Sickle Cell Maculopathy: Microstructural Analysis Using OCTA and Identification of Genetic, Systemic, and Biological Risk Factors

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SHORT TITLE: Risk factors of Sickle cell maculopathy

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Introduction:

Sickle cell disease (SCD) is the most common genetic disease affecting around 300,000 to 400,000 newborns every year.[1,2] SCD is caused by a mutation in the β-globin gene resulting in the production of an abnormal hemoglobin called hemoglobin S (HbS). Hemoglobin S polymerizes under hypoxic conditions, which causes a mechanical distortion of red blood cells into a sickle-like shape. These sickled red blood cells are fragile and rigid, resulting in chronic hemolytic anemia and frequent vaso-occlusive like complications in multiple organs, including the eyes.[3] SCD is a generic term encompassing different syndromes according to genotype: homozygotes HbSS or compound heterozygotes (HbSvariant).

Sickle cell retinopathy (SCR) has been well described, with proliferative sickle cell retinopathy (PSR) being a major sight-threatening complication.[4] Since the development of Spectral Domain Optical Coherence Tomography (SD-OCT), macular vascular abnormalities in SCD are now commonly detected.[5] Sickle cell maculopathy (SCM) is defined as patchy areas of severe retinal thinning in the temporal macula. The exact etiology of macular thinning remains unclear but it may be related to the temporal macula ending along the horizontal raphe making it a watershed zone between the vascular arcades of the retinal circulation and thus more susceptible to vascular occlusions.[6] By allowing depth-resolved visualization of macular vascular network with high resolution, Optical Coherence Tomography Angiography (OCTA) recently detected much more macular vascular alterations than previously and provided additional features, including enlargement and irregularities of the foveal avascular zone (FAZ), hairpin venular loops and areas of flow loss responsible for irreversible macular thinning.[7,8]

Previous studies showed that HbSC genotype, male gender, low fetal hemoglobin (HbF), high hemoglobin levels and high blood viscosity (particularly in HbSS genotype) were associated with severe PSR.[9–11] However, no study has identified genetic or biological risk factors of SCM. Identifying risk factors could lead to reliable prediction models for SCM management and minimize the onset of irreversible macular scotoma, decreased retinal sensitivity or vision loss described in eyes with severe macular thinning.[12,13]

The primary purpose of this study was to identify the systemic, biological and genetic risk factors of SCM on OCT. The secondary purpose was to analyze macular microvascular network using OCTA and test if FAZ area and macular ischemia could be useful to identify more specific risk factors of SCM.

Materials and Methods:

Patients: This study received the ethical approval of the Regional Ethics Committee (CPP Sud-Ouest Outre-Mer III, Bordeaux, France) and adhered to the principles set by the Declaration of Helsinki. Written informed consent was obtained from each participant. This cross-sectional monocentric study concerned 117 patients with confirmed SCD diagnosis using standard methods and undergoing regular ophthalmological evaluation at the Ophthalmology Department of the University Hospital of Guadeloupe (French West Indies) between January 1st, 2019 and January 1st, 2020. Exclusion criteria were age under 18, history of other confounding retinal vascular disease such as hypertension, diabetes or retinal vein occlusion, prior retinal surgery, spherical equivalent (SE) > ± 1 diopter, retinal involvement such as

vitreomacular traction syndrome, epiretinal membrane or druses, poor quality images or ocular media opacities preventing detailed imaging. Sixty-nine eyes of 37 patients met these criteria and were excluded, so that 151 eyes of 78 patients were analyzed. An ethnic-matched control group of 20 healthy African Caribbean subjects were also recruited and underwent the same ocular procedures.

Multimodal imaging: Ophthalmic examination included best corrected visual acuity (BCVA) measured with Snellen chart, slit-lamp evaluation, dilated fundus examination. SCR was graded according to the Goldberg classification including peripheral arteriolar occlusions (stage I), peripheral arteriovenous anastomoses (stage II), preretinal neovascularization (stage III), vitreous hemorrhage (stage IV) and retinal detachment (stage V).[14] Eyes with no retinopathy were graded as Goldberg stage 0. Patients with previously performed focal laser photocoagulation for peripheral neovascularization were classified as stage III. Analyses were performed using three categories of retinopathy: no retinopathy (stage 0), non-proliferative retinopathy (stage I and II) and proliferative retinopathy (stage III, IV and V).
All patients and healthy controls underwent a macular SD-OCT and OCTA using the Spectralis HRA+OCT (Heidelberg Engineering, Heidelberg, Germany). For each eye, the standard scanning protocol included an automated « posterior pole » 30x25 cuboid volume scan of 61 high resolution OCT scans and 10° and 20° OCTA scans centered on the fovea. Multiple scans were acquired as needed to secure adequate quality criteria and the best quality scans were selected by the lead grader for analysis. Images were anonymized and analyzed by two independent retina fellowship trained graders in a blinded fashion. In case of disagreement, scans were reviewed and arbitrary graded together. Sickle cell maculopathy was identified as patchy retinal thinning areas on OCT (i.e., blue areas of markedly decreased thickness on retinal thickness color-coded map). The first step involved manual delineation of the full thickness retina FAZ border taken from 10° OCTA scans using the « area » function of the software (Figure 1). Scans were then qualitatively graded by the investigators for macular ischemia from no (0) to severe (3) macular ischemia (including extension of flow loss areas, vessel density, enlargement of the FAZ, presence of perifoveal venular loops and focal disruption of the perifoveal anastomotic capillary arcade). Figure 2 illustrates our macular ischemia graduation.

Clinical and biological data collection: Clinical data were retrospectively collected from patient's charts at the last routine clinical visit to the Sickle cell disease center of Guadeloupe and included : SCD type (genotype, α-thalassemia status), medical and surgery history (other systemic condition, spleen status, cholecystectomy), current treatment (hydroxyurea, chelation therapy, chronic transfusion, anticoagulants) and history of SCD systemic complications (acute chest syndrome, cerebrovascular accident, severe vaso-occlusive crisis frequency, osteonecrosis). Frequency of severe vaso-occlusive crises was defined using Duan et al's method, i.e. as rare if once a year or less, occasional if twice a year and frequent if three times a year or more, averaged over the two years prior to the ocular examination.[10] Severe vaso-occlusive crises were defined as requiring acute Emergency Department visit or inpatient admission.
We also collected laboratory results including fetal hemoglobin (HbF) level, baseline hemoglobin level, hematocrit, C-reactive protein (CRP), prothrombin ratio (PR), reticulocytes, leucocytes, neutrophils and platelets count, lactate dehydrogenase

(LDH), total bilirubin, serum ferritin and fibrinogen levels and G6PD deficiency. Biological parameters were obtained at steady-state.

Statistical analysis: Mann-Whitney tests were used to compare the different demographic, clinical and ocular characteristics between the two groups. A chi^2 was used for qualitative data. For the three groups comparisons, a Kruskal-Wallis test was used followed by a Dunn post-hoc test when appropriate. P-value < 0.05 was considered as significant. Three ocular criteria were included in the analysis: the occurrence of maculopathy, the FAZ enlargement and the severity of the macular ischemia. Because simple comparison of the distribution of systemic and biological factors between patients can be misleading as certain factors are interdependent or age related, we used multiple logistic regression analysis, adjusted by genders, genotypes and factors showing a p-value < 0.1 in univariate analysis to identify risk factors independently associated with sickle cell maculopathy, FAZ enlargement and macular ischemia in an ordinal Generalized Estimating Equations (GEE) model.
Complete data were not available for all patients, so we used a simple imputation with a predictive mean matching (PMM) method for the missing data. Matched controls were used to compare FAZ measures with SCD patients using a Mann-Whitney test. Statistical analyses were performed using R statistical software. Data are expressed in mean ± SD or percentages for quantitative and qualitative parameters, respectively.

