Optic Nerve Head Vessel Density Assessment in Recovered COVID-19 Patients: A Prospective Study Using Optical Coherence Tomography Angiography

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Precis: Vascular diseases have been linked to alterations in optic nerve head perfusion.

Purpose: The main objective was to investigate the changes in peripapillary vessel density (VD) in post coronavirus disease (COVID-19) patients.

Methods: In this prospective pilot exploratory study, patients with COVID-19 that were attended in the Emergency Department of Hospital Clinico San Carlos (Madrid) were included. All patients underwent optic nerve head optical coherence tomography angiography using the Cirrus HD-OCT 500 with AngioPlex OCTA (Zeiss, Dublin, CA) 4 and 12 weeks after diagnosis by positive reverse transcriptase-polymerase chain reaction test from nasopharyngeal swab at the Emergency Department. Sociodemographic data, medical history, disease severity, and laboratory work-up were collected.

Results: One hundred and eighty eyes of 90 patients with severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) infection were included. None of the patients reported visual changes. Severe patients were older, more frequently hispanic, dyslipidemic, and presented lower lymphocytes counts, as well as increased ferritin, D-dimer, fibrinogen, and international normalized ratio levels. No changes in optic nerve head vascularization were observed when both visits were compared. No correlation was found between VD and clinical parameters, disease severity and laboratory work-up.

Conclusions: Changes to peripapillary VD were not observed in patients with COVID-19 in the early months following diagnosis.

Key Words: coronavirus, COVID, optic nerve head, optical coherence tomography angiography

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Severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2), a novel betacoronavirus, is responsible for coronavirus disease (COVID-19), which was first reported in December 2019 in Wuhan, China. Since then, this disease has spread quickly through most countries around the world with a high mortality rate.[1,2]

COVID-19 typically presents as a viral pneumonia and symptoms include fever, fatigue, dry cough, anorexia, and myalgia. Acute respiratory distress syndrome is the most common severe manifestation, occasionally leading to death. In addition, evidence suggests that COVID-19 presents important vascular involvement with an increased incidence of disseminated intravascular coagulation, thromboembolic events, and coagulopathy. Reports reveal that many patients develop vascular involvement with systemic coagulopathy and an increase of thromboembolic events. In fact, up to one third of critical patients may present thrombotic events.[3,4]

Many mechanisms have been postulated to be responsible for this vascular damage in COVID-19 patients. SARS-CoV-2 specifically binds to the angiotensin-converting enzyme (ACE) 2 receptor to enter the cells, which is present at high concentrations in the lower respiratory tract. More importantly, ACE2 is also expressed in the vascular endothelium. When the virus binds to the receptor, ACE levels are diminished while angiotensin II increases, stimulating fibrosis, apoptosis, vasoconstriction, inflammation, and proliferation.[5] The characteristic hypoxia in severe COVID-19 can also stimulate thrombosis by increasing blood viscosity and vasoconstriction. A hypoxia-inducible transcription factor-dependent signaling pathway may also play a role in increasing coagulability.[6] Yet another mechanism for inflammation and vascular remodeling is the release of cytokines characteristic in the inflammatory phase of the disease. All these mechanisms converge in an inflammatory, oxidative, and hypoxic environment, which are responsible for endothelial dysfunction, microthrombosis, and vasculitis.[5] The endothelitis and vasculitis provoke severe endothelial injury and consequently, may result in higher thrombin levels and fibrinolysis shutdown, increasing the risk of thrombosis.[7]

The eye acts as a relevant biomarker of the neurological system that also allows analysis of the optic nerve and retinal vascular network.[8–10] Since an homology between the retinal and the cerebral microcirculations exists, retinal microvascular changes may reflect cerebral microvascular changes. In fact, there is evidence of retinal vascular alterations related to aging, as well as to diseases such as vascular dementia and stroke. Interestingly, retinal microvascular changes may reflect cumulative microcirculatory changes.[9,11,12] Moreover,

vascular diseases have been linked to alterations in optic nerve perfusion.[13] In this sense, there is increasing evidence supporting that the retinal vasculature plays a central role in the development of glaucoma.[14]

