Monitoring retinal pathology and cerebral injury in sickle cell disease using spectral-domain optical coherence tomography in pediatric patients

Jing Jin\textsuperscript{1} | Vinay Kandula\textsuperscript{2} | Robin E. Miller\textsuperscript{3}

\textsuperscript{1}Department of Pediatric Ophthalmology, Nemours/Alfred I. duPont Hospital for Children, 1600 Rockland Road, Wilmington, Delaware 19803, USA
\textsuperscript{2}Department of Radiology, Nemours/Alfred I. duPont Hospital for Children, Wilmington, Delaware, USA
\textsuperscript{3}Center for Cancer and Blood Disorders, Nemours/Alfred I. duPont Hospital for Children, Wilmington, Delaware, USA

Correspondence
Jing Jin, Department of Pediatric Ophthalmology, Nemours/Alfred I. duPont Hospital for Children, 1600 Rockland Rd, Wilmington, DE 19803, USA.
Email: jing.jin@nemours.org

Funding information:
National Institute of General Medical Sciences, National Institutes of Health, Grant numbers: U54-GM104941, P20GM109021, and P20GM103446

Abstract

Purpose: This study aimed to confirm the correlation between sickle cell disease (SCD) genotype and retinal damage identified by spectral-domain optical coherence tomography (SD-OCT), and examine a potential link between hypoxic ischemic injury in the retina and brain.

Methods: In this prospective, observational case series, 117 patients (56 males) aged 5–20 years with SCD (36 SC, 68 SS, eight S\textsuperscript{$\beta^+}$ thalassemia, five S\textsuperscript{$\beta^0$} thalassemia) underwent ophthalmologic examination including funduscopy and SD-OCT imaging. Comparison of SCD genotypes and association between ocular findings and cerebrovascular disease (CVD) in subjects with SS/S\textsuperscript{$\beta^0$} genotype were investigated.

Results: Visual acuity ranged from 20/20 to 20/40. On funduscopic exam, 16 of 117 (13.7\%) had retinopathy; 69 of 117 (59.0\%) showed inner retina thinning on SD-OCT. Patients with SS/S\textsuperscript{$\beta^0$} showed a higher frequency of sickle cell retinopathy (SCR) change (68.5\% vs. 47.2\%), bilateral SCR (49.9\% vs. 25.0\%), and foveal involvement (15.1\% vs. 0) than the SC genotype. While funduscopic findings in our cohort with SS/S\textsuperscript{$\beta^0$} genotype showed no correlation with CVD, 20 of 21 patients with CVD had abnormal SD-OCT. Elevated reticulocyte percentage and aspartate aminotransferase are associated with SD-OCT changes and CVD.

Conclusions: SD-OCT was better than funduscopy in detecting retinal changes, higher frequency, and more extensive retinal changes in the more severe SCD genotypes SS and S\textsuperscript{$\beta^0$} as compared with SC. The correlation between abnormal SD-OCT and CVD strongly suggests that retinal exam using SD-OCT may aid in detection and monitoring SCD-related CVD. Retinopathy may be another component of the hemolytic subphenotype of SCD.

Keywords: cerebrovascular disease, OCT, sickle cell retinopathy, silent cerebral infarct

Abbreviations: AST, aspartate aminotransferase; CI, confidence interval; CVD, cerebrovascular disease; FA, fluorescein angiography; MRA, magnetic resonance angiogram; MRI, magnetic resonance imaging; NAIDHC, Nemours/Alfred I. duPont Hospital for Children; OCT, optical coherence tomography; SC, hemoglobin sickle cell disease; SCD, sickle cell disease; SCI, silent cerebral infarct; SCR, sickle cell retinopathy; SD-OCT, spectral-domain optical coherence tomography; SS, hemoglobin SS disease; S\textsuperscript{$\beta^+}$, sickle/\textbeta+/thalassemia; S\textsuperscript{$\beta^0$}, sickle/\textbeta/-thalassemia; TCD, transcranial Doppler; VEGF, vascular endothelial growth factor; WBC, white blood cell.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

© 2021 The Authors. Pediatric Blood & Cancer published by Wiley Periodicals LLC

https://doi.org/10.1002/pbc.29028
Sickle cell disease (SCD) is an inherited group of hemoglobinopathies with numerous associated systemic and ocular complications related to occlusion of small blood vessels by sickled red cells. One of the main factors influencing the clinical presentation of SCD is the genotype. Patients with hemoglobin SS (SS) and S/β-0 thalassemia (Sβ0) have a higher rate of complications including stroke, vaso-occlusive pain episodes, and earlier mortality than those with hemoglobin SC (SC) and S/β+ thalassemia (Sβ+). Genotype also affects the manifestation of sickle cell retinopathy (SCR). Current standard of care for SCR is annual funduscopic retinal examination. The general understanding is that patients with the SC genotype, although usually exhibiting less severe clinical manifestations, were more frequently diagnosed with SCR by funduscopy and fluorescein angiography (FA), and tended to develop vision-threatening proliferative SCR more often than those with SS disease. In the past 20 years, optical coherence tomography (OCT) retinal imaging has been widely used in ophthalmology clinics and offers new information on retinal microanatomy change in SCR. OCT is a noninvasive imaging technique that uses light waves to take cross-sectional pictures of the retina with micrometer resolution. With OCT, each of the retina’s distinctive layers can be seen, allowing ophthalmologists to map and measure their thickness. SCR and other vaso-occlusive diseases cause selective thinning of the inner retinal layers following retinal infarction. This is consistent with findings on histopathological studies. Our previous work showed that inner retinal thinning detected by OCT was more frequently present in children with the SS/Sβ0 genotypes than in those with SC. This is consistent with a published study on adult patients.

