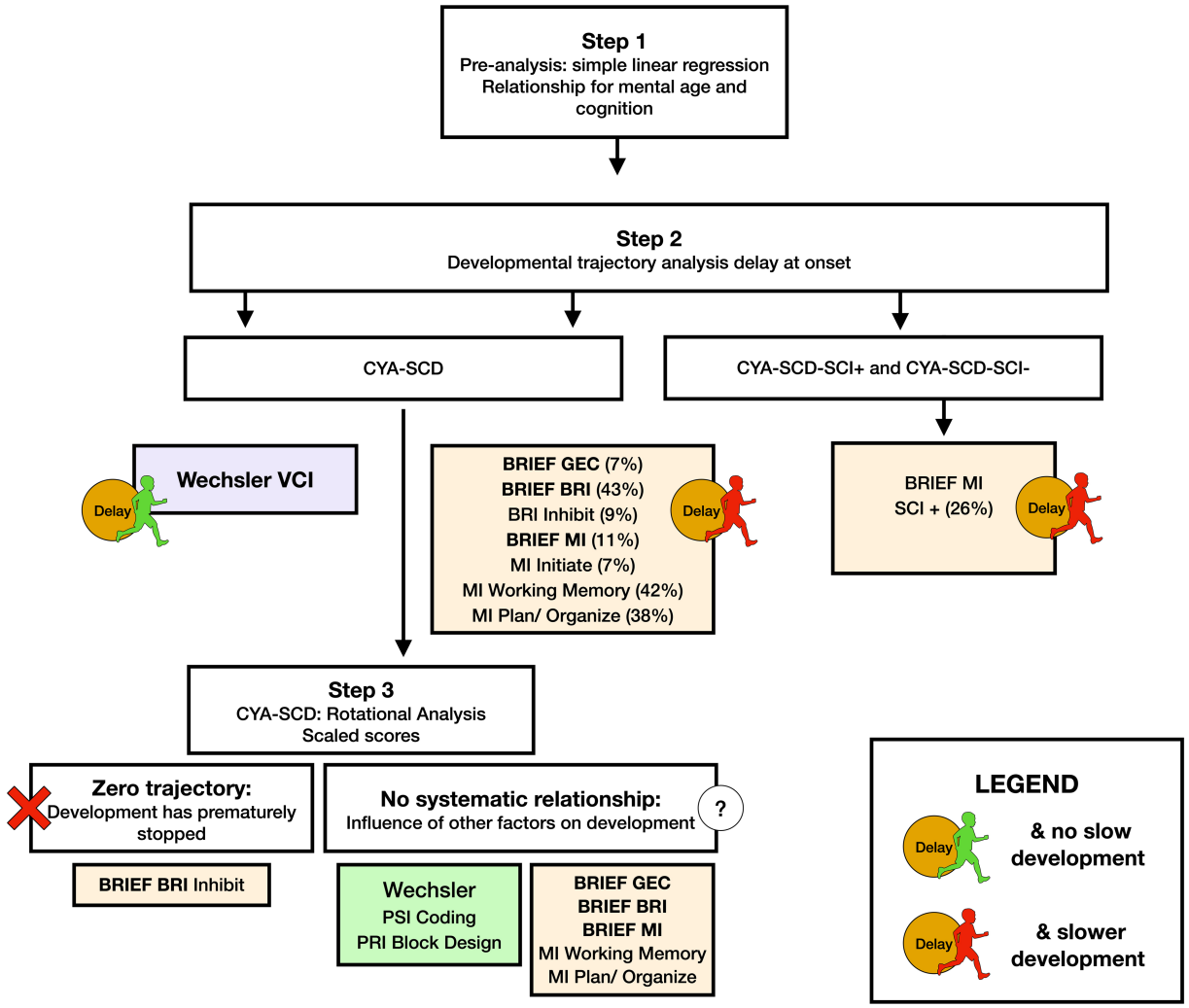


FIGURE 8
Summary findings of developmental analysis for mental age and cognition in CYA-SCD, and CYA-SCD with and without silent cerebral infarct. CYA-SCD, children and young adults living with sickle cell disease; CYA-SCD-SCI+, children and young adults with sickle cell disease with silent cerebral infarcts; CYA-SCD-SCI–, children and young adults with sickle cell disease without silent cerebral infarcts; BRIEF, Behavior Rating Inventory of Executive Function; BRIEF MI, Metacognitive Index; VCI, Verbal Comprehension Index.

findings indicate that EF difficulties continue into young adulthood. Most difficulties seem to relate to the child's ability to control and regulate emotions and behaviour, important for self-regulation (measured on the BRIEF BRI). Our research is the first to show that CYA-SCD plateau prematurely on the development impulsivity and inhibitory control (measured on the BRIEF BRI Inhibition). Recent research in children living with SCD aged 8–15 years found that impulsivity (Conners' Continuous Performance Test) was negatively associated with health-related quality of life (Paediatric Quality of Life Inventory Sickle Cell Disease Module) (66). There is also a high incidence of ADHD diagnosis (25%) in children living with SCD aged 8–16 years, which may contribute to EF difficulties in these patients (67).

Parents of children living SCD observe behavioural difficulties already at a young age. We found that they have difficulties anticipating an activity or task, develop ideas and problem-solving strategies, which are crucial for efficient time management and planning (measured on the BRIEF MI). Similar findings were observed in 8- to 12-year old children living with SCD compared to typically developing controls (68). Berg et al. (68) found higher scores on the parent and teacher BRIEF for MI and GEC. The authors mention that parents of children living with SCD observe EF difficulties in daily behaviour such as organizing and remembering things (i.e., homework materials, daily chores) and initiating behaviour to start tasks.