Results:

Demographic results: Table 1 summarizes patients' demographical and clinical characteristics. All participants were of African Caribbean origin. 151 eyes of 78 patients (25 men and 53 women) with a mean age of 37 years (range 18 – 61 years old) were enrolled in this study. Five eyes were excluded due to unilateral high myopia (1 eye), phthisis bulbi (1 eye), poor acquisition due to no fixation (2 eyes) and a large foveal vascular pigmentary epithelium drusen (1 eye). Several sickle cell syndromes were analyzed including homozygotes HbSS (43 patients, 55%) and compound heterozygotes HbSvariant (35 patients, 45%) including HbSC (30 patients), HbS/β+ (4 patients) and HbSLepore (1 patient). Visual acuity ranged from 20/25 to 20/20 for all patients. The most frequent systemic events in our population were painful vaso-occlusive crisis (49%), acute chest syndrome (42%) and osteonecrosis (31%). There was no statistical difference in age, gender, functional α-globin gene number, vaso-occlusive crisis, osteonecrosis, anticoagulant medication and chronic transfusion between HbSS and HbSvariant groups. However, HbSS patients exhibited more ulcers (p = 0.019), more acute chest syndrome (p < 0.01) and more cerebrovascular accidents (p < 0.01). Lower hemoglobin and hematocrit (p < 0.001), higher LDH and total bilirubin level (p < 0.001) were found in the HbSS group. Finally, HbSS patients seemed to exhibit a pro-inflammatory state: higher leucocytes and neutrophils levels (p < 0.001 and <0.02, respectively) and a trend through higher level of CRP (p=0.087).

Sickle cell maculopathy: SCM was present in 66 eyes (43.7%), especially in HbSS patients (71.2%, p=0.004). Univariate analysis (Table 2) showed that patients with SCM had lower hemoglobin (p = 0.001), lower hematocrit (p = 0.01), lower PR (p = 0.038) and higher LDH levels (p = 0.007) than those without. In addition, we

observed higher occurrence of SCM in eyes with proliferative sickle cell retinopathy ($p = 0.04$). Chronic transfusion tended to be associated with the occurrence of SCM ($p = 0.073$). As expected, maculopathy was associated with FAZ enlargement ($p = 0.021$) and more severe macular ischemia ($p < 0.001$). A cut-off level of HbF > 15% based on the prior studies about sickle cell retinopathy did not show any association with maculopathy.[15] Multivariate regression analysis revealed that HbSS genotype and lower prothrombin ratio (PR) were the most predictive risk factors of SCM ($p = 0.01$).

Faz area: On OCTA, FAZ was measured in 142 eyes (94%) of SCD patients and in all healthy control eyes. In 9 eyes, it was not detectable by the software used related to extensive macular ischemia (Figure 3) due to central arterial occlusion (4 eyes) and extensive perifoveal capillary remodeling with a poorly defined FAZ (5 eyes). For these 9 eyes, the quantitative value of their surely enlarged FAZ could be misleading in the analysis. Accordingly, we used a censored statistical model that assigned 1.0 to larger FAZ area than 1.0. Univariate analysis showed that FAZ enlargement was associated with HbSS genotype ($p = 0.04$), hydroxyurea treatment ($p = 0.04$), anticoagulant medication ($p = 0.05$), positive history of acute chest syndrome ($p = 0.02$), occasional vaso-occlusive crises ($p = 0.01$), lower hematocrit and hemoglobin levels ($p = 0.01$) and higher LDH level ($p = 0.02$). Unexpectedly, we also found that male SCD patients had a smaller FAZ size ($p = 0.01$). In multivariate analysis only high LDH level was independently associated with FAZ enlargement (a rise of 0.03 μm^2 for every 100 units of LDH, $p = 0.02$). Interestingly, positive history for acute chest syndrome was marginally associated with FAZ enlargement ($p = 0.07$). Comparison between SCD patients with or without SCM and age, ethnic and sex – matched controls revealed that the FAZ area was larger in our patients than controls (respectively 0.52 ± 0.19 vs 0.38 ± 0.17, $p < 0.01$).

Macular ischemia: Macular ischemia was subjectively identified using SD-OCT and OCTA with quantitative and qualitative criteria: irregularity of the FAZ, the extension of no flow areas and the general vascular density. We graded ischemia from no (0) to severe (3) for all 151 eyes of the study. The size of the grade 3 (severe) group of macular ischemia was not large enough to perform statistical analysis and was regrouped with the grade 2 (moderate) group for the analysis. Thus, we had 49 eyes (32.5%) with no ischemia, 52 with mild ischemia (34.4%) and 50 with moderate or severe ischemia (33.1%). Areas of no flow were more frequent in the temporal superior (36%) subfield then the temporal inferior (27%), nasal superior (9%) and nasal inferior (5%) subfields. Seventy eyes had irregularities of the FAZ borders (46%). Thirty-eight eyes had perifoveal venular loops (25%). Abnormal macular flow loss was visible even in SCD eyes with no macular thinning on OCT (36 eyes, 42%). To have sufficient number of patients in groups for the identification of potential risk factors of macular ischemia, we regrouped moderate (2) and severe (3) eyes. Univariate analysis revealed that 80% of the eyes with moderate and severe macular ischemia are detected in HbSS patients ($p < 0.001$). We also showed a significant association between the severity of macular ischemia and hemoglobin level ($p < 0.001$), hematocrit and PR ($p = 0.01$ and < 0.001, respectively), platelet level ($p = 0.01$) and LDH ($p = 0.003$). In multivariate analysis, low hemoglobin level ($p = 0.004$) and low PR ($p = 0.01$) remained independently associated with macular ischemia severity.

Discussion:

The current study is the largest study to date evaluating SCD patients with OCTA. The analysis revealed that HbSS genotype, lower PR and proliferative sickle cell retinopathy are independently associated with sickle cell maculopathy. Correlation between proliferative sickle cell retinopathy and maculopathy is an expected outcome. However, the association between genotype and sickle cell maculopathy has been poorly described. Even though it is well accepted that sickle cell retinopathy is more common and more severe in HbSC patients than in HbSS,[14,16] Mathew et al. reported a higher rate of retinal thinning in HbSS patients than in HbSC patients (48% vs 35%),[5] whereas other studies found no statistical difference.[8,17] Our study demonstrated that retinal thinning occurs more frequently in HbSS eyes compared to the other syndromes of SCD, especially in HbSC eyes. This result is in agreement with prior studies suggesting that higher rate of vaso-occlusive crisis in HbSS patients could lead to repeated ischemic episodes in the eyes and thus the development of extensive retinal atrophy.[9,18,19] On the other hand, milder degree of retinal ischemia in HbSC genotype would be not enough to infarct the retina but could result in the production of angiogenic factors and a higher risk of peripheral neovascularization.[22,23] Natural history of sickle cell retinopathy is unique because peripheral neovessels can infarct over time.[18] In this study, we graded as PSR the few eyes with focal photocoagulation for a prior neovascularization, so PSR eyes may have been overrepresented in our analysis. However, our results suggest a strong relationship between maculopathy and retinopathy. This relationship may be explained by the fact that the temporal macular area and the retinal periphery are both supplied by small caliber terminal arterioles, therefore vascular occlusion can easily damage these areas.[7] Interestingly, PR was detected at lower level in patients with maculopathy. Low PR means a prolonged prothrombin time which is common in SCD even if the reason is not well established.[24,25] It is likely that prolonged prothrombin time is a marker of chronic vascular inflammation among others like platelets activation and high plasma levels of adhesion molecules. Prolonged PR could be related to several conditions such as liver dysfunction that decreases the synthesis of clotting factors or dysfunctional coagulation factors on vitamin K deficiency due to cholestasis for example.

