Insights into Sickle Cell Disease through the Retinal Microvasculature

Adaptive Optics Scanning Light Ophthalmoscopy Correlates of Clinical OCT Angiography

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Purpose: Clinical OCT angiography (OCTA) of the retinal microvasculature offers a quantitative correlate to systemic disease burden and treatment efficacy in sickle cell disease (SCD). The purpose of this study was to use the higher resolution of adaptive optics scanning light ophthalmoscopy (AOSLO) to elucidate OCTA features of parafoveal microvascular compromise identified in SCD patients.

Design: Case series of 11 SCD patients and 1 unaffected control.

Participants: A total of 11 eyes of 11 SCD patients (mean age, 33 years; range, 23–44; 8 female, 3 male) and 1 eye of a 34-year-old unaffected control.

Methods: Ten sequential 3 × 3 mm parafoveal OCTA full vascular slab scans were obtained per eye using a commercial spectral domain OCT system (Avanti RTVue-XR; Optovue). These were used to identify areas of compromised perfusion near the foveal avascular zone (FAZ), designated as regions of interest (ROIs). Immediately thereafter, AOSLO imaging was performed on these ROIs to examine the cellular details of abnormal perfusion. Each participant was imaged at a single cross-sectional time point. Additionally, 2 of the SCD patients were imaged prospectively 2 months after initial imaging to study compromised capillary segments across time and with treatment.

Main Outcome Measures: Detection and characterization of parafoveal perfusion abnormalities identified using OCTA and resolved using AOSLO imaging.

Results: We found evidence of abnormal blood flow on OCTA and AOSLO imaging among all 11 SCD patients with diverse systemic and ocular histories. Adaptive optics scanning light ophthalmoscopy imaging revealed a spectrum of phenomena, including capillaries with intermittent blood flow, blood cell stasis, and sites of thrombus formation. Adaptive optics scanning light ophthalmoscopy imaging was able to resolve single sickled red blood cells, rouleaux formations, and blood cell–vessel wall interactions. OCT angiography and AOSLO imaging were sensitive enough to document improved retinal perfusion in an SCD patient 2 months after initiation of oral hydroxyurea therapy.

Conclusions: Adaptive optics scanning light ophthalmoscopy imaging was able to reveal the cellular details of perfusion abnormalities detected using clinical OCTA. The synergy between these clinical and laboratory imaging modalities presents a promising avenue in the management of SCD through the development of noninvasive ocular biomarkers to prognosticate progression and measure the response to systemic treatment. Ophthalmology Science 2022;2:100196 © 2022 by the American Academy of Ophthalmology. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Supplemental material available at www.ophthalmologyscience.org.

Sickle cell disease (SCD) has been present in areas endemic to malaria such as sub-Saharan Africa, the Middle East, and India for thousands of years, with some areas of Africa having a newborn incidence of up to 2% per year.1 In the present-day United States, SCD is the most common inherited blood disorder and has a population prevalence of approximately 100 000, with > 3 million people living with sickle cell trait.2 By 2050, the global number of people with SCD is expected to increase by 30%.3

Despite significant progress in diagnosis and management over the years, SCD continues to cause significant morbidity and mortality from a relatively young age.1 The
median survival of affected individuals in high-income countries remains low at 43 years. Sickle cell disease is associated with substantial economic cost to society and imposes significant financial burden to the patient due to treatment costs and productivity loss. Because the disease is multifactorial and its effects on the body are complex, it is difficult to understand the full scope of the cost of illness, and there is still a gap in estimating the total healthcare expenditure in the United States and globally.

As novel therapeutics are being developed for this debilitating disease, strong clinical end points are being sought after to aid in the assessment of disease severity and treatment efficacy. The intrinsic transparency of the ocular media and visibility of the retinal tissue provide a rare window into the human body, enabling noninvasive study of the living retinal microvascular milieu. OCT angiography (OCTA) is a recent clinical advance in noninvasive, contrast-free retinal microvascular imaging, providing higher lateral and axial resolution than conventional intravenous fluorescein angiography (FA). OCT angiography uses movement of red blood cells (RBCs) as intrinsic “contrast” to depict capillary perfusion within discrete retinal layers presented in the en face perspective.