Interestingly, older CYA-TD in our sample had more EF difficulties compared to CYA-SCD. There are multiple factors which need to be considered when understanding EF development. In addition to changes in adolescence which may be prolonged into early adult life, the impact of psychosocial factors (i.e., family environment, parental support and engagement) (69) and even school environment (70) could contribute to individual differences in EF-skills development. Studies have shown that family function plays an important role in the development of EF skills early in life (71). Downes et al. (71) noted that a positive family environment and

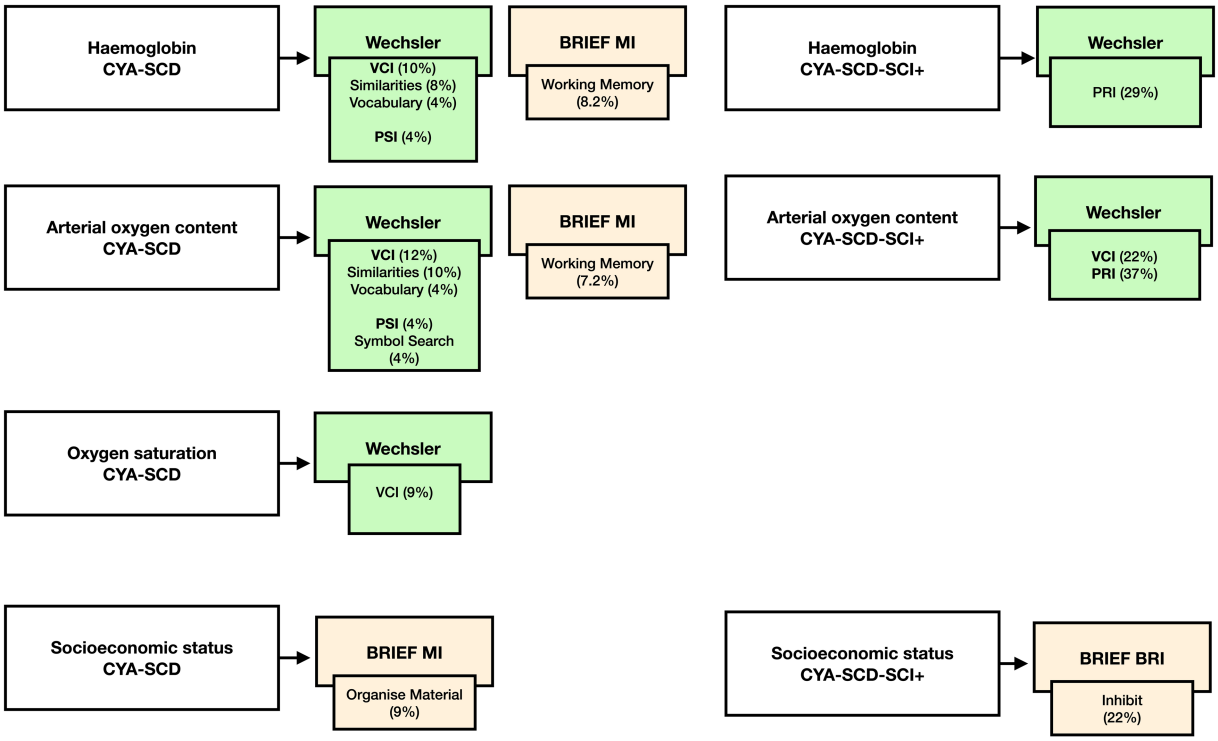


FIGURE 9
Summary findings of the effect of haemoglobin, CaO_2, SpO_2 and SES on cognition CYA-SCD and CYA-SCD-SCI+. CYA-SCD-SCI+, children and young adults with sickle cell disease with silent cerebral infarcts; CYA-SCD-SCI–, children and young adults with sickle cell disease without silent cerebral infarcts; BRIEF, Behavior Rating Inventory of Executive Function; VCI, Verbal Comprehension Index; PSI, Processing Speed Index; RRI, Perceptual Reasoning Index.

functioning significantly impacted attention control and cognitive flexibility in pre-schoolers living with SCD. It is likely that these functions, especially attention control, may have a cascading effect on the development of other EF skills, suggesting that efforts to support families during the early years could benefit EF development later in life. However, health plays a crucial role in children with SCD, which needs to be considered as well. Current research in people living with SCD in a similar age range to our study (6–31 years) has shown that this group is vulnerable to EF deficits because of cerebral haemodynamic stress related to chronic anaemia and oxygen desaturation (52). For example, cerebral blood flow increases to maintain adequate oxygen delivery to the cortex and is an indicator of cerebral hemodynamic stress, which is associated with worse EF, as measured by the D-KEFS Tower Test (72). One neuroimaging study in the SCD population showed reduced resting state activity in the default mode network (important for cognitive processing) but increased activity in the pain processing regions suggesting allocation of cognitive resources towards pain management which may hinder normal EF development (73).

Besides multiple bio-psychosocial factors that affect EF development, it is also possible that our results could be attributed to recruitment methods. Other cohort participants have attended a neuropsychological evaluation based on a medical referral for academic concerns, whereas we recruited participants from clinics and community settings regardless of any history of cognitive difficulties, so it is probable that our sample has a broader range of EF profiles, making our results applicable in a wider clinical context.