Optical coherence tomography angiography (OCTA) is a novel, noninvasive technique, which has proven effective in quantifying retinal vascularization without the need of a contrast agent. Several studies have assessed the macular and peripapillar perfusion in ophthalmologic and systemic diseases, reporting interesting differences. Patients with vascular diseases affecting the microvasculature such as diabetes mellitus (DM) and arterial hypertension (AHT) show a clear decrease in perfusion and flow of retinal vascularization, detectable by OCTA.[15–17]

Recently a decrease in central retinal vessel density (VD) in patients with moderate and severe SARS-CoV-2 pneumonia compared with paucisymptomatic cases or control subjects has been reported.[18] In addition, a lower peripapillary VD has been found in post-COVID patients 1 month after the disease compared with healthy subjects, with similar peripapillary vessel flow index in both groups.[19] However, no studies have been performed to date to investigate optic nerve vascularization in COVID-19 patients and its changes with time. Such knowledge of the peripapillary vascularization in this disease would be useful to address possible short-term and medium-term vascular complications related to SARS-CoV-2 infection.

The aim of this pilot exploratory study was to depict optic nerve head vascularization in COVID-19 patients and evaluate changes in perfusion, analyzing possible factors linked to the latter.

METHODS

Subjects

In this prospective, observational study, COVID-19 patients diagnosed at the Hospital Clinico San Carlos Emergency Department in Madrid (Spain) were included. The hospital's Clinical Research Ethics Committee approved the study protocol, which was in adherence with the tenets of the Declaration of Helsinki. Written informed consent was obtained from each participant before inclusion in the study.

Patients diagnosed with SARS-CoV-2 infection by positive reverse transcriptase-polymerase chain reaction (RT-PCR) test from nasopharyngeal swab in the hospital's Emergency Department from March 23 to March 29, 2020 were included in the study.

As in a previous study by our group,[20] the inclusion criteria were (1) <65 years of age, (2) laboratory-confirmed SARS-CoV-2 infection, (3) blood test, (4) chest x-ray, and (5) ability to give written consent. Patients with previous history of a thromboembolic event (or during follow-up), still presenting COVID-19 symptoms, on quarantine or unable to attend the hospital because of general health status were excluded. Those patients with ophthalmic diseases, including high myopia, maculopathies, optic nerve diseases, retinal vascular disorders, and previous ocular surgery other than uneventful cataract surgery performed more than 6 months before inclusion were excluded.

The patient's age, sex, race, past medical history [AHT, DM, dyslipidemia (DL), smoking], blood oxygen saturation, and laboratory tests results were recorded. Laboratory work-up included the levels of hemoglobin (Hb), total lymphocytes, platelet count, ferritin, D-dimer, international normalized ratio (INR), activated partial thromboplastin time, and fibrinogen. In each patient, all the blood tests performed were reviewed and the results of the blood test that represented the greatest severity were noted.

Patients were classified according to their clinical severity as mild, moderate, and severe, following CURB-65 score, physical examination, respiratory assessment (respiratory rate, dyspnea, blood oxygen saturation, and ventilation system requirements) or organ failure as classified in previous studies by our group.[20]

The patients were examined at 4 and 12 weeks after SARS-CoV-2 infection diagnosis was made with PCR-confirmed SARS-CoV-2, symptom onset being between 2 and 21 days before laboratory confirmation. Before the OCTA exam, all participants underwent an ophthalmologic examination including a slit lamp exam and ophthalmoscopy through dilated pupils to confirm the inclusion and exclusion criteria.

A small control group of hospital health personnel with negative serology and RT-PCR test were included. In this group the same exclusion criteria were applied and the following data was recorded: age, sex, race, and past medical history (AHT, DM, DL, and smoking). These subjects underwent the same ophthalmologic examination and OCTA exam.

OCTA

Peripapillary scans were obtained using the Cirrus HD-OCT 500 with AngioPlex OCTA (Zeiss, Dublin, CA). All the scans were performed by the same operator with pupil dilation in a dark room.

The OCTA Zeiss 5000 provides a VD map and an automated measurement of the vascularization in the scanned region. To do so, the device uses an algorithm that identifies changes in the phase and intensity information of the OCT scans to quantify motion contrast. To minimize motion artifacts, eye tracking is used.

For this study, a scan area of 4.5×4.5 mm area centered on the optic nerve head was used. The device's software automatically quantifies perfusion VD and flux index (FI) for the global and quadrant peripapillary regions (superior, temporal, inferior, and nasal) limited to an annulus with an inner diameter of 2 mm and outer diameter of 4.5 mm. VD is the total area of perfused radial peripapillary capillary vasculature per unit area in a specific region of interest, while FI is the total area of perfused vasculature per unit area in a specific region.