OCTA enabled us to determine valuable findings of maculopathy and to identify or confirm specific risk factors for macular damages in SCD. FAZ area and macular ischemia were correlated with several biological factors. Indeed, high serum LDH level was significantly associated with FAZ area enlargement in multivariate analysis with a rise of 0.03 μm^2 for every 100 units of LDH. Also, lower hemoglobin level was significantly associated with more severe macular ischemia in multivariate analysis with a rise of 0.36 μm^2 for every unit of hemoglobin. Two mechanisms have been proposed to be involved in the pathophysiology of SCD: chronic hemolysis that could promote the development of several complications (such as leg ulcers, stroke, pulmonary hypertension, priapism) and blood hyper-viscosity that would increase the risks for vaso-occlusive like complications.[26] Few studies found that severe sickle cell retinopathy was more frequent in patients with high hemoglobin and hematocrit levels, suggesting blood hyperviscosity could be a potential risk factor.[11,27] These studies served as a basis to use phlebotomy to treat blood hyperviscosity and prevent retinopathy. In our study, we failed to found any statistical association

between SCM and complications belonging to the “blood hyperviscosity” phenotype. Moreover, the association found between hemolysis and FAZ area enlargement in our study suggest that hemolysis, and not blood hyperviscosity, could play a leading role in the development of sickle cell maculopathy.[26]

Concerning hydroxyurea use, this compound induces an increase of HbF preventing HbS polymerization and red blood cells sickling.[26] Prior studies suggest a protective effect of HbF on retinopathy, in particular when HbF is higher than 15%.[17,27] Even if Dell‘Arti et al. showed a correlation between lower HbF and sickle cell maculopathy, the current study failed to show any statistically significant association with hydroxyurea use. [19]

On OCT, the occurrence of maculopathy in patients with SCD was 43.7%, which is in agreement with prior studies showing that maculopathy frequency varied between 44% to 60%.[7,28,29] A great advantage of OCTA is to detect microvascular abnormalities before retinal thinning occurs. For instance, Minvielle et al already detected perifoveal abnormalities in fluorescence angiography in only 50% of eyes whereas OCTA detected in 100% of cases.[9] Our results are in agreement with prior studies.[7,30] Areas of no flow on OCTA were present in 36/85 eyes without maculopathy on OCT. This observation suggests that OCTA may detect early signs of macular changes in patients and can likely be considered as a clinically useful marker in the screening and the evaluation of SCD progression or even for preventive treatment.

As expected, FAZ area was found larger in SCD patients than controls. However, this finding should be read with caution because the loss of capillaries may have been the result of blood flow velocity below the detection threshold of the OCTA rather than no perfusion. In addition, it is important to acknowledge that FAZ area can vary with ethnicities.[31] An enlarged FAZ area is not specific of patients with SCD but also a physiological variation for healthy subjects from African origin. To avoid any bias related to ethnicity effect, the control group was selected in the same population studied. We also noticed a substantial variability in FAZ area and irregularity between patients and controls. Some studies performed on healthy eyes suggest that this parameter can be ambiguous and thus may be insufficient for discriminating the occurrence and severity of maculopathy in SCD.[32,33] Because of the extended disruption in the FAZ of 9 concerned eyes beyond the borders of the OCTA scans, causing misleading quantitative values with clinical implication, we did not exclude them from analysis but used a censored statistical model instead. As such, eyes with more severe macular vascular pathology have been included in this study in order to establish association with potential risk factors. We did not detect statistical difference between male and female in the occurrence of maculopathy and the macular ischemia, but men exhibited a lower FAZ area than women. Interestingly, some studies have questioned a protective female effect in SCR. Indeed, Fox et al. found that HbSS men had a 2.5-fold higher risk of developing severe SCR than women.[11] They attributed the female protective effect to estrogen levels through a vascular protective effect by increasing endothelial nitric oxide synthase activity. Further studies are warranted to address this issue.

Finally, our analysis also revealed that the occurrence of the maculopathy or the severity of macular ischemia can vary significantly between two eyes of the same

patient. Future prospective studies may elucidate the ocular risk factors that may predispose the injury of one eye compared to the other.

Our study had several limitations. First, it was a cross-sectional study, so we could not estimate the incidence and the evolution of the sickle cell maculopathy. Further studies are warranted to evaluate macular microvascular changes with OCTA in the same patients over time in a longitudinal manner. Secondly, we used strict inclusion and exclusion criteria resulting in a modest cohort of patients, which limited the multivariate analysis for potential risk factors. Yet, we did not exclude patients with prior photocoagulation because we believe that it reflects a real-life situation and their exclusion can underestimate a number of severe patients in the present analysis. Another limitation concerned image acquisition and the software used. OCTA has the advantage to be a non-invasive dye-free technique and to provide structural microvascular information. However, it requires a precise and stable ocular fixation from the patients for several seconds. Furthermore, the software we used could not provide any vascular density, so we could not have any quantitative criteria for ischemia. For this reason, we subjectively graded macular ischemia because we suspect that OCTA is a better tool to reveal maculopathy than OCT. However, the study may underestimate the frequency of macular abnormalities in patients with more severe systemic events.

In conclusion, the identification of systemic, genetic and biological factors associated with the occurrence and the severity of maculopathy confirms the complexity of this disease. Association between sickle cell maculopathy and other organ damages in SCD is a significant question. OCTA is a highly sensitive method to detect microvascular macular abnormalities before the onset of macular thinning that commonly define the maculopathy status. Avoiding the occurrence of these abnormalities is critical to minimize and to manage the functional implication of retinal thinning in these young patients. Prospective studies are needed to determine the natural history of SCM and its relevance regarding preventive strategies for SCD patients. Furthermore, the emergence of novel technology such as OCTA could provide valuable information to contribute in a public health screening and follow up of patients with SCD.