The capability to visualize retinal microanatomy greatly enhances early detection, diagnosis, and treatment of retinal diseases. Furthermore, for many systemic illnesses involving the vasculature, including SCD and various neurologic and systemic conditions such as multiple sclerosis, Alzheimer’s disease, diabetes, renal diseases, coronary heart disease, and more, the value of noninvasive examinations is not limited to detecting vision-threatening complications. The eye provides a unique window into pathological changes in small vessel circulation and could aid in the diagnosis and monitoring of overall disease progression. Retinal vascular events are associated with increased risk of stroke. The close embryonic origin, high energy demand, and blood supply shared by the brain and retina may explain why they are often affected by similar disease processes.

One of the most devastating complications of SCD is brain injury. Improvements in detecting and monitoring retinal ischemia may advance both the surveillance of SCR and damage to the cerebral vasculature in SCD. Silent cerebral infarcts (SCIs) are the most common form of neurologic injury in SCD affecting up to 37% of children with SCD by the age of 14 years. Patients with SCI are at increased risk for stroke, progressive SCIs, and neurocognitive difficulties. Early detection of SCI can have significant clinical impact because interventions such as chronic transfusions can halt progression. While increased risk of stroke can be predicted using the noninvasive technique of transcranial Doppler (TCD), currently, the only available means to detect SCI is magnetic resonance imaging (MRI), which is very expensive and often requires sedation in young children (a significant risk for children with SCD). The ability to identify children at high risk for SCI would allow more targeted MRI screening.

This prospective study confirms the correlation between SCD genotype and retinal damage identified by OCT using an expanded dataset. Furthermore, we have compared eye examination findings and brain imaging from patients with the genotypes (SS/Sβ0) associated with the highest incidence of stroke and cerebrovascular disease (CVD). These data suggest a potential link between hypoxic ischemic injury in the retina and brain. Additionally, we also collected data on baseline hematologic results and laboratory markers of hemolysis from patients with SCD to examine associations between these values and SCR, as well as stroke, SCI, and cerebral vasculopathy.

2 METHODS

This study was approved by the Nemours/Alfred I. duPont Hospital for Children’s (NAIDHC) Institutional Review Board Office of Human Subject Protection and conformed to the requirements of the U.S. Health Insurance Portability and Accountability Act of 1996. The study was conducted from July 2015 to March 2020. After providing informed consent, consecutive African American patients aged 5–20 years with SCD receiving care at NAIDHC were enrolled prospectively. Patients with SCD were grouped based on their sickle genotype (SS, SC, Sβ+, Sβ0). Patients with SS and Sβ0 were grouped together because of their similar clinical presentation and the limited number of patients with Sβ0 type. All patients were recommended for yearly eye examination, with more frequent tests performed due to significant findings and/or new ocular symptoms.

Following a comprehensive eye examination, including a careful fundus examination through dilated pupils for comparison, spectral-domain OCT (SD-OCT) images were acquired using the Heidelberg SPECTRALIS (Heidelberg Engineering Inc., Carlsbad, CA, USA). The posterior pole volume scan was obtained using the same protocol as described in our earlier report. It involves a 30° × 25° cuboid, centered at approximately 3 mm temporal to the foveal center. Data from both eyes were collected and analyzed. The areas of visible thinning on OCT color-coded retinal thickness map appear as blue or magenta patches where the normal retina is colored green-yellow (Figure 1). Each thickness map is generated from 31 cross-sectional images, also known as B-scan, and ischemic damages are associated with loss of definition of the inner retinal layers.

The locations of retinal thinning spots were determined using the Early Treatment Diabetic Retinopathy Study circle grid function on the thickness map. The grid is constructed with three concentric circles with diameters 1, 3, and 6 mm. The origin of the circles was placed at the fovea, a small, central pit in the retina that is responsible for sharp central vision. Thickness measurement within the inner-most 1-mm diameter circle was defined as foveal thickness. Any thinning present in this circle was defined as involving the fovea. Inner retinal thickness was measured from the internal limiting membrane to the
FIGURE 1 Retinal optical coherence tomography thickness maps from patients with sickle cell disease. (A) normal; (B) dark blue areas indicate retinal thinning from prior ischemic injuries

eexternal/outer limiting membrane, and outer retinal thickness as measured from the external limiting membrane to Bruch’s membrane.

The ophthalmologic data collected and used in the analysis included absence or presence of retinal disease, unilateral or bilateral, proximity of retinal thinning to foveal center, and foveal thickness. For patients with unilateral disease, measurement from the eye with retinopathy was used for analysis. For patients with symmetric bilateral disease, average foveal thicknesses were used. For patients with asymmetric bilateral disease, the eye with lesion(s) closer to the fovea was used for lesion location and foveal thickness.

Data on the presence or absence of CVD were collected, including history of completed stroke, findings of SCI by MRI, findings of abnormal TCD, and cerebral arteriopathy as documented by magnetic resonance angiogram (MRA). Additional clinical data were collected including number of vaso-occlusive crises requiring hospitalization or outpatient visit during the 3 years prior to each eye exam, history of other sickle cell complications including acute chest syndrome (more than one episode), priapism, splenectomy, avascular necrosis, renal disease/proteinuria, and hypertension as well as history of chronic transfusion therapy and hydroxyurea use. Laboratory data including baseline white blood cell (WBC) count, platelet count and hemoglobin, indirect bilirubin, aspartate aminotransferase (AST), and reticulocyte percentage were collected. The average values of the three tests obtained closest to the eye examination date were calculated and used for analysis.