Examinations with signal strength <7 (of 10), poor image quality from motion artifacts, significant decentration, segmentation errors or media opacities, such as vitreous floaters were excluded. Both eyes of each patients were included, unless one eye did not meet the above inclusion-exclusion criteria.

Statistics

All statistical tests were performed using the software package IBM SPSS (version 21.0; IBM Corp., Somers, NY). Statistical significance was set at $P < 0.05$.

Data are presented as means ± SD and qualitative variables are presented as frequencies. The Kolmogorov-Smirnov test was used to confirm the normal distribution of the quantitative data. Parameters were normally distributed. The quantitative parameters that followed a normal distribution were studied with analysis of variance using Bonferroni correction. The Chi-square test was used to determine if there were differences in AHT, diabetes and DL between groups.

OCTA data from the first (4 wk after PCR-confirmed diagnosis) and second visit (3 mo after diagnosis) were compared among each severity group (mild, moderate, and severe stage disease) using the paired-samples *t* Student test.

To study the relationship between vascular density parameters and the demographic variables, Pearson correlation was used.

Multiple regression analysis was used to determine clinical parameters and blood test values influence on VD values. Those considered clinically relevant were chosen as independent variables in the model. A backward step strategy was used to obtain the best final predictive model.

RESULTS

The study population comprised 180 eyes of 90 patients with laboratory-confirmed SARS-CoV-2 infection. Twenty nine patients were excluded because of concomitant ophthalmological diseases. No patients were excluded because of poor image quality.

Table 1 summarizes the demographic, medical history, and clinical characteristics in the study group according to severity groups. Mean visual acuity was 0.76 ± 0.16 in the patients included. Severe patients were older, more frequently hispanic, dyslipidemic and presented lower lymphocytes counts, as well as increased ferritin D-dimer, fibrinogen and INR levels. No visual impairment or alterations were found in the ocular or fundus examination attributable to SARS-CoV-2.

As represented in Table 2, 29 healthy subjects were included, finding only significant differences in the VD of the temporal peripapillary sector (Temporal VD). The distribution of AHT, DM, and DL was lower in the control group, the percentage of smokers was higher in the group of COVID patients and as for race, the control group only included Caucasian subjects.

The results on VD as well as the differences between vascularization at one and 3 months in COVID-19 patients are shown in Table 3. No statistically significant differences were found in VD or FI globally or by papillary sectors in any of the groups of patients classified by disease severity.

Regarding correlation analysis, no correlation was noted between VD and SARS-CoV-2 parameters when clinical parameters, disease severity and blood test were analyzed. Hb levels presented a negative correlation with baseline VD and at 3 months (Baseline global VD $r = -0.193$, $P = 0.014$; 3-month global VD $r = -0.307$, $P < 0.0001$). Age showed a negative correlation with VD at 3 months, but not in the baseline analysis (3-month global VD $r = -0.239$, $P = 0.004$; 3-month global FI $r = -0.313$, $P < 0.0001$).

The results and the variables included of the three regression models are shown in Table 4. A greater effect of the parameters included in the regression models has been found on peripapillary VD (global VD) than on vascular flow (global FI). Of all the registered parameters of the disease, Hb levels had an effect on the peripapillary VD, but not over flow (RM1). Adjusting the regression model by Hb, age and sex (RM2), the negative effect on global peripapillary VD is greater. In fact, in RM2, the relevant parameters affecting global peripapilar VD the most are age and Hb, although the effect of age is minimal. Of the parameters evaluated in RM3, Hb and diabetes are the factors that affect most peripapillary VD. Indeed, the presence or absence of diabetes in COVID patients is the factor that affects peripapillary VD the most. Diabetic patients presented a mean reduction of -1.4% in global peripapillary VD compared with nondiabetic patients (RM3).