4.1.3. Presence of SCI and cognition

We found that CYA-SCD-SCI+ showed more delay at onset and a slower rate of development for BRIEF MI. These results are in line with previous literature showing an association between poor EF and SCI status (74). Although we did not examine the site of SCI lesions, previous studies looking at SCI presence have noted that frontal lobe SCI lesions could explain poor EF, especially metacognition, such as inhibition and working memory (74, 75). People living with SCD and SCI tend to have poorer white matter microstructural integrity as compared to people living with SCD without SCI which may affect EF as well (76). However, more diffusion tensor-based imaging studies as well as functional connectivity on MRI studies are needed to explore the multidimensional relationship between structural connectivity and EF in detail.

4.1.4. Factors affecting cognition

In addition to finding that verbal comprehension was delayed in participants with SCI (although not significantly), we also found that haemoglobin, SpO_2, and CaO_2 are all predictors of verbal comprehension in CYA-SCD. Haemoglobin and CaO_2 were also associated with processing speed. These results are in line with previous studies exploring effects of blood oxygenation measures on cognition in people living with SCD. Using a path-analysis model, Hogan et al. (34) found that reduced SpO_2 is compensated by increased cerebral blood flow to the brain which results in lowered verbal IQ in adolescents living with SCD (mean age 17.4 years). Several studies of blood oxygenation measures and cognition have found that reduced haemoglobin is associated with reduced IQ in adolescents and young adults (51) and also neurodevelopmental delay in 9- to 12-month old infants living with

SCD (77). Similarly, reduced processing speed was linked to lower CaO_2, poor white matter microstructural integrity in (35) and reduced white matter structural connectivity (78, 79) in CYA-SCD. Taken together, these data suggest that cerebral haemodynamic stress, central to sickle cell anaemia pathology, may result in poor long-term cognitive outcomes.

4.2. Limitations

There were some limitations to our study, which should be considered for future research. We did not include other factors that have been shown to impact on cognition in people living with SCD, such as: sleep and cortisol (80) and medical interventions (i.e., hydroxyurea and blood transfusions) (81, 82), and cortical brain areas affected by stroke. We did not exclude CYA-TD with SCI. The cross-sectional developmental trajectory approach gives only an initial understanding as to how cognitive performance might develop with age but needs to be validated in longitudinal follow-up studies. Therefore, observing these children from birth, taking both disease-related (83), social and environmental factors (84) into account would be the ideal way to assess developmental changes and to control for potential confounding variables. However, this methodology is challenging due to funding restrictions and participants being lost to follow-up. Although the Wechsler tests are widely used they may underestimate the influence of general and fluid intelligence on the other cognitive domains (85). Various subtests of the Wechsler tests require intact EF although it is not measured directly, such as the subtest digit span backwards included in the Working Memory Index (86). We can find EF in most cognitive processing. For example, research in young and older adults (18–88 years) has shown that FSIQ, Verbal Comprehension Index and Working Memory Index on the WAIS-IV were strongly correlated with EF skills measured on the Neuropsychological Assessment Battery, suggesting an impact of EF on other cognitive domains (87). Future research should carefully select quantitative cognitive assessments, such as the NIH Toolbox assessing EF in CYA living with sickle cell disease (23, 88). Adolescents and young adults living with SCD and executive dysfunction may also have learning difficulties (89), which we did not screen for, although the participants in our cohorts had FSIQ within the average range and only 4 (4.4%) showed an FSIQ $\leq$69. Current research addresses this issue and recommends screening for neurodevelopmental disorders (e.g., autism) to implement early interventions, which can result in better treatment efficacy (90). Screening should also consider language, education, and culture bias, since most of the neuropsychological assessments are not language and culture free. Further, the BRIEF is a caregiver report which potentially makes it more vulnerable to bias. Future research could also incorporate a mixed method approach (i.e., using qualitative and quantitative measures). Teachers and caregiver perspective on cognitive abilities would provide some needed background to the current findings.

4.3. Conclusion

Our findings demonstrate that when considering cognitive developmental trajectories in CYA-SCD, their cognitive profiles are not impaired, but verbal comprehension is delayed with later catch-up and EF appears to develop at a slower rate. It is recommended that, in addition to initiation of medical treatment, early cognitive intervention and educational support could strengthen cognitive development.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving human participants were reviewed and approved by West London NHS (SAC; 05/Q0408/42, 11/EM/0084, 15/LO/0347), Yorkshire NHS (POMS; 15/YH/0213), and University College London (14475/001). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

MK conceptualised the paper, completed the review, and drafted the manuscript. MK and HS were involved in data collection. MK and SH analysed the data supervised by FK, AH, and DD. HS and AH contributed to the review and provided feedback on the manuscript. FK and DD conceptualised the paper and provided feedback on the manuscript. All authors contributed to the article and approved the submitted version.

Funding

MK and HS were funded by Action Medical Research (GN2509).