Many of the features of OCTA are particularly well suited for the study of sickle cell retinal disease. Its quantitative nature has allowed exploration of foveal avascular zone (FAZ) size, microvascular density, and vessel tortuosity. Cross-sectional OCTA studies have been used to detect the presence and stage of sickle cell maculopathy, and to correlate findings with genetics and systemic features. Zhou et al added sequential OCTA imaging and the concept of an intermittent perfusion index, which reflects systemic disease burden and is able to measure response to systemic therapy.

Despite these clinical advances, OCTA is still limited in its ability to resolve the details of cellular flow and vessel wall features due to the optical aberrations of the eye. Adaptive optics scanning light ophthalmoscopy (AOSLO) can compensate for these optical limitations and has demonstrated the ability to image retinal features at a cellular level in vivo, including photoreceptors, retinal pigment epithelial cells, vascular mural cells, hyalocytes, and ganglion cells and their nerve fibers. Backscattered light in AOSLO can be separated into confocal and nonconfocal components. Confocal AOSLO FA and reflectance imaging using singly scattered light have been used to visualize decreased macular microvascular density in eyes with sickle cell retinopathy (SCR). Nonconfocal imaging techniques using multiply scattered light have been shown to enhance edge contrast, improving the ability of AOSLO to reveal retinal vessel wall and blood flow features.

Nonconfocal detection schemes include using offset detection, split-detection with the annular detection area split into 2 halves, quadrant-detection with the annular detection area split into 4 sections, or multi-offset detection. An important limitation of these techniques for a given image is that edge contrast enhancement is limited to the respective detector’s direction, leading to suboptimal visualization of edges that run parallel to the detector’s
The OCTA imaging protocol was performed as previously described. Once the eye of interest was dilated, a commercial mydriatic agent was used to obtain 10 sequential 3 mm en face OCTA scans centered on the fovea. The 10 OCTA scans were collected over 10 minutes, with one scan collected approximately every minute. Imaging was performed using the standard device wavelength of 840 nm, bandwidth of 45 nm, and axial line rate of 70 kHz. The OCTA imaging protocol was performed as previously described.

### Ancillary Ocular Imaging

Color fundus photography (Topcon DRI OCT Triton, Topcon Medical Systems Inc) and macular OCT raster scans (Heidelberg Spectralis HRA+OCT, Heidelberg Engineering Inc) were performed on the day of imaging. OCT macula scans with ETDRS thickness measurements centered at the fovea were used to assess for macular thinning. Axial length measurements were taken using an IOLMaster (Carl Zeiss Meditec, Inc) and were used to adjust for the retinal magnification of the OCTA with the Littmann formula and of the AOSLO images as previously published.

### OCTA Image Processing

The 10 OCTA scans were registered and averaged into one scan, and the OCTA full vascular slab was segmented, extending from the inner limiting membrane to 3 mm below the posterior boundary of the outer plexiform layer. The 10 sequential OCTA scans were compared sequentially to identify regions of interest (ROIs) adjacent to the foveal avascular zone (FAZ) with poorly perfused capillary segments. Poorly perfused capillary segments were non-perfused or intermittently perfused. Intermittently perfused capillary segments were defined as vessels showing perfusion in ≥ 1 OCTA scan with nonfilling during a previous or subsequent scan. Nonperfused capillary segments, although not directly visible on OCTA scans, were inferred to be present in areas of grossly irregular FAZ borders and rarified surrounding capillary beds.

### AOSLO Image Acquisition

The AOSLO imaging of ROIs was performed immediately after OCTA imaging. The custom-built AOSLO simultaneously collected confocal and nonconfocal quadrant-detection images using a circular pinhole ~1 Airy disk diameter (ADD) and an annular pinhole with an inner diameter of 2 ADD and an outer diameter of 20 ADD. During AOSLO video capture of an ROI, frames from the 4 quadrant detectors were used to create 4 split-detection frames at 180°, 135°, 90°, and 45° as shown in the Supplementary Methods Figure (available at www.ophthalmologyscience.org) and as previously described.

During imaging, each participant’s head was stabilized using a customized bite bar. Participants were instructed to direct their gaze to a green internal fixation target. The imaging light source was a superluminescent diode with a wavelength centered at 790 nm, and imaging was performed at a frame rate of 16.6 Hz. Each video recording segment contained approximately 200 frames. A few videos were taken at each ROI at each time point. Participants were given frequent breaks in between video segment acquisitions. During breaks, participants were encouraged to blink, and artificial tear drops were given as needed to help maintain good tear film coverage throughout the imaging session. The AOSLO imaging sessions lasted approximately 1 hour.