DISCUSSION

While SARS-CoV-2 is becoming better understood for its effects, the sequelae are yet to be thoroughly investigated. Our study provides valuable insight on the superficial peripapillary microcirculation measured with OCTA in patients with SARS-CoV-2 resolved infection. The results showed no changes in peripapillary vascularization when 1 month and

TABLE 1. Clinical Characteristics of Recovered COVID-19 Patients Divided by Disease Severity

	Total (n = 90)	Mild Stage Disease (n = 31)	Moderate Stage Disease (n = 23)	Severe Stage Disease (n = 36)	*P* (Mild and Moderate)	*P* (Moderate and Severe)	*P* (Mild and Severe)
Age (y)	55.48 ± 8.93	53.29 ± 9.38	52.52 ± 10.07	59.25 ± 6.09	0.684*	**0.0001***	**0.0001***
Sex (females), n (%)	46 (51.1)	19 (61.3)	10 (43.4)	17 (47.2)	0.067†	0.694†	0.105†
Race (Caucasic/ Hispanic/Black), n (%)	60 (60.7)/29 (32.2)/1 (1.1)	24 (77.4)/7 (22.6)/0	17 (73.9)/5 (21.7)/1 (4.34)	19 (52.8)/17 (47.2)/0	0.457†	0.091†	**0.004†**
AHT (yes), n (%)	26 (28.9)	7 (22.6)	6 (26.1)	13 (36.1)	0.677†	0.260†	0.089†
DM (yes), n (%)	8 (8.9)	2 (6.4)	2 (8.7)	4 (11.1)	0.633†	0.675†	0.350†
DL (yes), n (%)	25 (27.8)	6 (31.3)	4 (17.4)	15 (41.7)	0.797†	**0.006†**	**0.005†**
Smoking (yes), n (%)	17 (18.9)	4 (12.9)	4 (17.4)	9 (25%)	0.769†	0.143†	0.058†
Hemoglobin (mg/dL)	14.22 ± 1.30	14.29 ± 1.20	14.14 ± 1.65	14.21 ± 1.11	0.596*	0.770*	0.718*
Total lymphocytes (/μL)	1.13 ± 0.53	1.45 ± 0.61	1.16 ± 0.41	1.16 ± 0.41	**0.006***	**0.0001***	**0.0001***
Platelet count (/μL)	246.01 ± 96.35	256.39 ± 99.95	240.35 ± 109.99	241.43 ± 83.82	0.443*	0.952*	0.363*
Ferritin (ng/mL)	804.51 ± 856.95	384.33 ± 450.76	900.04 ± 965.57	1085.92 ± 908.38	**0.001***	0.928*	**0.0001***
D-Dimer (ng/mL)	1920.52 ± 6673.27	723.77 ± 603.61	777.78 ± 510.58	3681.14 ± 10322.50	0.625*	0.059*	**0.026***
INR (ratio)	1.13 ± 0.14	1.06 ± 0.08	1.13 ± 0.09	1.18 ± 0.18	**0.0001***	0.056*	**0.0001***
APTT (ratio)	1.01 ± 0.1	1.01 ± 0.08	1.02 ± 0.08	1.01 ± 0.11	0.641*	0.604*	0.904*
Fibrinogen (mg/dL)	739.86 ± 180.51	637.40 ± 167.52	748.78 ± 152.97	811.27 ± 173.32	**0.001***	0.052*	**0.0001***

All above measurements are represented by mean ± SD and frequency.
Statistical significant differences are shown in bold values.
*Analysis of variance test and Bonferroni correction.
†χ² test.
AHT indicates arterial hypertension; APTT, activated partial thromboplastin time; DL, dyslipidemia; DM, diabetes mellitus; INR, international normalized ratio.

TABLE 2. Healthy and COVID-19 Patients' Comparison

	Healthy Controls (n = 29)	COVID-19 at 1 mo (n = 90)	P
Age	52.83 ± 8.49	55.48 ± 8.93	0.137*
Sex (females) n (%)	16 (55.2)	46 (51.1)	0.165†
Race (Caucasian) n (%)	29 (100)	59 (65)	**0.001†**
AHT, n (%)	5 (17)	25 (27.8)	0.232†
DM, n (%)	2 (6.9)	8 (8.9)	1.0†
DL, n (%)	5 (17)	24 (26.7)	0.279†
Smoking, n (%)	1 (3.4)	15 (16.7)	0.088†
Visual acuity	0.88 ± 0.14	0.76 ± 0.16	**0.005***
External region VD (%)	47.57 ± 33.13	44.93 ± 1.49	0.789*
External region FI (ratio)	0.44 ± 0.04	0.44 ± 0.03	0.707*
Superior VD (%)	42.91 ± 4.06	43.24 ± 2.04	0.214*
Superior FI (ratio)	0.43 ± 0.06	0.42 ± 0.03	0.881*
Nasal VD (%)	45.69 ± 2.56	45.78 ± 2.62	0.680*
Nasal FI (ratio)	0.45 ± 0.04	0.45 ± 0.04	0.928*
Inferior VD (%)	45.02 ± 2.67	45.14 ± 2.84	0.547*
Inferior FI (ratio)	0.43 ± 0.04	0.43 ± 0.03	0.921*
Temporal VD (%)	45.27 ± 2.75	45.43 ± 2.68	**0.0001***
Temporal FI (ratio)	0.45 ± 0.04	0.44 ± 0.03	0.215*