Acknowledgments

We would like to thank the families who took part in the study.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2023.1087054/full#supplementary-material

References

1. Aygun B, Odame I. A global perspective on sickle cell disease. *Pediatr Blood Cancer*. (2012) 59:386–90. doi: 10.1002/pbc.24175

2. Kirkham FJ, Lagunju IA. Epidemiology of stroke in sickle cell disease [Internet]. *J Clin Med*. (2021) 10:4232. doi: 10.3390/jcm10184232

3. Nawaiseh M, Shaban A, Abualia M, Haddadin R, Nawaiseh Y, AlRyalat SA, et al. Seizures risk factors in sickle cell disease. The cooperative study of sickle cell disease. *Seizure*. (2021) 89:107–13. doi: 10.1016/j.seizure.2021.05.009

4. Schatz J, McClellan CB. Sickle cell disease as a neurodevelopmental disorder. *Dev Disabil Res Rev*. (2006) 12:200–7. doi: 10.1002/mrdd.20115

5. Kawadler JM, Clayden JD, Clark CA, Kirkham FJ. Intelligence quotient in paediatric sickle cell disease: a systematic review and meta-analysis. *Dev Med Child Neurol*. (2016) 58:672–9. doi: 10.1111/dmcn.13113

6. Armstrong FD, Thompson RJ Jr, Wang W, Zimmerman R, Pegelow CH, Miller S, et al. Cognitive functioning and brain magnetic resonance imaging in children with sickle cell disease. *Pediatrics*. (1996) 97:864–70. doi: 10.1542/peds.97.6.864

7. Prussien KV, Jordan LC, Debaun MR, Compas BE. Cognitive function in sickle cell disease across domains, cerebral infarct status, and the lifespan: a meta-analysis. *J Pediatr Psychol*. (2019) 44:948–58. doi: 10.1093/jpepsy/jsz031

8. Stotesbury H, Kawadler JM, Clayden JD, Saunders DE, Hood AM, Koelbel M, et al. Quantification of silent cerebral infarction on high-resolution FLAIR and cognition in sickle cell Anemia. *Front Neurol [Internet]*. (2022) 13:867329. doi: 10.3389/fneur.2022.867329

9. Karmiloff-Smith A, D'Souza D, Dekker TM, van Herwegen J, Xu F, Rodic M, et al. Genetic and environmental vulnerabilities in children with neurodevelopmental disorders. *PNAS Nexus*. (2012) 109:17261–5. doi: 10.1073/pnas.1121087109

10. Jacob M, Stotesbury H, Kija E, Saunders D, Mtei RJ, Tutuba H, et al. Effect of age, cerebral infarcts, vasculopathy and haemoglobin on cognitive function, in Tanzanian children with sickle cell anaemia. *Europ J Paediat Neurol*. (2022) 37:105–13. doi: 10.1016/j.ejpn.2022.01.010

11. Kreutzner JC, DeLuca J, Caplan B. WISC-iv In: . *Encyclopedia of clinical neuropsychology*. New York: Springer (2011). 2719.

12. Drozdick LW, Wahlstrom D, Zhu J, Weiss LG. The Wechsler adult intelligence scale—fourth edition and the Wechsler memory scale—fourth edition [Internet] In: DW Flanagan and PL Harrison, editors. *Contemporary intellectual assessment: theories, tests, and issues. 3rd* ed. New York City: Guilford Press (2012). 223–926.

13. Happé F. Fluid intelligence In: FR Volkmar, editor. *Encyclopedia of autism spectrum disorders [Internet]*. New York, NY: Springer (2013). 1310–1.

14. Cattell R. *Abilities: their structure, growth, and action [Internet]*. New York: Houghton Mifflin (1971).

15. Happé F. Crystallized intelligence In: FR Volkmar, editor. *Encyclopedia of autism spectrum disorders [Internet]*. New York, NY: Springer (2013). 824–5.

16. Rottschy C, Langner R, Dogan I, Reetz K, Laird AR, Schulz JB, et al. Modelling neural correlates of working memory: a coordinate-based meta-analysis. *NeuroImage*. (2012) 60:830–46. doi: 10.1016/j.neuroimage.2011.11.050

17. Roger C, Núñez Castellar E, Pourtois G, Fias W. Changing your mind before it is too late: the electrophysiological correlates of online error correction during response selection. *Psychophysiology*. (2014) 51:746–60. doi: 10.1111/psyp.12224

18. McAlister C, Schmitter-Edgecombe M. Executive function subcomponents and their relations to everyday functioning in healthy older adults. *J Clin Exp Neuropsychol*. (2016) 38:925–40. doi: 10.1080/13803395.2016.1177490

19. Garon N, Bryson SE, Smith IM. Executive function in preschoolers: a review using an integrative framework. *Psychol Bull [Internet]*. (2008) 134:31–60. doi: 10.1037/0033-2909.134.1.31

20. Thompson A, Steinbeis N. Sensitive periods in executive function development. *Curr Opin Behav Sci*. (2020) 36:98–105. doi: 10.1016/j.cobeha.2020.08.001

21. Hackman DA, Gallop R, Evans GW, Farah MJ. Socioeconomic status and executive function: developmental trajectories and mediation. *Dev Sci [Internet]*. (2015) 18:686–702. doi: 10.1111/desc.12246

22. Hoyt CR, Varughese TE, Erickson J, Haffner N, Luo L, L'Hotta AJ, et al. Developmental delay in infants and toddlers with sickle cell disease: a systematic review [Internet]. *Dev Med Child Neurol*. (2022) 64:168–75. doi: 10.1111/dmcn.15048

23. Hood AM, King AA, Fields ME, Ford AL, Guilliams KP, Hulbert ML, et al. Higher executive abilities following a blood transfusion in children and young adults with sickle cell disease. *Pediatr Blood Cancer [Internet]*. (2019) 66:e27899. doi: 10.1002/pbc.27899