### Table 1. Patient Demographics

| Patient | Age, yrs/ Sex | Genotype | Sickle Cell Sequelae | Treatment | Eye | SCR Type |
|---------|---------------|----------|----------------------|-----------|-----|----------|
| 1       | 44/F          | HbSS     | Pain crises, ACS, asthma, anemia | Hydroxyurea, FA, Vitamin D chronic RBC transfusions | OS | NP       |
| 2       | 23/F          | HbSS     | Pain crises, ACS     | Hydroxyurea, FA | OD | NP       |
| 3       | 31/M          | HbSC     | PE                   | None (stopped warfarin) | OS | P/H (6 mos prior) |
| 4       | 36/F          | HbSC     | Pain crises, anemia  | FA, history of RBC transfusions during pregnancy | OD | P |
| 5       | 23/M          | HbSS     | Pain crises, history of cholecystectomy | Vitamin D | OD | NP |
| 6       | 31/F          | HbSS     | Anemia               | None, history of RBC transfusions | OD | NP |
| 7       | 25/F          | HbSS     | Pain crises          | FA, Vitamin B-12, Vitamin D, Vitamin E, history of RBC transfusions | OS | NP |
| 8       | 42/F          | HbSS     | Repaired mitral valve regurgitation | Hydroxyurea, FA, Vitamin D, ASA, metoprolol, levothryoxine | OD | P status post SLP |
| 9       | 39/F          | HbSS     | Pain crises          | Hydroxyurea, FA, amlodipine, metoprolol | OS | NP |
| 10      | 37/F          | HbSS     | Pain crises, asthma  | Hydroxyurea, FA, montelukast | OS | NP |
| 11      | 31/M          | HbSS     | Pain crises, PE, ACS, intubations ×6 | History of BMT 2 yrs prior, rivaroxaban, Vitamin D | OD | NP |

ACS = acute chest syndrome; ASA = acetylsalicylic acid; BMT = bone marrow transplant; F = female; FA = folic acid; HbSC = hemoglobin SC; HbSS = hemoglobin SS; M = male; NP = nonproliferative; OD = right eye; OS = left eye; P = proliferative; PE = pulmonary embolism; SLP = scatter laser photocoagulation; RBC = red blood cell; SCR = sickle cell retinopathy; VH = vitreous hemorrhage.

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**Initial Visit** Patients 4 and 6 were imaged prospectively 2 months after the initial visit to study compromised capillary segments across time and with treatment.

### Imaging Protocol

Mydriasis and cycloplegia were induced at the beginning of each imaging session with 1 drop each of 2.5% phenylephrine hydrochloride ophthalmic solution (Bausch & Lomb Inc) and 1% tropicamide ophthalmic solution (Akorn Inc). Mydriasis and cycloplegia were induced at the beginning of each imaging session with 1 drop each of 2.5% phenylephrine hydrochloride ophthalmic solution (Bausch & Lomb Inc) and 1% tropicamide ophthalmic solution (Akorn Inc).
AOSLO. The OCTA superficial layer was defined as having boundaries from the inner limiting membrane to 10 \( \mu \)m above the posterior boundary of the inner plexiform layer.

**AOSLO Image Processing**

Frames from confocal and nonconfocal quadrant-detection AOSLO videos were registered and processed offline to create averaged images and videos.\(^{38,39}\) To optimize edge contrast along any edge in the 4 split-detection frames per ROI, a directional difference filtering approach using an emboss filter was used, as illustrated in Supplementary Methods Figure A–D (available at www.ophthalmologyscience.org). This approach was similar to the one previously described by Migacz et al,\(^{24}\) which used a directional difference filtering approach with an emboss filter to enhance edge contrast of the cell perimeters of vitreous cortex hyalocytes, improving visualization of these cells compared with the original split-detection images. The directional difference filtering approach used in this article had the following differences: The kernel size used was 5 \( \times \) 5 pixels, instead of 3 \( \times \) 3 pixels, with pixel values of +1.0 and −1.0, instead of +1.85 and −1.85; the emboss filters were applied on single frames instead of averaged images; and the 4 emboss-filtered frames were merged using minimum intensity projection instead of maximum intensity projection. These parameters were decided in a subjective and qualitative manner, adapting the hyalocyte image processing method to optimize visualization of blood vessel and blood cell structures on individual frames (Supplementary Methods Figure D vs. E, available at www.ophthalmologyscience.org). For the remainder of this article, nonconfocal quadrant-detection AOSLO is referred to as “nonconfocal AOSLO.”