Chi-square tests of proportions were used to compare frequency of abnormal findings across subpopulations within the study, and Fisher’s exact test was used when the number of cases was below five in certain subgroups. For continuous variables, two-tailed, unpaired t-tests were used for age, and Mann–Whitney U tests were used for blood test results. P-values of <.05 were considered statistically significant for all statistical analyses, which were performed using IBM SPSS Statistics 26 (IBM, Armonk, NY, USA).

3 RESULTS

A total of 117 consecutive African American patients with SCD were included. Patient age ranged from 5 to 20 years. All participants had best-corrected visual acuity of at least 20/40 at the time of examination. Patient demographics are listed in Table 1.

The frequency of retinal change detected by funduscopic examination and SD-OCT in different SCD genotypes were compared (Table 2).

Funduscopic findings included retinal hemorrhage, salmon patches, areas of hyper- or hypopigmentation, and evidence of nonproliferative retinopathy. One patient had episodes of vitreous hemorrhage, indicating proliferative retinopathy. Consistent with our earlier report, SD-OCT had a higher rate of detecting retinal pathology from a prior ischemic event.14 Although not statistically significant, funduscopic examination had a slightly higher rate of diagnosing retinopathy in the SC group than in the SS/Sβ0 group. Conversely, SS/Sβ0 patients had a significantly higher rate (p = .032) of inner retinal thinning than SC patients by SD-OCT study. The differences between the mean ages when retinal change was initially identified with either method were not statistically significant. For the SC group, on average, retinal findings on SD-OCT were presented at younger ages (13.4 ± 4.73 years) than on funduscopic examination (14.3 ± 4.53 years).

Using SD-OCT, the extent of retinal damage from SCD was quantified as unilateral versus bilateral involvement and by the proximity of damages to the foveal center. By both measures, the SS/Sβ0 group showed more frequent involvement of both eyes and the pathology invading fovea.
Based on review of medical history and diagnostic testing results, information on CVD including history of completed stroke, findings of SCI by MRI, findings of abnormal TCD and cerebral arteriopathy as documented by MRA, and TCD results were analyzed (Table 3). Fifty-two of 73 patients in the SS/Sβ0 group had MRI studies within 3 years of OCT imaging, 49 of the 52 patients also had MRA. These patients were included in analysis for SCI and cerebral vasculopathy by MRA. Among them, 42 patients had MRI study before OCT imaging and 10 patients had MRI study after OCT imaging.

The results demonstrated an association between abnormal OCT findings with the presence of CVD including stroke, cerebral vasculopathy by MRA, and SCI (Table 3). Among the patients who had brain MRI/MRA studies (n = 52), a positive OCT finding as compared with a negative OCT finding increased the odds of SCI in a proportional-odds logistic regression model (odds ratio 12.63; 95% confidence interval [CI] 1.49–106.77; p = .0199). The odds increase when OCT-demonstrated retinal damages reach the parafovea, an area less than 1.5 mm from the foveal center (odds ratio 16.50; 95% CI 1.77–154.08; p = .0139). In this dataset, the outcome of OCT imaging detecting retinal injury from SCD had 95.24% sensitivity (95% CI 76.18–99.88%) and 38.71% specificity (95% CI 21.85–57.81%) of showing lesions consistent with SCI by MRI. There was no significant correlation between OCT findings and abnormal TCD.

History of greater than one episode of acute chest syndrome was significantly associated with SCR by OCT (p = .0025). There was no association between retinal examination (funduscopic and OCT) and hypertension, renal disease, frequency of vaso-occlusive crisis, avascular necrosis, splenectomy, or priapism. There was no significant association between CVD and clinical markers including incidence of acute chest syndrome (more than one episode), renal disease/proteinuria, hypertension frequency of vaso-occlusive crisis, avascular necrosis, and splenectomy.

As expected, significant differences in baseline hematologic values were present between the SC and SS/Sβ0 groups. There was no association between OCT findings and baseline hematologic values in the SC genotype. In the SS/Sβ0 group, higher reticulocyte count and AST were significantly associated with SCR by OCT (p = .001 and .028; Table 4), but no significant correlations between OCT findings and baseline WBC count, hemoglobin, or platelet count were seen. Some hematologic values were also associated with CVD (Table 4). Higher baseline WBC, reticulocyte count, indirect bilirubin, and AST were all associated with the presence of SCI and stroke. Higher baseline WBC and lower hemoglobin were also associated with presence of cerebral vasculopathy by MRA (Table 4).

A logistic regression was performed to ascertain the effects of OCT results, WBC count, and markers of hemolysis including

---

### TABLE 1 Patient demographics for each genotype

| Demographic | Sβ+ | SC | SS | Sβ0 | Total |
|-------------|-----|----|----|-----|-------|
| No. of patients | 8 | 36 | 68 | 5 | 117 |
| Male | 5 | 13 | 36 | 2 | 56 |
| Female | 3 | 23 | 32 | 3 | 61 |
| Age in years, mean ± SD | 13.17 ± 4.41 | 11.91 ± 4.30 | 12.35 ± 4.10 | 13.08 ± 5.09 | 12.30 ± 4.18 |
| (min, max) | (7.32, 18.22) | (5.42, 19.71) | (5.25, 20.26) | (6.66, 19.64) | (5.25, 20.26) |

Abbreviation: SD, standard deviation.