24. Jacob M, Stotesbury H, Kija E, Saunders D, Mtei RJ, Tutuba H, et al. Effect of age, cerebral infarcts, vasculopathy and haemoglobin on cognitive function, in Tanzanian children with sickle cell anaemia. *Eur J Paediatr Neurol*. (2022) 37:105–13. doi: 10.1016/j.ejpn.2022.01.010

25. Karmiloff-Smith A. Development itself is the key to understanding developmental disorders [Internet]. *Trends Cogn Sci*. (1998) 2:389–98. doi: 10.1016/s1364-6613(98)01230-3

26. D'Souza D, Karmiloff-Smith A. Why a developmental perspective is critical for understanding human cognition. *Behav Brain Sci [Internet]*. (2016) 39:e122. doi: 10.1017/S0140525X15001569

27. Wang W, Enos L, Gallagher D, Thompson R, Guarini L, Vichinsky E, et al. Neuropsychologic performance in school-aged children with sickle cell disease: a report from the cooperative study of sickle cell disease. *J Pediatr*. (2001) 139:391–7. doi: 10.1067/mpd.2001.116935

28. Ampomah MA, Drake JA, Anum A, Amponsah B, Dei-Adomakoh Y, Anie K, et al. A case-control and seven-year longitudinal neurocognitive study of adults with sickle cell disease in Ghana. *Br J Haematol [Internet]*. (2022) 199:411–26. doi: 10.1111/bjh.18386

29. Annaz D, Remington A, Milne E, Coleman M, Campbell R, Thomas MSC, et al. Atypical development of motion processing in children with autism. *Dev Sci*. (2010) 13:826–38. doi: 10.1111/j.1467-7687.2009.00939.x

30. Van Herwegen J, Ranzato E, Karmiloff-Smith A, Simms V. The foundations of mathematical development in Williams syndrome and down syndrome. *J Appl Res Intellect Disabil [Internet]*. (2020) 33:1080–9. doi: 10.1111/jar.12730

31. Hamdule SI, Kölbel M, Stotesbury H, Murdoch R, Clayden JD, Sahota SS, et al. Effects of regional brain volumes on cognition in sickle cell anaemia: a developmental perspective. *Front Neurol [Internet]*. (2023) 14:143. doi: 10.3389/fneur.2023.1101223

32. Thomas MSC, Annaz D, Ansari D, Scerif G, Jarrold C, Karmiloff-Smith A. Using developmental trajectories to understand developmental disorders. *J Speech Lang Hear Res*. (2009) 52:336–58. doi: 10.1044/1092-4388(2009/07-0144)

33. Hood AM, Crosby LE, Stotesbury H, Kölbel M, Kirkham FJ. Considerations for selecting cognitive endpoints and psychological patient-reported outcomes for clinical trials in pediatric patients with sickle cell disease. *Front Neurol*. (2022) 13:1221. doi: 10.3389/fneur.2022.835823

34. Hogan AM, Pit-ten Cate IM, Vargha-Khadem F, Prengler M, Kirkham FJ. Physiological correlates of intellectual function in children with sickle cell disease: hypoxaemia, hyperaemia and brain infarction. *Dev Sci*. (2006) 9:379–87. doi: 10.1111/j.1467-7687.2006.00503.x

35. Stotesbury H, Kirkham FJ, Kölbel M, Balfour P, Clayden JD, Sahota S, et al. White matter integrity and processing speed in sickle cell anemia. *Neurology*. (2018) 90:e2042–50. doi: 10.1212/WNL.0000000000005644

36. Rosen CL, Debaun MR, Strunk RC, Redline S, Seicean S, Craven DI, et al. Obstructive sleep apnea and sickle cell anemia. *Pediatrics [Internet]*. (2014) 134:273–81. doi: 10.1542/peds.2013-4223

37. Howard J, Slee AE, Skene S, Inusa B, Kawadler J, Downes M, et al. Overnight auto-adjusting continuous airway pressure+standard care compared with standard care alone in the prevention of morbidity in sickle cell disease phase II (POMS2b): study protocol for a randomised controlled trial. *Trials*. (2018) 19:1–10. doi: 10.1186/s13063-017-2419-0

38. Kölbel M, Kirkham F, Iles RK, Stotesbury H, Halstead E, Brenchley C, et al. Exploring the relationship of sleep, cognition, and cortisol in sickle cell disease. *Compr Psychoneuroendocrinol [Internet]*. (2022) 10:100128. doi: 10.1016/j.cpnec.2022.100128

39. DeBaun MR, Gordon M, McKinstry RC, Noetzel MJ, White DA, Sarnaik SA, et al. Controlled trial of transfusions for silent cerebral infarcts in sickle cell anemia. *N Engl J Med*. (2014) 371:699–710. doi: 10.1056/NEJMoa1401731

40. Kölbel M, Kirkham FJ, Dimitriou D. Developmental profile of sleep and its potential impact on daytime functioning from childhood to adulthood in sickle cell anaemia. *Brain Sci [Internet]*. (2020) 10:1–22. doi: 10.3390/brainsci10120981

41. Heald A, Laing I, McLernon DJ, Donn R, Hartland AJ, Fryer AA, et al. Socioeconomic deprivation as measured by the index of multiple deprivation and its association with low sex hormone binding globulin in women. *Open Biochem J [Internet]*. (2017) 11:1–7. doi: 10.2174/1874091X01711010001

42. Ministry of Housing C and LG. English indices of deprivation [Internet]. Ministry of Housing, Communities & Local Government (2019). Available at: https://www.google.com/search?client=firefox-b-e&q=Index+of+Multiple+Deprivation+Rank (Accessed October 30, 2020).