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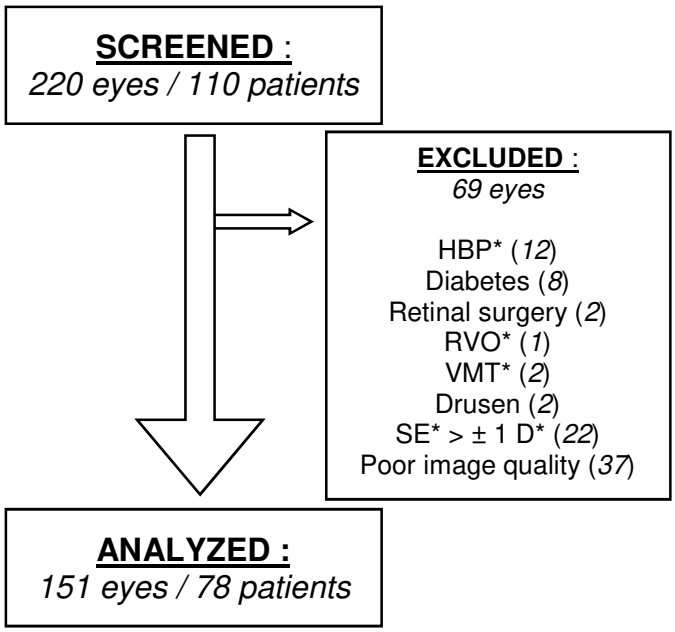
Figure captions:

Figure 1. Manual delineation of FAZ area.

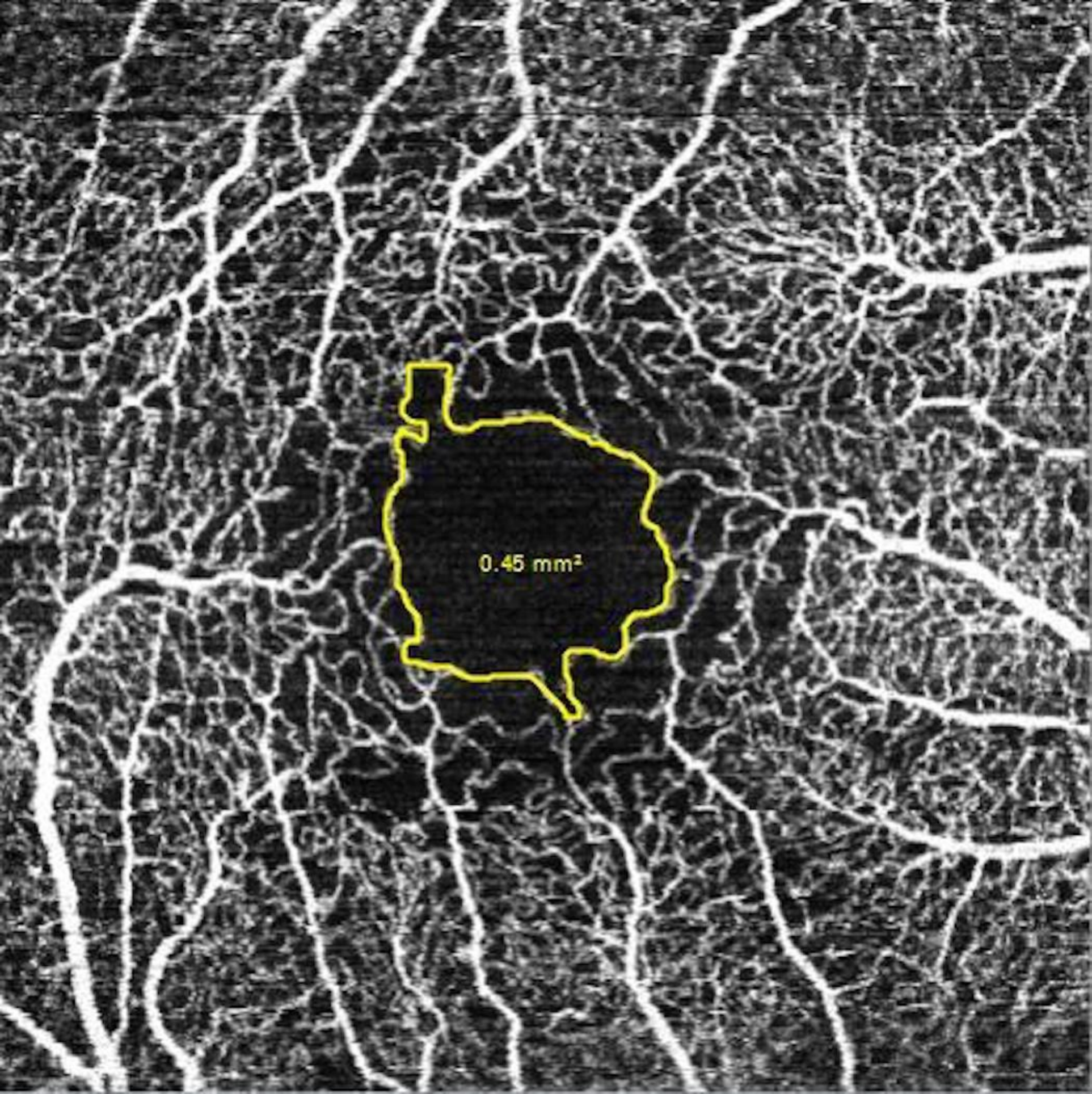
Figure 2. Subjective graduation of macular ischemia based on SD-OCT and OCTA using the following criteria: extension of blue area in retinal thickness map, the areas of flow loss in OCTA 10° and 20°, the irregularity and enlargement of the FAZ. A. No ischemia, B. mild ischemia, C. moderate ischemia, D. severe ischemia.

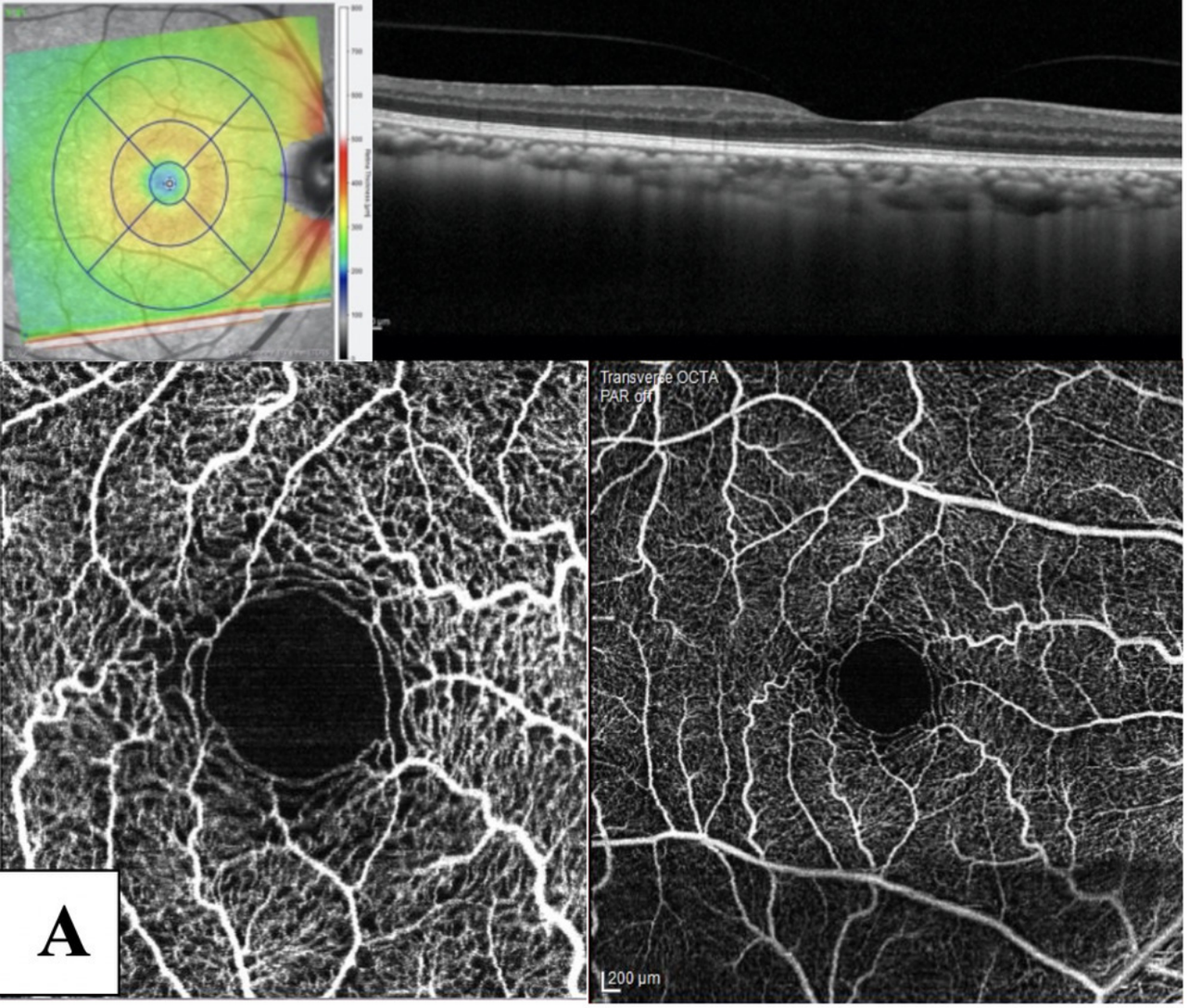
Figure 3. Example of difficulty to manually delineate FAZ area due to extensive macular ischemia.