**Results**

Imaging results from each of the 12 participants (11 SCD patients and 1 unaffected control) are presented in a case series fashion. All participants were imaged once at the initial imaging visit, and 2 patients (patients 4 and 6) were imaged prospectively at baseline and 2 months later. We found evidence of abnormal blood flow patterns on OCTA and AOSLO in all imaged SCD patients. Results from patients 8 to 11 are shown in the Appendix (available at www.ophthalmologyscience.org). Figures 1 to 8 and Fig S9 (unaffected control and patients 1–8) include accompanying Videos 1 to 9 (available at www.ophthalmologyscience.org). The frame rate of all videos (both OCTA and AOSLO) was 16 frames per second, which is similar to the original unregistered AOSLO videos recorded at a frame rate of 16.6 Hz.

**Unaffected Control**

Figure 1 shows the left eye of the 34-year-old unaffected control. Video 1 (available at www.ophthalmologyscience.org) demonstrates dynamic features of blood flow and the vessel walls. Identification of the arterioles and venules was based on correlation to fundus photographs and confirmed by the presence of peripapillary capillary free zones within the superficial OCTA layers.

In the unaffected control, the color fundus photograph and macular OCT appeared normal (Fig 1A, D). The OCTA revealed an intact FAZ and normal paravascular microvascular perfusion. An ROI was chosen with capillary and noncapillary blood vessels (Fig 1B; Video 1B) for AOSLO imaging, which confirmed normal vessel wall features and blood flow (Fig 1C; Video 1C). A confocal AOSLO image (Fig 1C1; Video 1C1) was included for comparison with a nonconfocal AOSLO image (Fig 1C2; Video 1C2) to show its limitations in revealing vascular details compared with nonconfocal imaging. Intravascular blood cells appeared as highly reflective objects under confocal AOSLO visualization (Fig 1C1; Video 1C1). With nonconfocal AOSLO, individual blood cell boundaries were more easily discernible (Fig 1C2; Video 1C2).

**Patient 1**

Figure 2 and Video 2 (available at www.ophthalmologyscience.org) show the left eye of patient 1, a 44-year-old woman with hemoglobin SS (HbSS) disease and nonproliferative SCR. The color fundus photograph revealed increased vascular tortuosity with angioid streaks (Fig 2A), and the macular OCT showed moderate thinning of the temporal macula (Fig 2D, white arrow). OCT angiography showed widening of the intercapillary spaces in the parfoveal region (Fig 2B; Video 2B), with an intermittently perfused capillary segment at the temporal border of the FAZ (Fig 2B; Video 2B, box).

Adaptive optics scanning light ophthalmoscopy imaging of the intermittently perfused capillary showed a pattern of poor forward flow followed by restoration of perfusion within the capillary segment (Video 2C). During an episode of slowed forward flow, the nonconfocal images and Video 2 revealed a single torpedo-shaped sickled RBC within the capillary segment (Fig 2C3, C4, yellow arrows; Video 2C2), and a trailing acellular plasma space preceded by an RBC rouleau (Fig 2C3, C5, red arrows; Video 2C2).

The sickled morphology of the isolated cell and the rouleau formation are consistent with the behavior of RBCs from SCD patients in the laboratory setting.\(^{40,41}\) A rouleau (plural is rouleaux) is a stack of RBCs that forms when RBCs stick to each other along their flat surfaces. Although nonspecific and not always pathological, the increased presence of rouleaux signals the presence of a pathologic state.\(^{42}\) In patient 1, the RBC rouleau appeared responsible for the congestion, moving forward haltingly as it navigated the surrounding capillary wall. Individual RBCs can be seen within the stack of the rouleau (Fig 2C5; Video 2C2).