43. Wechsler D. *WASI: Wechsler abbreviated scale of intelligence*. San Antonio, TX: Psychological Corporation (1999).

44. Delis DC, Kaplan E, Kramer JH. *Delis–Kaplan executive functioning system: Examiner's manual*. San Antonio, TX: The Psychological Corporation (2001).

45. Gioia GA, Isquith PK, Guy SC, Kenworthy L. Behavior rating inventory of executive function. *Child Neuropsychol*. (2000) 6:235–8. doi: 10.1076/chin.6.3.235.3152

46. Roth RM, Isquith PK, Gioia GA. *BRIEF-A: Behavior rating inventory of executive function — adult version*. Lutz, FL: Psychological Assessment Resources (2005).

47. Pino MC, Mazza M, Mariano M, Peretti S, Dimitriou D, Masedu F, et al. Simple mindreading abilities predict complex theory of mind: developmental delay in autism spectrum disorders. *J Autism Dev Disord [Internet]*. (2017) 47:2743–56. doi: 10.1007/s10803-017-3194-1

48. Panoscha R. Developmental delay In: FR Volkmar, editor. *The 5-minute pediatric consult, 8th edition [Internet]*. New York, NY: Springer (2018)

49. Drazen CH, Abel R, Gabir M, Farmer G, King AA. Prevalence of developmental delay and contributing factors among children with sickle cell disease. *Pediatr Blood Cancer [Internet]*. (2016) 63:504–10. doi: 10.1002/pbc.25838

50. NICHD Early Child Care Research Network. Pathways to reading: the role of oral language in the transition to reading. *Dev Psychol*. (2005) 41:428–42. doi: 10.1037/0012-1649.41.2.428

51. Prussien KV, Siciliano RE, Ciriegio AE, Anderson AS, Sathanayagam R, Debaun MR, et al. Correlates of cognitive function in sickle cell disease: a meta-analysis. *J Pediatr Psychol [Internet]*. (2020) 45:145–55. doi: 10.1093/jpepsy/jsz100

52. Prussien KV, Compas BE, Siciliano RE, Ciriegio AE, Lee CA, Kassim AA, et al. Cerebral hemodynamics and executive function in sickle cell Anemia. *Stroke [Internet]*. (2021) 52:1830–4. doi: 10.1161/STROKEAHA.120.032741

53. Kastner JW, May W, Hildman L. Relationship between language skills and academic achievement in first grade. *Percept Mot Skills [Internet]*. (2001) 92:381–90. doi: 10.2466/pms.2001.92.2.381

54. Deniz Can D, Richards T, Kuhl PK. Early gray-matter and white-matter concentration in infancy predict later language skills: a whole brain voxel-based morphometry study. *Brain Lang*. (2013) 124:34–44. doi: 10.1016/j.bandl.2012.10.007

55. Steen RG, Emudianughe T, Hunte M, Glass J, Wu S, Xiong X, et al. Brain volume in pediatric patients with sickle cell disease: evidence of volumetric growth delay? *Am J Neuroradiol*. (2005) 26:455–62.

56. Hidese S, Ota M, Matsuo J, Ishida I, Hiraishi M, Yokota Y, et al. Corrigendum: correlation between the Wechsler adult intelligence scale-3rd edition metrics and brain structure in healthy individuals: a whole-brain magnetic resonance imaging study. *Front Hum Neurosci*. (2020) 14, 14:211. doi: 10.3389/fnhum.2020.00211

57. Smith KE, Schatz J. Working memory in children with neurocognitive effects from sickle cell disease: contributions of the central executive and processing speed. *Dev Neuropsychol*. (2016) 41:231–44. doi: 10.1080/87565641.2016.1238474

58. Shields RH. The role of cognitive control in language comprehension. *Undergrad J Psychol [Internet]*. (2014) 27. Available at: https://journals.charlotte.edu/ujop/article/view/285.

59. Downes M. The development and assessment of executive functioning in preschool children with and without sickle cell anaemia. Doctoral dissertation, UCL (2016).

60. Cabrera C, Harcourt S, Golden C. The relationship between verbal and executive function measures. *Arch Clin Neuropsychol [Internet]*. (2018) 33:703–94. doi: 10.1093/arclin/acy061.126

61. Bills SE, Schatz J, Hunt E, Varanasi S, Johnston J, Bradshaw J. Neurodevelopmental outcomes in preterm children with sickle cell disease. *J Int Neuropsychol Soc*. (2022) 28:1039–49. doi: 10.1017/S1355617721001338

62. IPS C, Viana MB. Cognitive profile of children with sickle cell anemia compared to healthy controls. *J Pediatr (Rio J) [Internet]*. (2019) 95:451–7. doi: 10.1016/j.jped.2018.04.012

63. Sigalla J, Alegria ND, Le Roux E, Toumazi A, Thiollier AF, Holvoet L, et al. Neuropathic pain in children with sickle cell disease: the hidden side of the vaso-occlusive crisis. *Children [Internet]*. (2021) 8:84. doi: 10.3390/children8020084

64. Purvis LN. Establishing validity for measures of executive functioning and behavior in children and adolescents with sickle cell disease [Internet]. ProQuest Dissertations and Theses (2013). Available at: http://proxy.bc.edu/login?url=http://search.proquest.com/docview/1410924210?accountid=9673%5Cnhttp://bc-primo.hosted.exlibrisgroup.com/openurl/BCL/services_page??url_ver=Z39.88-2004&rft_val_fmt=info:ofi/fmt:kev:mtx:dissertation&genre=dissertations+%26+the (Accessed April 14, 2022).