All above measurements are represented by frequency or mean ± SD and frequency.

Statistically significant differences are shown in bold values.

*t Test.

†χ^2 and the Fisher exact test.

AHT indicates arterial hypertension; DL, dyslipidemia; DM, diabetes mellitus; FI, flux index; VD, vessel density.

3 months examinations were compared and no differences among severity groups.

Regarding our sample, this study reliably represents the spectrum of disease. Analytical and severity results support this, showing that severe patients are older, have more comorbidities, present lower lymphocyte counts and higher levels of ferritin, D-Dimer, INR and fibrinogen. This is in close agreement with the data reported.[21] There is evidence that thrombocyte count is key in both diagnosis and prognosis, low white blood cell and neutrophil counts also being important markers of COVID-19 infection. In addition, levels of lymphocytes, D-dimer and C-reactive protein are all linked to the severity of COVID-19 infection.[21,22]

Optic nerve head perfusion depends on multiple factors, including oxygen saturation, blood flow, and Hb content.[23] This is supported by the vascular hypothesis of glaucoma's pathophysiology, which suggests that the impairment of the blood supply to the optic nerve is largely responsible for the development of the disease. The high metabolic demands of the retina imply that the retinal tissue requires a constant supply of oxygen. Changes in retinal perfusion can result in the release of reactive oxygen species and impaired vascular autoregulatory mechanisms, and consequently alterations in optic nerve head vascularization.[24]

Since oxygen saturation and Hb have been described to be decreased in COVID-19 patients, particularly in severe patients, this might lead to a vascularization deficiency involving the optic nerve. In addition, there is evidence of vascular involvement with an increase incidence of thromboembolic events and coagulopathy in COVID-19 patients.[25] Endothelial dysfunction and direct damage from SARS-CoV-2 are some of the mechanisms that have been postulated to be responsible for this. Consequently, based on these data, the optic nerve head and its vascularization could be affected by diverse aspects associated with SARS-CoV-2 infection, involving the hypoxemia suffered during the acute phase of the disease, the inflammatory damage or vascular complications resulting from thromboembolic events.

When analyzing statistical models, the severity factors associated with the disease do not appear to greatly influence the peripapillary vascular network. No significant correlations were found between VD and the clinical parameters, except for Hb and age, which, although weakly, correlated negatively. Therefore, if there is retinal endothelial damage in our series of COVID-19 patients, it does not appear to become chronic in time, nor is said damage associated with the severity of the disease.

It should be noted that a previous study from our group reveled a decrease in macular perfusion in patients with high D-Dimer compared with COVID-19 patients who did not present increased levels.[26] This is plausible since D-Dimer has been highly linked to thrombosis. Zapata et al also found a lower fovea-centered VD in a case-control study that included patients with PCR-confirmed SARS-CoV-2 infection during the 3 months before patient's enrollment.[18] The absence of the same findings in the present study may be because of the lower presence of small vessels in the peripapillary region. This would imply that greater alterations in the microcapillary network are required to detect differences. The ability to detect differences in the macular region but not in the peripapillary region in systemic vascular diseases has been reported and a longer follow-up or a larger sample size may be needed to confirm this.[15] Savastano et al reported a slightly lower peripapillary VD in subjects recovered from SARS-CoV-2 infection at 1 month after hospital discharge (0.437 ± 0.031 vs. 0.441 ± 0.459, $P = 0.041$) although they found no differences in peripapillary flow index between groups (0.454 ± 0.017 vs. 0.456 ± 0.012).[19]

In contrast, age and vascular risk factors such as diabetes showed a significant influence on peripapillary vascular density, which have already been reported to affect the peripapillary vascular network.[27,28] Previous studies have shown that older patients have lower macular and peripapillary VD.[29,30] Our results agree with those reported by other authors who also found a weak negative correlation with age.[18,19] In our series, the presence of diabetes was the factor that affected peripapillary VD the most, with these patients presenting 1.4% less vascular density. Therefore, in view of our patients' results, it does not appear that the risk factors or severity of SARS disease are more relevant than age or DM in peripapillary VD.