**Patient 2**

Figure 3 and Video 3 (available at www.ophthalmologyscience.org) depict the right eye of patient 2, a 23-year-old woman with HbSS disease and nonproliferative SCR. The color fundus photograph and macula OCT appeared grossly normal (Fig 3A, D). OCT angiography revealed compromised perfusion along the superior-nasal and inferior border of the FAZ, raising suspicion for the presence of poor perfusion in this area (Fig 3B; Video 3B, box).

Confocal AOSLO imaging at the inferior border of the FAZ showed foci of RBC stasis (Fig 3C1; Video 3C1, yellow arrows) and sluggish blood cell flow (Fig 3C1;
Video 3C1, red arrows) within a perivenular capillary segment. Nonconfocal AOSLO imaging was able to define the outlines of the static blood cells better than confocal AOSLO imaging.

Patient 3

Figure 4 and Video 4 (available at www.ophthalmologyscience.org) show the left eye of patient 3, a 31-year-old man with hemoglobin SC (HbSC) disease and proliferative SCR. The fundus image demonstrated increased vascular tortuosity (Fig 4A), and the macular OCT showed severe thinning of the temporal macula (Fig 4D, white arrow). OCT angiography revealed an enlarged irregular FAZ surrounded by widened inter-capillary spaces, especially superior-temporally (Fig 4B; Video 4B). The irregular superior border of the FAZ was inferred to be due to nonperfused capillary segments in this area (Fig 4B; Video 4B, box).

Confocal AOSLO imaging revealed a dilated capillary segment containing multiple immobile reflective foci (Fig
Figure 2. Left eye of patient 1. A, Standard color fundus photograph. Line represents horizontal OCT scan through fovea. **White box** localizes the parafoveal area imaged with OCT angiography (OCTA). B, Averaged OCTA of 10 scans. Red A’s label the associated arterioles, and blue V labels the associated venule. **Green box** localizes the region of interest (ROI) imaged with adaptive optics scanning light ophthalmoscopy (AOSLO). Scale bar represents 200 μm. C1, Single frame from a confocal, and (C2, C3) 2 frames from a nonconfocal AOSLO imaging video. **White arrow** represents direction of blood flow in the capillary segment of interest. **Red arrow** points to a red blood cell (RBC) rouleau. **Yellow arrow** points to a single sickled RBC. Blue V labels the associated venule from (B). Scale bar represents 200 μm. C4, C5, Zoomed-in views from (C3) highlighting morphological features of the torpedo-shaped single sickled RBC (yellow arrow) and the RBC rouleau (red arrow). Scale bar represents 10 μm. D, Macular OCT. **White arrow** indicates temporal macular thinning. The associated Video 2 is available at www.ophthalmologyscience.org.
4C1; Video 4C1, yellow arrows), suggesting a blood cell aggregate. Nonconfocal AOSLO imaging confirmed the presence of a wall-to-wall immobile blood cell thrombus (Fig 4C2; Video 4C2, yellow arrows). Nonconfocal AOSLO was able to resolve a plasma-filled capillary segment interrupted by tumbling RBCs spilling over from the nearby venule (Fig 4C2; Video 4C2, red arrows) and an adjacent sclerotic “ghost” capillary segment inferior to the dilated capillary segment (Fig 4C2; Video 4C2, blue arrows). The thrombus in this area appeared fixed in place throughout the imaging session.

**Patient 4**

Figure 5 and Video 5 (available at www.ophthalmologyscience.org) show the right eye of patient 4, a 36-year-old woman with HbSC disease and proliferative SCR. The patient had no visual symptoms and no evidence of vitreous
hemorrhage, prompting the decision to monitor her retina closely without laser treatment or changes to her systemic management. The patient’s images were taken at baseline and 2 months thereafter as a natural history survey of her microvascular occlusive activity. The color fundus photograph and macula OCT appeared grossly normal (Fig 5A, D), which remained unchanged between the 2 visits. OCT angiography showed a relatively stable capillary bed between the 2 visits, with some poorly perfused capillaries at baseline appearing well perfused 2 months later, and a few initially well-perfused capillaries appearing poorly perfused at the later imaging session (Fig 5B1 vs. B2).