65. Hood AM, Reife I, King AA, White DA. Brief screening measures identify risk for psychological difficulties among children with sickle cell disease. *J Clin Psychol Med Settings [Internet]*. (2020) 27:651–61. doi: 10.1007/s10880-019-09654-y

66. Hood AM, Kölbel M, Stotesbury H, Kawadler J, Slee A, Inusa B, et al. Biopsychosocial predictors of quality of life in paediatric patients with sickle cell disease. *Front Psychol*. (2021) 12:1–13. doi: 10.3389/fpsyg.2021.681137

67. Acquazzino MA, Miller M, Myrvik M, Newby R, Scott JP. Attention deficit hyperactivity disorder in children with sickle cell disease referred for an evaluation. *J Pediatr Hematol Oncol [Internet]*. (2017) 39:350–4. doi: 10.1097/MPH.0000000000000847

68. Berg C, Edwards DF, King A. Executive function performance on the children's kitchen task assessment with children with sickle cell disease and matched controls. *Child Neuropsychol [Internet]*. (2012) 18:432–48. doi: 10.1080/09297049.2011.613813

69. Bell MA, Cuevas K. Psychobiology of executive function in early development In: JA Griffin, MC PD and L Freund, editors. *Executive function in preschool-age children: integrating measurement, neurodevelopment, and translational research*. Washington, DC: American Psychological Association (2015). 157–79.

70. Segundo-Marcos R, Carrillo AM, Fernández VL, González MTD. Development of executive functions in late childhood and the mediating role of cooperative learning: a longitudinal study. *Cogn Dev [Internet]*. (2022) 63:101219. doi: 10.1016/j.cogdev.2022.101219

71. Downes M, Kirkham FJ, Telfer PT, De Haan M. Assessment of executive functions in preschool children with sickle cell anemia. *J Int Neuropsychol Soc [Internet]*. (2018) 24:949–54. doi: 10.1017/S1355617718000255

72. Stotesbury H, Hales PW, Koelbel M, Hood AM, Kawadler JM, Saunders DE, et al. Venous cerebral blood flow quantification and cognition in patients with sickle cell anemia. *J Cereb Blood Flow Metab [Internet]*. (2022) 42:1061–77. doi: 10.1177/0271678X211072391

73. Case M, Zhang H, Mundahl J, Datta Y, Nelson S, Gupta K, et al. Characterization of functional brain activity and connectivity using EEG and fMRI in patients with sickle cell disease. *NeuroImage Clin [Internet]*. (2017) 14:1–17. doi: 10.1016/j.nicl.2016.12.024

74. Brown RT, Davis PC, Lambert R, Hsu L, Hopkins K, Eckman J. Neurocognitive functioning and magnetic resonance imaging in children with sickle cell disease. *J Pediatr Psychol [Internet]*. (2000) 25:503–13. doi: 10.1093/jpepsy/25.7.503

75. Hogan AM, Vargha-Khadem F, Saunders DE, Kirkham FJ, Baldeweg T. Impact of frontal white matter lesions on performance monitoring: ERP evidence for cortical disconnection. *Brain [Internet]*. (2006) 129:2177–88. doi: 10.1093/brain/awl160

76. Jacob M, Stotesbury H, Kawadler JM, Lapadaire W, Saunders DE, Sangeda RZ, et al. White matter integrity in Tanzanian children with sickle cell anemia: a diffusion tensor imaging study. *Stroke*. (2020) 51:1166–73. doi: 10.1161/STROKEAHA.119.027097

77. Hogan AM, Telfer PT, Kirkham FJ, De HM. Precursors of executive function in infants with sickle cell anemia. *J Child Neurol [Internet]*. (2013) 28:1197–202. doi: 10.1177/0883073812453495

78. Clayden JD, Stotesbury H, Kawadler JM, Slee A, Koelbel M, Saunders DE, et al. Structural connectivity mediates the relationship between blood oxygenation and cognitive function in sickle cell anemia. *Blood Adv [Internet]*. (2023) 7:2297–308. doi: 10.1182/bloodadvances.2021006751

79. Stotesbury H, Hales PW, Hood AM, Koelbel M, Kawadler JM, Saunders DE, et al. Individual watershed areas in sickle cell Anemia: an arterial spin labeling study. *Front Physiol*. (2022) 13:664. doi: 10.3389/fphys.2022.865391

80. Kölbel M, Kirkham F, Iles RK, Stotesbury H, Halstead E, Brenchley C, et al. Exploring the relationship of sleep, cognition, and cortisol in sickle cell disease. *Compr Psychoneuroendocrinol [Internet]*. (2022) 10:100128. doi: 10.1016/j.cpnec.2022.100128

81. Puffer E, Schatz J, Roberts CW. The Association of Oral Hydroxyurea Therapy with improved cognitive functioning in sickle cell disease. *Child Neuropsychol [Internet]*. (2007) 13:142–54. doi: 10.1080/09297040600584626