Our results reveal that the most serious forms of the disease do not appear to produce alterations in the peripapillary capillary network compared with the other groups included in the present study. Thromboembolic complications up to 3 months have been described, so this study has covered the period of possible complications. None of the patients presented a thromboembolic event but it would be interesting to analyze changes in this group in the future. The lack of changes between the examination at one month and at three months suggests that if changes in peripapillary vascularization do occur, they do not worsen at 3 months,

TABLE 3. Four and 12 Weeks Optic Nerve Vascularization Comparison

	4 wk				3 mo							
	Total (n = 90)	Mild Stage Disease (n = 31)	Moderate Stage Disease (n = 23)	Severe Stage Disease (n = 36)	Total (n = 90)	Mild Stage Disease (n = 31)	Moderate Stage Disease (n = 23)	Severe Stage Disease (n = 36)	P Total	P Mild Stage	P Moderate Stage	P Severe Stage
External región VD (%)	47.57 ± 33.13	44.83 ± 1.21	44.50 ± 1.97	44.98 ± 1.41	44.93 ± 1.49	45.06 ± 1.17	44.86 ± 1.81	44.85 ± 1.53	0.342	0.101	**0.024**	0.429
External region FI (ratio)	0.44 ± 0.04	0.44 ± 0.03	0.44 ± 0.03	0.44 ± 0.05	0.44 ± 0.03	0.44 ± 0.03	0.44 ± 0.03	0.44 ± 0.03	0.394	0.996	0.661	0.242
Superior VD (%)	42.91 ± 4.06	43.00 ± 2.14	43.32 ± 2.33	42.56 ± 5.87	43.24 ± 2.04	43.24 ± 2.04	43.56 ± 2.26	43.04 ± 1.89	0.291	0.303	0.210	0.532
Superior FI (ratio)	0.43 ± 0.06	0.42 ± 0.03	0.43 ± 0.07	0.43 ± 0.07	0.42 ± 0.03	0.43 ± 0.03	0.42 ± 0.03	0.42 ± 0.03	0.335	0.251	0.442	0.310
Nasal VD (%)	45.69 ± 2.56	45.81 ± 2.57	45.33 ± 2.44	45.83 ± 2.65	45.78 ± 2.62	45.85 ± 2.41	45.58 ± 2.51	45.84 ± 2.90	0.525	0.860	0.302	0.946
Nasal FI (ratio)	0.45 ± 0.04	0.44 ± 0.03	0.44 ± 0.04	0.45 ± 0.04	0.45 ± 0.04	0.45 ± 0.05	0.45 ± 0.03	0.44 ± 0.03	0.886	0.501	0.871	0.308
Inferior VD (%)	45.02 ± 2.67	45.06 ± 2.28	44.38 ± 3.60	45.40 ± 2.20	45.14 ± 2.84	45.20 ± 3.06	44.83 ± 3.31	45.29 ± 2.28	0.406	0.634	0.055	0.645
Inferior FI (ratio)	0.43 ± 0.04	0.43 ± 0.02	0.43 ± 0.03	0.44 ± 0.06	0.43 ± 0.03	0.43 ± 0.03	0.43 ± 0.03	0.43 ± 0.02	0.247	0.332	0.600	0.279
Temporal VD (%)	45.27 ± 2.75	45.74 ± 2.59	45 ± 2.95	45.29 ± 2.56	45.43 ± 2.68	45.74 ± 2.59	45.37 ± 2.94	45.19 ± 2.61	0.291	0.212	0.254	0.711
Temporal FI (ratio)	0.45 ± 0.04	0.44 ± 0.04	0.45 ± 0.06	0.44 ± 0.04	0.44 ± 0.03	0.44 ± 0.04	0.45 ± 0.03	0.44 ± 0.03	0.752	0.870	0.836	0.865

All above measurements are represented by mean ± SD.
Statistically significant differences are shown in bold values.
Analysis of variance test and Bonferroni correction.
FI indicates flux index; VD, vessel density.