At the baseline visit, OCTA revealed an intermittently nonperfused periarteriolar capillary segment at the temporal border of the FAZ (Fig 5B1, box; Video 5C2, inset). Confocal AOSLO imaging revealed a reflective

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Figure 4. Left eye of patient 3. A, Standard color fundus photograph. Line represents horizontal OCT scan through fovea. White box localizes parafoveal area imaged with OCT angiography (OCTA). B, Averaged OCTA of 10 scans. Red A’s label the associated arterioles, and blue V labels the associated venule. Green box localizes region of interest (ROI) imaged with adaptive optics scanning light ophthalmoscopy (AOSLO). Scale bar represents 200 μm. Single frames from (C1) a confocal and (C2) a nonconfocal AOSLO video. White arrows represent direction of blood flow in the capillary segments of interest. Yellow arrows point to an immobile thrombus containing blood cells. Red arrows point to an area of plasma with occasional tumbling red blood cells. Blue arrows represent a sclerotic capillary segment. Blue V’s label the venule from (B). Scale bar represents 20 μm. D, Macular OCT. White arrow indicates temporal macular thinning. The associated Video 4 is available at www.ophthalmologyscience.org.
intravascular mass blocking entry into the capillary segment as it bifurcated off its arteriole (Fig 5C1, yellow arrow). Nonconfocal AOSLO imaging was able to resolve the mass as an RBC caught against the vessel wall (Fig 5C2; Video 5C2, yellow arrows). Blockage of the capillary lumen was only partial, as sparse RBC flow was visualized (Video 5C2, red arrow).

At the 2-month visit, OCTA showed restored perfusion through the capillary segment (Fig 5B2, box; Video 5C4, inset). Adaptive optics scanning light ophthalmoscopy imaging explained the OCTA findings, confirming the presence of restored capillary perfusion, which was better visible in the nonconfocal AOSLO Video (Video 5C4, yellow and red arrows) compared with confocal and nonconfocal single frames (Fig 5C3, C4). This case demonstrates the dynamic changes that can occur naturally from month to month in sickle cell disease.

**Patient 5**

Figure 6 and Video 6 (available at www.ophthalmologyscience.org) show the right eye of patient 5, a 23-year-old man with HbSS disease and nonproliferative SCR. Fundus appearance was grossly normal (Fig 6A), and OCT revealed a broad foveal depression and moderate thinning of the nasal macula (Fig 6D, white arrow). OCT angiography showed an enlarged FAZ with widened parafoveal intercapillary spaces (Fig 6B; Video 6B, box).

Adaptive optics scanning light ophthalmoscopy confocal imaging revealed the presence of a nonperfused capillary
segment not visible on OCTA (Fig 6C1; Video 6C1, black arrows). A reflective immobile intravascular mass was visible at its bifurcation from an upstream feeder capillary (Fig 6C1; Video 6C1, yellow arrows). Nonconfocal AOSLO imaging confirmed that there were no blood cells flowing through the capillary segment of interest (Fig 6C2, C3; Video 6C2, black arrows). Nonconfocal AOSLO imaging also revealed that the immobile mass at the capillary bifurcation consisted of a cellular thrombus stuck to the capillary wall (Fig 6C2, C3; Video 6C2, yellow arrows). Pulsatile spillover and backflow of blood cells into the nonperfused capillary segment from a downstream perfused capillary segment was also visible (Fig 6C2, C3; Video 6C2, red arrows). In the parallel perfused capillary
segment, a transient RBC rouleau was visible (Fig 6C3, Video 6C2, blue arrows). The lack of blood cell flow through the capillary segment of interest was consistent throughout AOSLO imaging.

**Patient 6**

Figure 7 and Video 7 (available at www.ophthalmologyscience.org) show the right eye of patient 6, a treatment-
naïve 31-year-old woman with HbSS disease and non-proliferative SCR, at baseline and 2 months after initiation of oral hydroxyurea therapy. Fundus exam at the initial visit showed increased arteriolar tortuosity (Fig 7A), and macular OCT showed a broad foveal depression (Fig 7D). OCT angiography revealed widened parafoveal capillary spacing (Fig 7B1; Video 7B). There was an intermittently nonperfused capillary segment near the superior-temporal border of the FAZ (Fig 7B1; Video 7B, box). Patient 6 started oral hydroxyurea therapy a few days after her initial imaging visit. Two months after initiation of therapy, her dilated fundus exam and macular OCT appeared unchanged. However, OCTA revealed quantitative improvement in overall perfusion of the