Figure 1. Flow chart of the study.

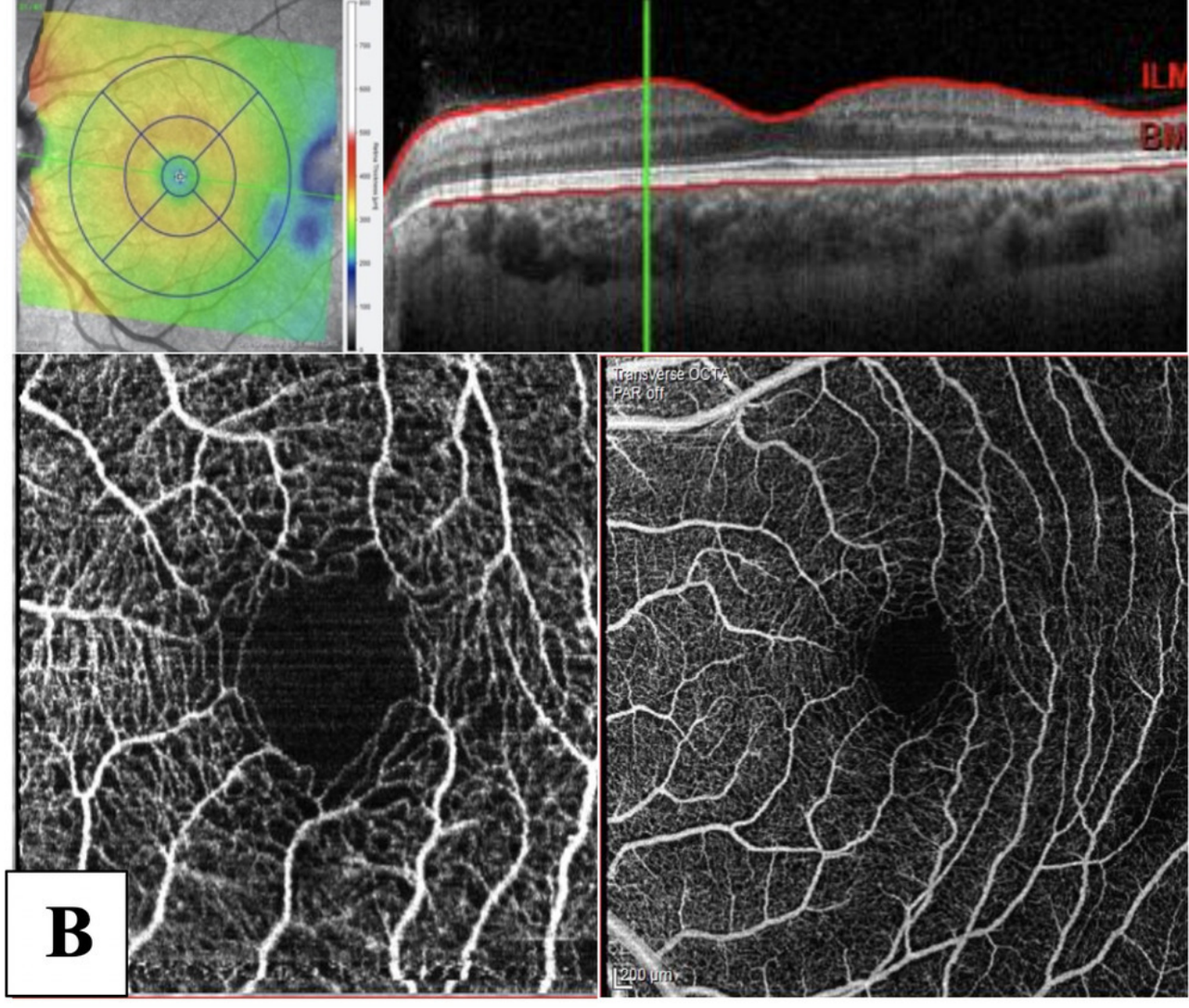


Sixty-nine eyes of 32 patients were excluded because of one or multiple criteria of exclusion.
* HBP = High Blood Pressure; RVO = Retinal Venous Occlusion; VMT = Vitreomacular Traction; SE = Spherical Equivalent; D = Diopter.