82. Wang WC, Zou P, Hwang SN, Kang G, Ding J, Heitzer AM, et al. Effects of hydroxyurea on brain function in children with sickle cell anemia. *Pediatr Blood Cancer [Internet]*. (2021) 68:e29254. doi: 10.1002/pbc.29254

83. Wang W, Freeman M, Hamilton L, Carroll Y, Kang G, Moen J, et al. Developmental screening of three-year-old children with sickle cell disease compared to controls. *Br J Haematol [Internet]*. (2021) 195:621–8. doi: 10.1111/bjh.17780

84. Schatz J, Reinman L, Bills SE, Johnston JD. Sociodemographic and biomedical correlates of developmental delay in 2- and 4-year-olds with sickle cell disease. *J Dev Behav Pediatr*. (2022) 43:224–32. doi: 10.1097/DBP.0000000000001011

85. Canivez GL, Kush JC. WAIS-IV and WISC-IV structural validity: alternate methods, alternate results. Commentary on Weiss et al. (2013a) and Weiss et al. (2013b). *J Psychoeduc Assess*. (2013) 31:157–69. doi: 10.1177/0734282913478036

86. Holdnack JA. The development, expansion, and future of the WAIS-IV as a cornerstone in comprehensive cognitive assessments In: G Goldstein, DN Allen and J DeLuca, editors. *Handbook of psychological assessment*. Amsterdam: Elsevier Academic Press (2019). 103–39.

87. Buczylowska D, Petermann F. Age-related commonalities and differences in the relationship between executive functions and intelligence: analysis of the NAB executive functions module and WAIS-IV scores. *Appl Neuropsychol [Internet]*. (2017) 24:465–80. doi: 10.1080/23279095.2016.1211528

88. Downes M, Keenan L, Duane Y, Duffy K, Fortune G, Geoghegan R, et al. Executive function in children with sickle cell anemia on transfusion: NIH toolbox utility in the clinical context. *Clin Neuropsychol [Internet]*. (2022) 36:1573–88. doi: 10.1080/13854046.2020.1847325

89. Longoria JN, Pugh NL, Gordeuk V, Hsu LL, Treadwell M, King AA, et al. Patient-reported neurocognitive symptoms influence instrumental activities of daily living in sickle cell disease. *Am J Hematol*. (2021) 96:1396–406. doi: 10.1002/ajh.26315

90. Aguwa CJ, Cannon AD, Casella JF, Shapiro BK, Lance EI. Current developmental screening practices in young children with sickle cell disease. *Br J Haematol [Internet]*. (2023) 200:377–80. doi: 10.1111/bjh.18575

Glossary

BRIEF	Behavior Rating Inventory of Executive Function
BRIEF GEC	BRIEF Global Executive Composite
BRIEF MI	Metacognitive Index
BRIEF RI	Regulation Index
CaO_2	Arterial oxygen content
CYA-SCD	children and young adults living with SCD
EF	Executive function
FSIQ	Full Scale IQ score
Hb	Haemoglobin
MA	Mental age
MRT	Magnetic resonance imaging
PSI	Processing Speed Index
PRI	Perceptual Reasoning Index
SCA	Sickle cell anaemia
SCD	Sickle cell disease
SCI	Silent cerebral infarction
SES	Socioeconomic status
SpO_2	Oxygen saturation
CYA-TD	Typically developing children and young adults
VCI	Verbal Comprehension Index
WMI	Working Memory Index

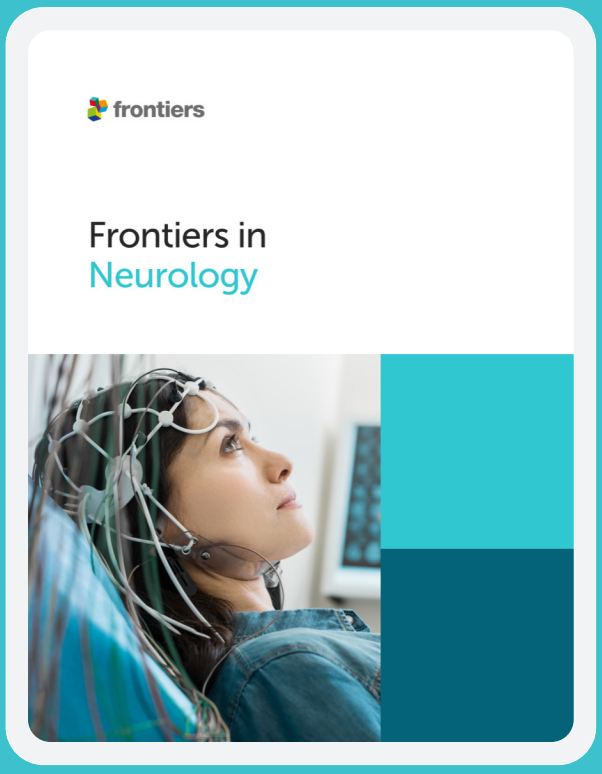
Frontiers in Neurology

Explores neurological illness to improve patient care

The third most-cited clinical neurology journal explores the diagnosis, causes, treatment, and public health aspects of neurological illnesses. Its ultimate aim is to inform improvements in patient care.

Discover the latest Research Topics

See more →



Frontiers

Avenue du Tribunal-Fédéral 34
1005 Lausanne, Switzerland
frontiersin.org

Contact us

+41 (0)21 510 17 00
frontiersin.org/about/contact