### TABLE 2 Eye examination findings by sickle cell disease genotype

| Exam Finding | SC (n = 36) | SS/Sβ0 (n = 73) | p-Value |
|--------------|-------------|-----------------|--------|
| Funduscopy | Number and frequency of SCR diagnosis (%) | 5 (14) | 9 (12) | .819 |
| Age at SCR diagnosis (years) mean ± SD | 14.3 ± 4.53 | 13.3 ± 4.30 | .968 |
| SD-OCT | Number and frequency of SCR diagnosis (%) | 17 (47) | 50 (69) | .032* |
| Age at SCR diagnosis (years) mean ± SD | 13.3 ± 4.73 | 13.2 ± 3.95 | .329 |
| Number and frequency of bilateral SCR (%) | 9 (25) | 35 (50) | .024* |
| Number and frequency of SCR involving fovea (%) | 0 (0) | 11 (15) | .0149* |
| Foveal thickness for all subjects (µm) mean ± SD | 254.28 ± 23.45 | 245.00 ± 19.62 | .237 |
| Foveal thickness among those with SCR (µm) mean ± SD | 260.88 ± 23.81 | 244.54 ± 18.37 | .004* |

Note: χ² test was used to find association for categorical variables except “involving fovea among SCR,” which used Fisher’s exact test due to its small number among SC genotypic children. For continuous variables, t-test was used.

Abbreviations: SCR, sickle cell retinopathy; SD, standard deviation; SD-OCT, spectral-domain optical coherence tomography.

*p < .05.
TABLE 3  Correlation between eye examination findings and cerebral vascular disease in SS/S$\beta$0

| Manifestation of CVD                  | Funduscopy | SD-OCT |
|---------------------------------------|------------|--------|
|                                       | Negative   | Positive | p-Value | Negative | Positive | p-Value |
| Complete stroke                       | Negative   | 53      | 9       | 23       | 39       |         |
|                                       | Positive   | 11      | 0       | .338     | 0        | 11      | .013*   |
| Cerebral vasculopathy by MRA$^a$      | Negative   | 31      | 7       | 12       | 26       |         |
|                                       | Positive   | 11      | 0       | .325     | 0        | 11      | .045*   |
| Transcranial Doppler$^b$               | Negative   | 53      | 9       | 20       | 42       |         |
|                                       | Positive   | 11      | 0       | .338     | 3        | 8       | 1.000   |
| Silent infarcts by MRI                | Negative   | 25      | 6       | 12       | 19       |         |
|                                       | Positive   | 20      | 1       | .219     | 1        | 20      | .008*   |

Abbreviations: CVD, cerebrovascular disease; MRA, magnetic resonance angiogram; MRI, magnetic resonance imaging; SD-OCT, spectral-domain optical coherence tomography.

$^a$Abnormal based on stroke-prevention trial in sickle cell anemia (STOP) criteria.$^{24}$

$^b$p < .05 based on Fisher’s exact test for each group.

Reticulocyte level, indirect bilirubin, and AST on the likelihood that patients have SCI. The logistic regression model was statistically significant, $\chi^2 (5) = 29.210, p = .000$. The model explained 58.0% (Nagelkerke $R^2$) of the variance in SCI and correctly classified 84.6% of cases as having or not having SCI. Reticulocyte percentage is the most important factor for predicting SCI among the laboratory values. For each unit increase of reticulocyte, there was a 1.43 times increase in the odds of SCI. Subjects with positive OCT findings were 11.5 times more likely to exhibit SCI on MRI. Increasing WBC count, reticulocyte count, higher indirect bilirubin, and AST were associated with an increased likelihood of having SCI.

4 | DISCUSSION

Results of the current study utilizing a larger sample size confirm our earlier findings that SD-OCT was more sensitive than funduscopic examination in detecting retinal ischemic change from SCD. Furthermore, the data confirm a correlation between the scope of retinal damage in SCR by SD-OCT imaging and SCD genotype.$^{14}$ A disparity between severity of overall systemic complications and ophthalmologic manifestations between different SCD genotypes has long been recognized. While homozygous SS disease, the most clinically severe form, frequently presents with severe vaso-occlusive crises and end-organ damage, the incidence of vision-threatening proliferative retinopathy is low. However, in the usually clinically milder heterozygous SC disease, a higher incidence of proliferative SCR has been documented.$^{25,26}$ Current understanding of the progression from often asymptomatic vaso-occlusive SCR to vision-threatening vaso-proliferative SCR is that chronic ischemia due to vascular occlusion may trigger the release of vascular endothelial growth factor (VEGF) by vascular endothelial cells. Excessive VEGF leads to development of neovascularization in proliferative SCR.$^{27-29}$ The retinal thinning observed on OCT is the result of tissue loss from retinal small arterial occlusion, a form of stroke. Patients with SS/S$\beta$0 genotypes have a greater number of small retinal strokes on OCT$^{14,15,30,31}$ indicating that the retina, like other end organs, suffers from higher frequency of circulatory interruption in this group than in those with SC. However, the extent of retinal infarct does not appear to unequivocally predict the degree of retinal neovascularization. This raises the question of whether different mechanisms are responsible for the progression from vaso-occlusion to vaso-proliferation in different SCD genotypes. Hypotheses to account for this disparity include the longer life expectancy of patients with SC genotype provides more time for the development of vascular proliferation; however, a more likely explanation is that vaso-occlusive events in the SS/S$\beta$0 genotype may be more extensive and complete. Retinal stroke leads to cell death, which results in loss of the source of VEGF, vascular endothelia, while chronic ischemia in patients with the SC genotype promotes release of VEGF.