TABLE 4. Multiple Regression Analysis to Determine Most Relevant SARS-CoV-2 Disease Parameters Influence on Peripapillary Vessel Density and Peripapillary Flux Index at 3 Months

	RM1			RM2			RM3		
	β slope	95% CI	P	β slope	95% CI	P	β slope	95% CI	P
Global VD (%)	−0.306 (Hb)	−0.493 to −0.119	0.002	−0.355 (Hb)	−0.534 to −0.177	<0.0001	−1.397 (DM)	−2.176 to −0.618	0.001
				−0.039 (Age)	−0.065 to −0.012	0.004	−0.323 (Hb)	−0.499 to −0.146	<0.0001
Global FI (ratio)	5.485×10^{-5} (platelets)	-2×10^{-6} to 1.12×10^{-4}	0.060	−0.001 (Age)	−0.002 to -4.87×10^{-4}	<0.0001	−0.025 (DM)	−0.042 to −0.008	0.004
							−0.011 (AHT)	−0.022 to −0.001	0.039

Variables included in regression model 1 (RM1): hemoglobin+ferritin+fibrinogen+APTT+INR+platelets+D-dimer+lymphocytes+disease severity.
Variables included in regression model 2 (RM2): hemoglobin+age+sex.
Variables included in regression model 3 (RM3): hemoglobin+AHT+DM+dyslipidemia.
The variable included in β slope is the resulting variable in the backward steps model.
AHT indicates arterial hypertension; APTT, activated partial thromboplastin time; CI, confidence interval; DM, diabetes mellitus; FI, flux index; Hb, hemoglobin; INR, international normalized ratio; RM, regression model; VD, vessel density.

suggesting that either there is no vascular damage or peripapillary vascular damage has been a reversible occurrence in our patients. Therefore, the risk of optic nerve head involvement in the form of glaucomatous damage appears unlikely. Nevertheless, long-term studies are needed to confirm our findings in the medium term given the long latency period of the disease.

Some limitations of this study should be addressed. The patients were included in the study in an established time window and not with a numerical sample size objective. Therefore, the available sample is too small to achieve 95% confidence and 80% statistical power. This is a pilot, exploratory study that aims to evaluate possible peripapillary vascular changes in patients who have recovered from COVID-19 disease, since it has not been previously described in this type of patient. Our study included a small control group of hospital health personnel, given that it is difficult and unethical to bring healthy patients to the hospital. Since they are relatively healthy people, there is a low prevalence of high blood pressure, diabetes, and DL. However, the main objective of our study is not to compare with a healthy population, but to determine the changes in vascularization over time and their relationship with the clinical parameters of the disease. Follow-up of COVID-19 patients is short in time to date, not including the acute phase of the disease because of ethical and public health reasons. Also, patients with the most severe forms of the disease have not been fully analyzed, in most cases because they remained hospitalized, symptomatic, isolated or had died. Patients older than 65 years were not included for 2 reasons: first, a higher incidence of ophthalmic disease; and second, because they represent population at risk that should avoid going to hospitals unless they require emergency care during this critical situation. Thus, the differences found in a sample with younger patients may be less evident and therefore require a larger group to find differences. Future studies should be undertaken to address these limitations, though to obtain a larger sample size including critical patients will be challenging.

The SARS-CoV-2 pandemic is one of the greatest threats to public health. As the world progressively recovers from the acute stages of the pandemic, we will face new challenges regarding the long-term consequences of COVID-19. In a noninvasive way, we analyzed the peripapillary vascular network, not finding changes prospectively nor an influence of the severity parameters on peripapillary vascularization. Our study provides a relatively large sample of patients compared with other studies in the literature. In addition, our patients have a 3-month follow-up, a detailed analysis and the relationship with the severity of the disease has been analyzed. In view of the results found in our sample, it appears that if alterations in optic nerve vascularization occur, no changes at 1 and 3 months occur, reversibility not being able to be ruled out. We will continue to evaluate these patients to assess if there are long-term changes, because of the possible risk of glaucoma and the biomarker factor of the eye.

In summary, the peripapillary vascular density at 3 months remains unchanged in the COVID-19 patients of our sample. Our results reveal that the amount of Hb in blood and the presence of DM are the parameters that most significantly influence peripapillary vascularization in these patients.

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