**Figure 8.** Left eye of patient 7. A, Standard color fundus photograph. Line represents horizontal OCT scan through fovea. White box localizes parafoveal area imaged with OCT angiography (OCTA). B, Averaged OCTA of 10 scans. Red A’s label the associated arterioles, and blue V labels the associated venule. Green box localizes region of interest (ROI) imaged with adaptive optics scanning light ophthalmoscopy (AOSLO). Scale bar represents 200 μm. C1, Single frame from a confocal AOSLO video. **White arrow** indicates the direction of blood flow through the capillary segment of interest. C2, C3, Single frames from a nonconfocal AOSLO video. **Yellow arrow** points to the formation of a cellular thrombus and subsequent nonperfusion of the capillary segment. **Red arrows** point to an adjacent poorly perfused capillary segment. Blue V labels the venule from (B). Scale bar represents 20 μm. D, Macular OCT. **White arrow** indicates temporal macular thinning. The associated Video 8 is available at www.ophthalmologyscience.org.
macula (Zhou et al., Fig 6). With treatment, many of the intermittently nonperfused capillary segments identified on the initial OCTA images were now consistently perfused (Fig 7B2).

At the initial baseline visit, confocal AOSLO imaging demonstrated blood cells within thrombi at 2 consecutive capillary bifurcations (Fig 7C1, C2, yellow arrows) and a poorly perfused capillary segment (Fig 7C1, C2, red arrows). Nonconfocal AOSLO imaging revealed more detail within the thrombi (Fig 7C4, C5; Video 7C4, C5, yellow arrows). Nonconfocal AOSLO also showed enlargement of a thrombus over a 5-minute interval (Fig 7C4, C5; Video 7C4, C5, yellow arrows).

In images from the second visit, 2 months after initiation of oral hydroxyurea therapy, AOSLO showed resolution of the thrombi (Fig 7C3 and C6, yellow arrows) and restored perfusion through the previously nonperfused capillary segments (Fig 7C3, C6, red arrows). The resolution of thrombi and restored perfusion are better visualized on the nonconfocal AOSLO Video (Video 7C6, yellow and red arrows).

**Patient 7**

Figure 8 and Video 8 (available at www.ophthalmology science.org) show the left eye of patient 7, a 25-year-old woman with HbSS disease and nonproliferative SCR. Fundus exam revealed peripapillary and macular atrophic changes (Fig 8A). Macular OCT showed severe temporal retinal thinning correlates to decreased microvascular density and that microvascular insults likely precede and cause structural thinning. OCT angiography has also confirmed that the majority of capillary pruning appears to be perivenular, supporting certain prior studies and refuting other animal studies implicating post-capillary venules, underscoring the importance of in vivo human study.

In our study, AOSLO imaging provided visual evidence that blood cell—blood cell and blood cell—endothelial interactions are at least partially responsible for microvascular occlusive events in SCD. A spectrum of phenomena at the cellular level was revealed with AOSLO imaging, including capillaries with intermittent blood cell flow, blood cell stagnation, and sites of thrombus formation containing cellular material. It is apparent that AOSLO imaging has enough resolution to visualize single sickled RBCs and RBC rouleaux formations. Figure 2 and Video 2 represent one of a few instances where we were able to conclusively distinguish a single sickled RBC flowing through a capillary segment based on its classic morphology. There was an RBC rouleau trailing behind that sickled RBC, forming a space of plasma in front and behind the sickled RBC, and slowing forward flow, making it possible to adequately visualize the cell’s morphology. There were likely multiple sickled RBCs captured in other patients with rapidly moving RBC columns, RBC rouleaux, and within thrombus formations. However, it was often difficult to conclusively identify cells as sickled RBCs in these instances.

Longitudinal imaging of patient 4 (Fig 5, Video 5) with AOSLO allowed study of the natural history of thromboocclusive events, showing that a thrombus may resolve on its own with time and lead to the return of normal capillary perfusion. Longitudinal OCTA imaging of patient 6 (Fig 7, Video 7) showed that macular perfusion improves with oral hydroxyurea therapy. As shown by longitudinal AOSLO imaging in patient 6, the improvement in perfusion with treatment was at least in part related to the resolution of cell-containing thrombi.