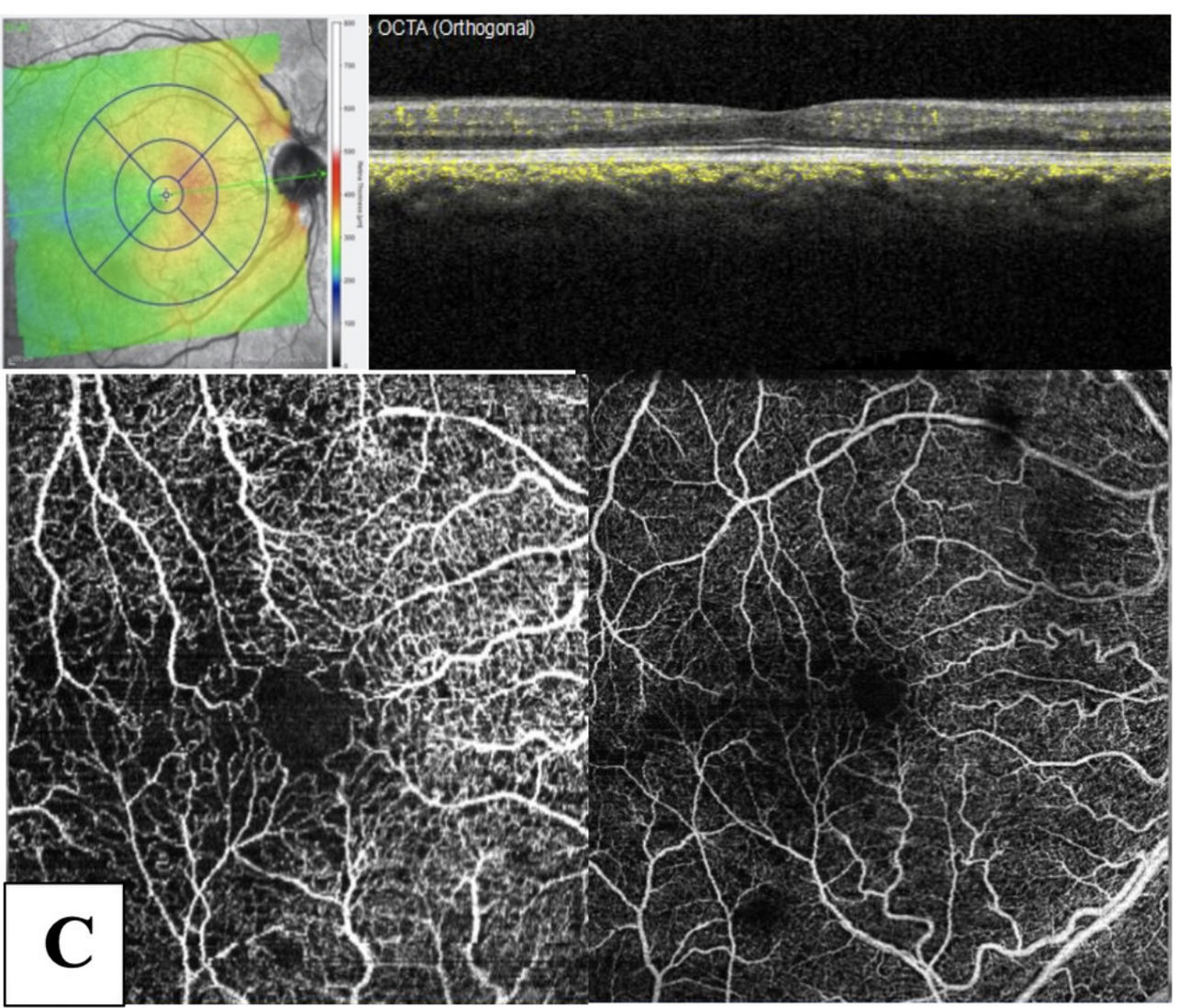

0.45 mm²


Transverse OCTA
PAR off
200 µm

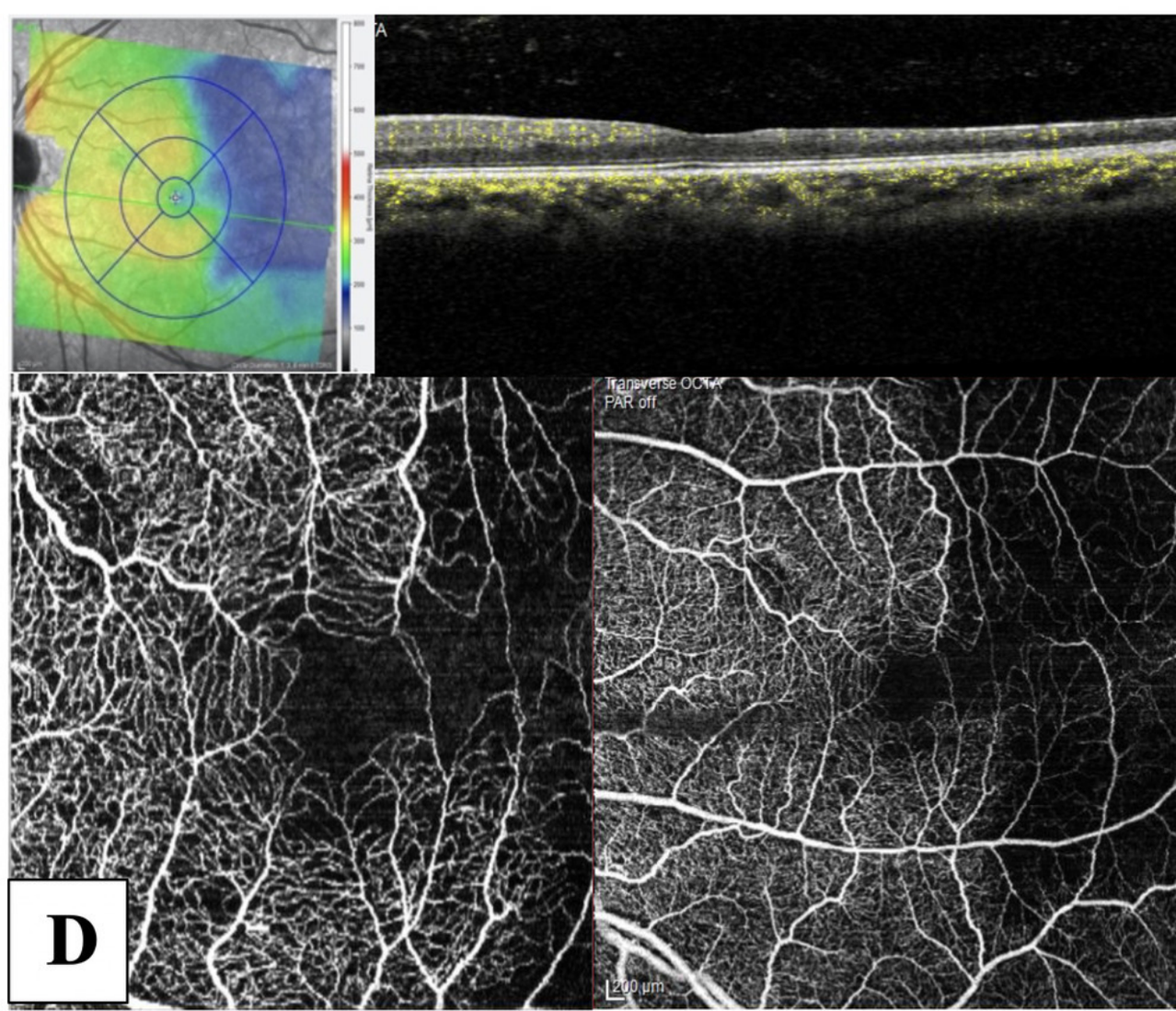
A


ILM
BM
Transverse OCTA
PAR off
200 µm

B


OCTA (Orthogonal)

C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PAR off
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D