The most important mechanism driving organ damage in SCD is vascular occlusion,$^{23,32}$ which leads to ischemia/reperfusion injury and resultant inflammation. In addition, the erythrocyte injury in SCD leads to excessive hemolysis, endothelial dysfunction, and vasculopathy.$^{23}$ Notably, the retina is considered to be part of the central nervous system because it forms from the out-pouches of the embryonic forebrain (optic vesicles). Additionally, as neuronal tissue, the brain and retina both have a high demand for energy and are very sensitive to hypoxia.$^{34}$ The brain and retina share blood supply from the internal carotid artery; their capillaries are situated in the neuronal-glial matrix and have similar capillary wall thickness.$^{35}$ These similarities explain why they are often affected by similar disease processes. Among adult non-SCD patients, ocular stroke (retinal vessel occlusion) usually presents with painless vision loss and is associated with a higher risk for cerebral vascular events.$^{18,19}$

In SCD, higher levels of baseline hemolysis have been associated with certain vasculopathic complications of SCD including stroke, leg ulcers, pulmonary hypertension, priapism, renal failure,$^{23}$ and elevated TCD velocities.$^{36,37}$ This has been attributed to intravascular...
**TABLE 4** Correlation of baseline hematologic values with optical coherence tomography and cerebrovascular disease in SS/Sβ0 group

|                | WBC       | Platelet  | Hb        | Reticulocyte | IndBili   | AST        |
|----------------|-----------|-----------|-----------|--------------|-----------|------------|
| **Funduscopy** |           |           |           |              |           |            |
| Negative, n = 64 | 11.73 ± 3.58 | 409.97 ± 114.62 | 8.93 ± 1.15 | 8.67 ± 3.49 | 3.24 ± 3.19 | 50.08 ± 18.73 |
| (min, max)     | (4.87, 19.53) | (210.33, 659.33) | (6.73, 12.10) | (1.60, 15.67) | (0.67, 23.27) | (23.00, 124.00) |
| Positive, n = 9 | 8.45 ± 2.78  | 321.22 ± 117.44  | 9.56 ± 1.50  | 7.59 ± 4.56  | 2.80 ± 2.96  | 49.09 ± 14.26  |
| (min, max)     | (5.17, 12.27) | (179.67, 488.67) | (7.83, 12.17) | (2.13, 17.10) | (0.60, 10.50) | (30.00, 70.33) |
| [p-value]      | [.012*]     | [.046*]     | [.275]     | [.330]       | [.237]     | [.763]     |
| **Optical coherence tomography** |           |           |           |              |           |            |
| Negative, n = 23 | 10.52 ± 3.56 | 391.32 ± 102.63 | 9.29 ± 1.15 | 6.51 ± 2.68 | 3.16 ± 4.50 | 43.25 ± 12.71 |
| (min, max)     | (5.00, 19.53) | (224.00, 573.00) | (7.27, 12.10) | (1.60, 13.30) | (0.73, 23.27) | (23.00, 81.67) |
| Positive, n = 50 | 11.69 ± 3.69 | 402.58 ± 125.06 | 8.88 ± 1.22 | 9.47 ± 3.64 | 3.20 ± 2.32 | 53.04 ± 19.52 |
| (min, max)     | (4.87, 19.33) | (179.67, 659.33) | (6.73, 12.17) | (2.13, 17.10) | (0.60, 10.70) | (25.33, 124.00) |
| [p-value]      | [.221]      | [.735]      | [.240]     | [.001*]      | [.254]    | [.028*]    |
| **Stroke**     |           |           |           |              |           |            |
| Negative, n = 62 | 10.79 ± 3.48 | 399.86 ± 122.13 | 8.92 ± 1.25 | 8.13 ± 3.53 | 3.02 ± 3.22 | 48.22 ± 16.37 |
| (min, max)     | (4.87, 19.53) | (179.67, 659.33) | (6.73, 12.17) | (1.60, 17.10) | (0.60, 23.27) | (23.00, 103.67) |
| Positive, n = 11 | 14.32 ± 3.12 | 394.36 ± 94.84  | 9.50 ± 0.76  | 10.83 ± 3.40 | 4.13 ± 2.59 | 60.97 ± 24.12  |
| (min, max)     | (9.23, 19.33) | (253.00, 588.00) | (8.57, 10.73) | (4.20, 14.80) | (1.93, 10.07) | (37.33, 124.00) |
| [p-value]      | [.004*]     | [.841]      | [.090]     | [.023*]      | [.025*]     | [.048*]     |