Grading systemic disease burden in SCD, especially at early stages of the disease, has proven challenging for clinicians. Development of therapeutic interventions has run...
into some challenges as well, especially in determining how effective a medication is for a given individual. OCT angiography and AOSLO imaging offer a powerful combination for evaluating disease activity and response to systemic therapeutic interventions. The intermittent perfusion index biomarker, based on serial OCTA imaging as described by Zhou et al., is able to quantify the ischemic burden of the retina in SCD patients. Intermittent perfusion index shows potential as a quantifiable surrogate for measuring systemic disease burden and response to systemic therapy.

As a tradeoff to its magnification capability, AOSLO imaging provides a much smaller field of view compared with that of OCTA, limiting its clinical utility for global quantitative analysis. The level of magnification and enhanced resolution provided, however, is most helpful for explaining OCTA findings from a cellular perspective. This use of AOSLO imaging may help reclassify SCD patients according to predominant mechanisms of microvascular occlusion, including RBC sickling, thrombus formation, and blood cell–vessel wall interactions, allowing for a more individualized therapeutic approach.

Study Limitations

Limitations in this study included the subjective method of optimizing the directional difference filtering approach for blood vessel and blood cell visualization. Future studies are warranted to systematically assess emboss filter parameters for visualization of various retinal structures. Furthermore, although the apparent sizes and shapes of blood cells and vascular structures in the merged emboss filtered frames looked comparable to those in the corresponding split-detection frames, future quantitative studies rigorously exploring the effect of directional difference filtering on apparent size and shape are needed.

Footnotes and Disclosures

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Limitations in this study also included the small sample size of patients with sickle cell and the mostly cross-sectional nature of the study. Because of the small sample size, we were unable to differentiate AOSLO findings in SCD patients with nonproliferative versus proliferative SCR or between HbSC and HbSS patients. Future studies in planning will expand on the participant numbers and longitudinal observations, and will aim toward development of quantitative metrics of blood flow patterns to improve assessment of disease activity and response to therapies. Hopefully, useful correlation of these ocular metrics to current clinical and laboratory findings in SCD can be achieved.

Conclusions

Sickle cell disease is a peculiar disease process that has provided several significant opportunities for advancement of medical knowledge, including in the fields of molecular and genetic disease. We hope that our exploration of in vivo retinal microvascular features in SCD contributes in a small way to the blossoming field of omics. We hope to advance the understanding of this systemic disease process through the insights gained from combining AOSLO and OCTA retinal imaging.

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In Memory of Peter N. Gillette, MD (1932-2020). Dr. Gillette lived a life of service. He was a pioneer and an advocate in the sickle cell community, and devoted his entire life to the care of his patients. Dr. Gillette’s life is well captured by Sir William Osler, who said in 1907, “To serve the art of medicine as it should be served, one must love his fellow man.”

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No animal subjects were used in this study.

Author Contributions:
Conception and design: Pinhas, Migacz, Zhou, Sredar, Dubra, Glassberg, Rosen, Chui
Data collection: Pinhas, Migacz, Zhou, Castanos Toral, Otero-Marquez, Israel, Sredar, Dubra, Rosen, Chui
Analysis and interpretation: Pinhas, Migacz, Zhou, Castanos Toral, Otero-Marquez, Israel, Sun, Gillette, Sredar, Dubra, Glassberg, Rosen, Chui
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In Memory of Peter N. Gillette, M.D. 1932-2020
Dr. Gillette’s life was a life of service. He was a pioneer and an advocate in the sickle cell community, and devoted his entire life to the care of his patients. Dr. Gillette’s life is well captured by Sir William Osler, who said in 1907, “To serve the art of medicine as it should be served, one must love his fellow man.”

Abbreviations and Acronyms:
ADD = air disk diameter; AOSLO = adaptive optics scanning light ophthalmoscopy; BCVA = best-corrected visual acuity; D = diopeters; FA = fluorescein angiography; FAZ = foveal avascular zone; HbSC = hemoglobin SC; HbSS = hemoglobin SS; IOP = intraocular pressure; OCTA = OCT angiography; RBC = red blood cell; ROI = region of interest; SCD = sickle cell disease; SCR = sickle cell retinopathy.

Keywords:
Adaptive optics, OCT angiography, Oculomics, Retinal microvasculature, Sickle cell disease.

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