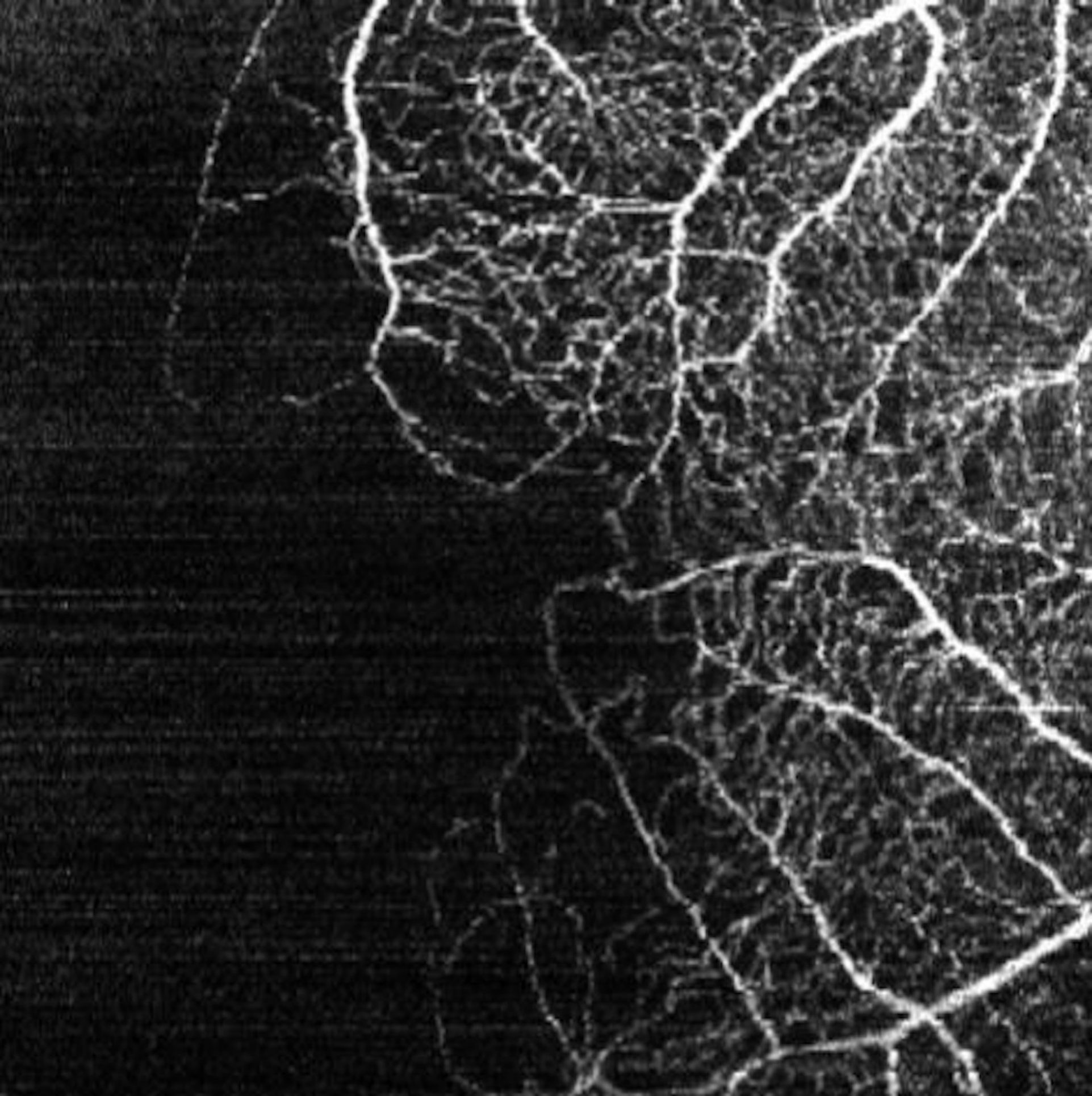

Table 1. Baseline demographic characteristics of the sickle cell disease population.

	Total	**Hb SS**	**Hb Svariant**	**P values**
Patients, n (eyes)	78 (151)	43 (85)	35 (66)	-
Age (years)	37 ± 11	35 ± 10	38 ± 12	0.21
Female, n (%)	53 (68%)	29 (6)	24 (69)	0.92
SYSTEMIC CHARACTERISTICS				
Hydroxyurea use, n (%)	27 (34.6)	27 (63)	0 (0)	**<0.001**
Anticoagulation, n (%)	12 (15.4)	9 (21)	3 (8,6)	0.13
Regular phlebotomy, n (%)	24 (31)	7 (16)	17 (49)	**<0.01**
Chronic transfusion, n (%)	6 (7.7)	5 (12)	1 (3)	0.22
Osteonecrosis, n (%)	24 (30.8)	16 (37)	8 (23)	0.17
ACS* history, n (%)	33 (42.3)	26 (60.5)	7 (20.0)	**<0.001**
VOC* frequency/2 years, n (%)				
Rare, n	62 (79)	33 (77)	29 (83)	0.51
Occasional, n	6 (7.7)	4 (9.3)	2 (5.7)	0.69
Frequent, n	10 (13)	6 (14)	4 (11)	1
CVA*, n (%)	10 (13)	10 (23)	0 (0)	**<0.01**
Cholecystectomy, n (%)	34 (44)	26 (60)	8 (23)	**<0.001**
BIOLOGICAL CRITERIA				
Hemoglobin (g/dL)	9.2 ± 1.7 (6-13)	8.1 ± 1.2	10.7 ± 1.0	**<0.001**
Baseline HbF* (%)	6.4 ± 7.0 (0-27.7)	9.0 ± 7.6	2.7 ± 3.9	**<0.001**
Hematocrit (%)	28 ± 5 (16-41)	24 ± 4	32 ± 3	**<0.001**
Platelets (G/L)	336 ± 155 (60-786)	388 ± 155	273 ± 130	**<0.001**
Reticulocytes (G/L)	198 ± 117 (7-582)	258 ± 120	124 ± 54	**<0.001**
Leucocytes (G/L)	8.1 ± 2.9 (3.9-16.2)	9.1 ± 3.0	6.8 ± 2.0	**<0.001**
CRP* (mg/L)	5.27 ± 6.05 (0-32)	6.28 ±7.0	3.97 ± 4.4	0.087
PR* (%)	83 ± 14 (23-100)	78 ± 14	88 ± 13	**<0.01**
LDH* (IU/L)	395 ± 178 (138-928)	507 ± 162	257 ± 60	**<0.001**
Total bilirubin (µmol/L)	36.4 ± 29.2 (6-184)	49.5 ± 32.9	20.6 ± 11.1	**<0.001**
GGT* (U/L)	59 +- 52 (10-248)	74 ± 60	40 ± 33	**<0.01**
G6PD* deficiency, n (%)	4 (5.3)	3 (7,.3)	1 (2.9)	0.62
αglobin gene number,n (%)	-	-	-	0.47
2	2 (2.6)	2 (5.3)	0 (0)	-
3	20 (25.6)	12 (32)	8 (26)	-
4	47 (60.3)	24 (63)	23 (74)	-

* ACS = acute chest syndrome; CVA = cerebrovascular accident; VOC = vaso-occlusive crisis; PR = Prothrombin ratio; HbF = hemoglobin F; CRP = C-reactive protein; LDH = lactate dehydrogenase; G6PD = Glucose-6-phosphate dehydrogenase; GGT = gamma-glutamyl transferase