(Continues)
|                      | WBC         | Platelet    | Hb          | Reticulocyte | IndBili     | AST         |
|----------------------|-------------|-------------|-------------|--------------|-------------|-------------|
| **Silent cerebral infarcts** |             |             |             |              |             |             |
| Negative, n = 31     | 10.11 ± 3.70| 377.69 ± 128.52| 9.29 ± 1.42 | 6.66 ± 2.53 | 3.01 ± 4.16 | 45.38 ± 14.66 |
| (min, max)           | (5.00, 19.53)| (179.67, 659.33)| (6.73, 12.17)| (1.60, 12.10)| (0.60, 23.27)| (23.00, 81.64)|
| Positive, n = 21     | 13.26 ± 3.42| 386.33 ± 109.66| 9.08 ± 0.93 | 10.77 ± 3.31| 3.87 ± 2.70 | 60.27 ± 21.39 |
| (min, max)           | (4.87, 19.53)| (225.00, 588.00)| (7.53, 11.03)| (1.43, 10.70)| (31.67, 124.00)|              |
| [p-value]            | .002*       | .016*       | .615*       | .000*        | .017*       | .004*       |
| **Cerebrovasculopathy** |             |             |             |              |             |             |
| Negative, n = 38     | 10.83 ± 3.93| 359.91 ± 113.69| 9.33 ± 1.29 | 8.03 ± 3.70 | 3.29 ± 4.01 | 49.37 ± 15.74 |
| (min, max)           | (4.87, 19.53)| (179.67, 618.33)| (7.00, 12.17)| (1.60, 15.67)| (0.60, 23.27)| (23.00, 81.67)|
| Positive, n = 11     | 14.08 ± 2.80| 453.54 ± 134.37| 8.84 ± 0.67 | 9.09 ± 3.05 | 3.77 ± 2.67 | 60.55 ± 28.10 |
| (min, max)           | (10.30, 19.33)| (225.00, 659.33)| (7.53, 9.50)| (4.77, 14.80)| (1.73, 10.70)| (34.33, 124.00)|
| [p-value]            | .009*       | .044*       | .292        | .332         | .101        | .369        |
| **Transcranial Doppler** |             |             |             |              |             |             |
| Negative, n = 62     | 11.03 ± 3.72| 394.47 ± 120.84| 8.99 ± 1.28 | 8.50 ± 3.69 | 2.71 ± 1.89 | 50.33 ± 18.81 |
| (min, max)           | (4.87, 19.53)| (179.67, 659.33)| (6.73, 12.17)| (1.60, 17.10)| (0.60, 10.50)| (25.33, 124.00)|
| Positive, n = 11     | 12.99 ± 2.74| 424.72 ± 100.39| 9.14 ± 0.60 | 8.73 ± 3.35 | 5.85 ± 6.33 | 47.85 ± 14.52 |
| (min, max)           | (8.07, 16.20)| (225.00, 573.00)| (8.13, 10.50)| (4.20, 14.80)| (1.73, 23.27)| (23.00, 70.00)|
| [p-value]            | .064        | .388        | .441        | .865         | .815        | .939        |

Abbreviations: AST, aspartate aminotransferase; Hb, hemoglobin; HbF, fetal hemoglobin; IndBili, indirect bilirubin; WBC, white blood cell count.

* Denotes statistical significance.
hemolysis promoting endothelial dysfunction, including nitric oxide depletion. Elevated baseline reticulocyte count is a strong marker of hemolytic rate. Interestingly, we have demonstrated a significant correlation between higher reticulocyte percentage and SCI in this analysis. Thus, patients with abnormal OCT, elevated baseline WBC count, and evidence of high hemolytic rate may be at significantly higher risk for SCI, thus warranting MRI screening and disease-modifying therapeutic interventions.

Earlier studies attempting to correlate findings from conventional eye examination and SCD clinical course have produced mixed results. The results of our study and published reports demonstrate a correlation between SCR detected by SD-OCT and the presence of CVD, strongly suggesting that retinal exam using SD-OCT may aid in detection and monitoring of SCD-related CVD. Ischemic brain damage is one of the most significant complications of SCD. The similarity of the course of SCR and CVD is that vascular occlusion in the retina and brain is often asymptomatic unless they involve a critical region and/or large areas. However, subclinical ischemic injuries are not truly silent. Their functional consequences are progressive and the “silence” is only temporary. Silent infarct indicates a high risk for further progression of cerebrovascular injury, which ultimately leads to cognitive decline; similarly, retinal vascular occlusions indicate a high potential for progressive worsening of SCR. Improvements in detecting and monitoring retinal ischemia may advance both the surveillance of SCR and cerebral vascular damage in SCD. Early detection of SCI is critical because interventions such as chronic transfusions can halt progression. Currently, the only reliable method to identify SCI is MRI, which is very expensive and often not covered by insurance without a clear indication. Furthermore, young children usually require sedation for MRI, which increases the risk for the patients. The possible association between OCT and SCI may improve our ability to identify children at high risk for SCI and would allow targeted MRI screening and early initiation of disease-modifying therapies to halt progression.

Using SD-OCT for diagnosis of SCR and monitoring SCD has several limitations. (1) SPECTRALIS SD-OCT used for this study scans an area of 6 × 9 mm, which is only 4% of the entire retina. Retinal changes outside of the scanned area visible by funduscopy may be missed by SD-OCT. (2) SD-OCT shows only the after-effects of retinal ischemic injury and does not provide direct information on retinal circulation. With the exception of one patient with SS disease who had a neovascular complex in the scanned area, SD-OCT images do not distinguish nonproliferative or proliferative SCR. (3) The high incidence of retinal change uncovered by SD-OCT, while attesting to its power in the early diagnosis of SCR, may also lower its positive predictive value for CVD. Our study showed that 20 of 21 patients with evidence of SCI had positive findings on SD-OCT. However, only 20 of 39 patients with abnormal SD-OCT had detectable SCI. A few factors need to be considered. First, one must evaluate the structural disparities of small vasculature between the brain and retina. The cerebral cortical capillaries have larger diameters, more abundant collateral channels, and lack the right angle branching of the precapillary arterioles characteristic of some retinal vessels, suggesting that retinal vessels may be more vulnerable to occlusion. A study of 389 children with SCD detected peripheral retinal arteriolar closure in 90% of patients by age 12 years. A review of neurologic injury in SCD stated that SCI (abnormal brain MRI with a normal neurologic examination without evidence of an overt stroke) occurs in 37% of pediatric patients by age 14 years. Second, SD-OCT has a resolution of micrometers, which far exceeds that of current MRI technology. Additionally, within our SS/β0 group, only 52 of the 73 patients had brain MRI within 3 years, mostly prior to the SD-OCT study. The incidence of SCI is likely higher than we were able to confirm from the medical records. It is also possible that abnormal OCT may predict the development of cerebral ischemic changes that are not yet detectable by MRI. If this is confirmed, finding OCT changes may be an indication to move forward with new disease-modifying agents or curative therapies like bone marrow transplantation or gene therapy. Pairing SD-OCT and brain MRI and serial examinations in a longitudinal study will offer more reliable information on the predictive value of retinal changes by SD-OCT on CVD.

This study focused on SCD retinal injury shown on SD-OCT and we did not stage SCR according to the Goldberg classification. Based on funduscopy and FA observations of 24 SC patients, Goldberg introduced the five stages of SCR. They are (1) peripheral arteriolar occlusion, (2) peripheral arteriolar-venular anastomoses, (3) neovascular and fibrous proliferation, (4) vitreous hemorrhage, and (5) retinal detachment. According to this system, stages 3 and above will define proliferative SCR. In our group of patients, one patient with SS disease had a neovascular complex in the SD-OCT scanned area, and one patient with SC disease had two episodes of vitreous hemorrhage. The rest of the enrollees showed no sign of neovascularization on funduscopy examination and/or OCT. A limitation of this study is that we did not have the capacity to perform FA. Adding this gold standard methodology for SCR staging to our future studies will likely provide invaluable information on retinal vasculature circulation, its possible association with OCT findings, and cerebral injury in SCD.

Another limitation of this study is that we were not able to include lactate dehydrogenase, a strong marker of hemolysis, in this analysis as this was not collected routinely in most patients. This will be included in future studies. To summarize, SD-OCT is a sensitive, fast, and noncontact imaging modality for ophthalmology. It is well tolerated by preschool age children. Adding SD-OCT to the conventional eye examination for children with SCD has improved our understanding of the natural history and retinal pathology of SCR. The capability to assess small vascular disease in the retina using SD-OCT could not only improve long-term visual outcome for patient but may also serve as proxy indicators of CVD progression.
REFERENCES
1. Platt OS, Thornton BD, Brambilla DJ, et al. Pain in sickle cell disease. Rates and risk factors. N Engl J Med. 1991;325:11-16.
2. Platt OS, Brambilla DJ, Rosse WF, et al. Mortality in sickle cell disease. Life expectancy and risk factors for early death. N Engl J Med. 1994;330:1639-1644.
3. Ohene-Frempong K, Weiner SJ, Sleeper LA, et al. Cerebrovascular accidents in sickle cell disease: rates and risk factors. Blood. 1998;91:288-294.
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;85:77-78.
5. Fox PD, Dunn DT, Morris JS, et al. Risk factors for proliferative sickle retinopathy. Br J Ophthalmol. 1990;74:172-176.
6. Condon PI, Hayes RJ, Serjeant GR. Retinal and choroidal neovascularization in sickle cell disease. Trans Ophthalmol Soc UK. 1980;100:434-439.
7. Condon PI, Serjeant GR. Behaviour of untreated proliferative sickle retinopathy. Br J Ophthalmol. 1980;64:404-411.
8. AlRyalat SA, Jaber BAM, Alzarea AA, et al. Ocular manifestations of sickle cell disease in different genotypes. Ophthalmic Epidemiol. 2020;1-6. https://doi.org/10.1080/09286586.2020.1801762
9. Vatansever E, Vatansever M, Dinç E, et al. Evaluation of ocular complications by using optical coherence tomography in children with sickle cell disease evaluated by optical coherence tomography (OCT) and OCT angiography. Am J Ophthalmol. 2017;28:623-628.
10. Chow CC, Genead MA, Anastasakis A, et al. Structural and functional correlation in sickle cell retinopathy using spectral-domain optical coherence tomography and scanning laser ophthalmoscope microperimetry. Am J Ophthalmol. 2011;152:704-711.
11. Pahl DA, Green NS, Bhatia M, et al. Optical coherence tomography angiography and ultra-widefield fluorescein angiography for early detection of adolescent sickle retinopathy. Am J Ophthalmol. 2017;183:91-98.
12. Foos RY. Regional ischemic infarcts of the retina. Albrecht Von Graefes Arch Klin Exp Ophthalmol. 1976;200:183-194.
13. Romayanan N, Goldberg MF, Green WR. Histopathology of sickle cell retinopathy. Trans Am Acad Ophthalmol Otolaryngol. 1973;77:OP642-OP676.
14. Jin J, Miller R, Salvin J, et al. Funduscopic examination and SD-OCT in detecting sickle cell retinopathy among pediatric patients. J AAPOS. 2018;22:197-201.e1.
15. Mathew R, Bafiq R, Ramu J, et al. Spectral domain optical coherence tomography in patients with sickle cell disease. Br J Ophthalmol. 2015;99:967-972.
16. Chhablani PP, Ambiya V, Nair AG, et al. Retinal findings on OCT in systemic conditions. Semin Ophthalmol. 2018;33:525-546.
17. Wang J, Jiang J, Zhang Y, et al. Retinal and choroidal vascular changes in coronary heart disease: an optical coherence tomography angiography study. Biomed Opt Express. 2019;10:1532-1544.
18. Woo SCY, Lip GYH, Lip PL. Associations of retinal artery occlusion and retinal vein occlusion to mortality, stroke, and myocardial infarction: a systematic review. Eye (Lond). 2016;30:1031-1038.
19. Hong JH, Sohn SI, Kwak J, et al. Retinal artery occlusion and associated recurrent vascular risk with underlying etiologies. PLoS One. 2017;12:e0177663.