Table 2. Univariate analysis for occurrence of sickle cell maculopathy

	Maculopathy	**No maculopathy**	**P value**
Number of eyes (%)	66 (43.7)	85 (56.3)	-
Female, n (%)	42 (63.6)	60 (70,6)	0.45
Age (years)	36.1 ± 9.5	36.8 ± 11.4	0.63
SYSTEMIC CHARACTERISTICS			
HbSS genotype	47 (71.2)	38 (44.7)	**0.004**
Hydroxyurea use, n (%)	28 (42.4)	26 (30.6)	0.19
Anticoagulation, n (%)	12 (18.2)	11 (12.9)	0.45
Regular phlebotomy, n (%)	15 (22.7)	30 (35.3)	0.15
Chronic transfusion, n (%)	8 (12.1)	3 (3.5)	0.073
ACS* history, eyes (%)	33 (50)	31 (36.5)	0.17
VOC* frequency/2 years, eyes (%)			
Rare	51 (77)	68 (80)	0.68
Occasional	5 (8.2)	7 (7.6)	0.88
Frequent	10 (15)	10 (12)	0.54
Osteonecrosis	20 (30.3)	26 (30.6)	0.95
OCULAR CHARACTERISTICS			
FAZ* area (ym^2)	0.56 ± 0.18	0.49 ± 0.13	**0.021**
Macular ischemia index, eyes (%)			
0	0 (0)	49 (58)	**<0.001**
1	18 (27)	34 (40)	-
2	33 (50)	2 (2)	-
3	15 (23)	0	-
Sickle cell retinopathy, eyes (%)			
Proliferative	20 (30.3)	14 16.5)	**0.04**
Non-proliferative	46 (69.7)	71 (83.5)	-
BIOLOGICAL CRITERIA			
Hemoglobin (g/dL)	8.8 ± 1.6	9.6 ± 1.7	**0.001**
HbF* > 15%, n (%)	10 (15.2)	13 (15.3)	0.97
Hematocrit (%)	26.1 ± 5.3	28.9 ± 5.0	**0.01**
Platelets (G/L)	373.0 ± 153.8	305.7 ± 147.9	**0.02**
Leucocytes (G/L)	8.3 ± 2.9	7.9 ± 2.9	0.40
CRP* (mg/L)	5.4 ± 4.9	6.2 ± 6.9	0.45
PR* (%)	79.2 ± 15.0	84.4 ± 12.9	**0.038**
LDH* (IU/L)	449.7 ± 183.6	355.7 ± 161.0	**0.007**
Total bilirubin (µmol/L)	42.3 ± 30.6	32.9 ± 28.2	0.12
GGT* (IU/L)	57 ± 52.6	59.6 ± 51.8	0.79
α globin gene number, n (%)	-	-	0.85
2	3 (4.5)	2 (2.4)	-
3	19 (28.8)	26 (30.6)	-
4	44 (66.7)	57 (67.1)	-

* ACS = acute chest syndrome; VOC = vaso-occlusive crisis; HbF = hemoglobin F; PR = Prothrombin ratio; CRP = C-reactive protein; LDH = lactate dehydrogenase; GGT = gamma-glutamyl transferase

Table 3. Univariate analysis of FAZ area

	FAZ area			
	Estimator	Std error	t	P value
SYSTEMIC CHARACTERISTICS				
Male	-0.09	0.03	7.30	**0.01**
HbSS Genotype	0.07	0.03	2.07	**0.04**
Hydroxyurea use	0.07	0.03	2.05	**0.04**
Anticoagulation	0.09	0.04	1.97	**0.05**
Chronic transfusion	0.12	0.06	1.91	0.06
Regular phlebotomy	-0.12	0.03	-3.53	**<0.01**
Osteonecrosis	0.04	0.04	1.28	0.20
CVA*	0.00	0.06	0.00	0.98
ACS* history	0.08	0.03	5.83	**0.02**
VOC* frequency/2 years				
Rare	-0.01	0.03	0.10	0.75
Occasional	0.09	0.03	6.75	**0.01**
Frequent	0.06	0.04	3.14	0.08
OCULAR CHARACTERISTICS				
Macular ischemia index				
1	0.001	0.018	0.00	0.94
2	0.028	0.027	1.10	0.29
3	0.036	0.037	0.94	0.33
Proliferative retinopathy	-0.03	0.02	1.24	0.27
BIOLOGICAL CRITERIA				
Hemoglobin	-0.03	0.01	- 2.73	**0.01**
HbF* < 15%	-0.03	0.03	0.99	0.32
Hematocrit	-0.01	0.00	6.84	**0.01**
Platelets	0.00	0.00	2.03	0.15
PR*	-0.0004	0.00	0.24	0.62
LDH*	0.0003	0.00	5.85	**0.02**
GGT*	0.00	0.00	2.11	0.15
α globin gene number	-0.01	0.03	- 0.21	0.83

* ACS = acute chest syndrome; CVA = cerebrovascular accident; VOC = vaso-occlusive crisis; PR = Prothrombin ratio; LDH = lactate dehydrogenase; GGT = gamma-glutamyl transferase

Table 4. Univariate analysis of risk factors in macular ischemia

	No ischemia	**Mild ischemia**	**Moderate or severe ischemia**	**P value**
Number of eyes (%)	49 (32.5)	52 (34.4)	50 (33.1)	-
Female, eyes (%)	33 (67.3)	41 (78.8)	28 (56.0)	0.40
HbSS genotype, eyes (%)	17 (34.7)	28 (53.9)	40 (80.0)	**<0.001**
Age	34.2 ± 11.2	41.0 ± 10.2	34.0 ± 9.0	0.912
SYSTEMIC CHARACTERISTICS				
Hydroxyurea use, eyes (%)	11 (22.4)	21 (40.4)	22 (44.0)	0.055
Regular phlebotomy, eyes (%)	17 (34.7)	18 (34.6)	10 (20.0)	0.16
Chronic transfusion, eyes (%)	2 (4.1)	1 (1.9)	8 (16.0)	0.09
Osteonecrosis, eyes (%)	11 (22.4)	22 (42.3)	13 (26.0)	0.66
ACS* history, eyes (%)	17 (34.7)	21 (40.4)	26 (52.0)	0.16
OCULAR CHARACTERISTICS				
Occurrence of maculopathy, eyes				
No maculopathy (%)	49 (100)	34 (65)	2 (4)	**< 0.001**
Maculopathy (%)	0 (0)	18 (35)	48 (96)	-
Proliferative retinopathy, eyes (%)	24 (49.0)	28 (53.9)	37 (74.0)	0.53
Photocoagulation, eyes (%)	12 (24.5)	19 (36.5)	21 (42.0)	0.123
BIOLOGICAL CRITERIA				
Hemoglobin (g/dL)	10.2 ± 1.7	9.0 ± 1.4	8.6 ± 1.6	**<0.001**
HbF* > 15% (%)	5 (10.2)	10 (19.2)	8 (16.0)	0.42
Hematocrit, mean ± SD	30.2 ± 5.1	27.3 ± 4.2	25.6 ± 5.6	**0.01**
Platelets (G/L)	298.8 ± 140.1	311.4 ± 141.4	395.3 ± 163.4	**0.01**
CRP* (mg/L)	5.9 ± 6.1	6.0 ±7.0	5.6 ±5.2	0.842
PR* (%)	86.2 ± 8.9	82.7 ± 17.1	77.6 ± 13.5	**<0.001**
LDH* (IU/L)	323.4 ± 140.5	399.8 ± 183.5	465.5 ± 176.9	**0.003**
Total bilirubin (µmol/L)	30.7 ± 27.1	33.4 ± 25.0	47.1 ± 33.7	0.156
GGT* (IU/L)	50.8 ± 46.5	68.7 ± 60.8	55.4 ± 46.2	0.597

* ACS = acute chest syndrome; HbF = hemoglobin F; PR = Prothrombin ratio; CRP = C-reactive protein; LDH = lactate dehydrogenase; GGT = gamma-glutamyl transferase