20. Chen YY, Yen YF, Lin JX, et al. Risk of ischemic stroke, hemorrhagic stroke, and all-cause mortality in retinal vein occlusion: a nationwide population-based cohort study. J Ophthalmol. 2018;2018:8629429.
21. Bernaudin F, Verhac S, Arnaud C, et al. Impact of early transcranial Doppler screening and intensive therapy on cerebral vasculopathy outcome in a newborn sickle cell anemia cohort. Blood. 2011;117:1130-1140 quiz 1436.
22. DeBaun MR, Gordon M, McKinstry RC, et al. Controlled trial of transusions for silent cerebral infarcts in sickle cell anemia. N Engl J Med. 2014;371:699-710.
23. Rees DC, Williams TN, Gladwin MT. Sickle-cell disease. Lancet. 2010;376:2018-2031.
24. Lee MT, Piomelli S, Granger S, et al. Stroke prevention trial in sickle cell anemia (STOP): extended follow-up and final results. Blood. 2006;108:847-852.
25. Friberg TR, Young CM, Milner PF. Incidence of ocular abnormalities in patients with sickle hemoglobinopathies. Ann Ophthalmol. 1986;18:150-153.
26. Duan XJ, Lanzkron S, Linz MO, et al. Clinical and ophthalmic factors associated with the severity of sickle cell retinopathy. Am J Ophthalmol. 2019;197:105-113.
27. Cao J, Mathews MK, McLeod DS, et al. Angiogenic factors in human proliferative sickle cell retinopathy. Br J Ophthalmol. 1999;83:838-846.
28. Kim SY, Mocanu C, Mcleod DS, et al. Expression of pigment epithelium-derived factor (PEDF) and vascular endothelial growth factor (VEGF) in sickle cell retina and choroid. Exp Eye Res. 2003;77:433-445.
29. Lee K, Rodrigues M, Kashiwabuchi F, et al. Expression of the angiogenic mediator, angiopoietin-like 4, in the eyes of patients with proliferative sickle retinopathy. PLoS One. 2017;12:e0183320.
30. Ong SS, Linz MO, Li X, et al. Retinal thickness and microvascular changes in children with sickle cell disease evaluated by optical coherence tomography (OCT) and OCT angiography. Am J Ophthalmol. 2020;209:88-98.
31. Lim JI, Cao D. Analysis of retinal thinning using spectral-domain optical coherence tomography imaging of sickle cell retinopathy eyes compared to age- and race-matched control eyes. Am J Ophthalmol. 2018;192:229-238.
32. Do BK, Rodger DC. Sickle cell disease and the eye. Curr Opin Ophthalmol. 2017;28:623-629.
33. Kato GJ, Steinberg MH, Gladwin MT. Intravascular hemolysis and the pathophysiology of sickle cell disease. J Clin Invest. 2017;127:750-760.
34. Wong-Riley MT. Energy metabolism of the visual system. Eye Brain. 2010;2:99-116.
35. Cogan DG, Kuwabara T. Comparison of retinal and cerebral vasculature in trypsin digest preparations. Br J Ophthalmol. 1984;68:10-12.
36. Bernaudin F, Verhac S. Stroke prevention in sickle-cell disease: results, hurdles and future perspectives. Bull Acad Natl Med. 2008;192:1383-1393.
37. Bernaudin F, Verhac S, Chevret S, et al. G6PD deficiency, absence of alpha-thalassemia, and hemolytic rate at baseline are independent risk factors for abnormally high cerebral velocities in patients with sickle cell anemia. Blood. 2008;112:4314-4317.
38. Gill HS, Lam WC. A screening strategy for the detection of sickle cell retinopathy in pediatric patients. Can J Ophthalmol. 2008;43:188-191.
39. Rosenberg JB, Hutcheson KA. Pediatric sickle cell retinopathy: correlation with clinical factors. J AAPOS. 2011;15:49-53.
40. Merritt JC, Risco JM, Pantell JP. Bilateral macular infarction in SS disease. J Pediatr Ophthalmol Strabismus. 1982;19:275-278.
41. Robert MP, Ingster-Moati I, Roche O, et al. Asymptomatic atrophy of the temporal median raphe of the retina associated with cerebral vasculopathy in homozygous sickle cell disease. *J AAPOS*. 2012;16:394-397.

42. Kolb H, Fernandez E, Nelson R, eds. Webvision: The Organization of the Retina and Visual System. Salt Lake City, UT: University of Health Sciences Center; 1995. [https://webvision.med.utah.edu/]. Accessed October 30, 2020.

43. Talbot JF, Bird AC, Maude GH, et al. Sickle cell retinopathy in Jamaican children: further observations from a cohort study. *Br J Ophthalmol*. 1988;72:727-732.

44. DeBaun MR, Armstrong FD, McKinstry RC, et al. Silent cerebral infarcts: a review on a prevalent and progressive cause of neurologic injury in sickle cell anemia. *Blood*. 2012;119:4587-4596.

45. Goldberg MF. Classification and pathogenesis of proliferative sickle retinopathy. *Am J Ophthalmol*. 1971;71:649-665.

46. O’Driscoll S, Height SE, Dick MC, et al. Serum lactate dehydrogenase activity as a biomarker in children with sickle cell disease. *Br J Haematol*. 2008;140:206-209.

**How to cite this article:** Jin J, Kandula V, Miller RE. Monitoring retinal pathology and cerebral injury in sickle cell disease using spectral-domain optical coherence tomography in pediatric patients. *Pediatr Blood Cancer*. 2021;68:e29028. [https://doi.org/10.1002/pbc.29028](https://doi.org/10.1002/pbc.29028)