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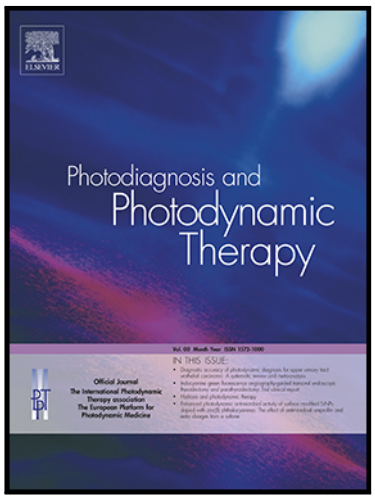

Retinal microvascular morphology versus COVID-19: What to anticipate?

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Highlights

- COVID-19 may be asymptomatic or impact many organs and tissues, including eyes; nevertheless, reports of ocular symptoms, as well as clinical manifestations and fatal results, are rare.
- Non-specific retinal manifestations, including micro-hemorrhages, etc., have been documented, and various investigations have lately been done to establish whether these findings are related to COVID-19 or are merely coincidental in this setting.
- Using a novel optical coherence tomography angiography (OCTA) that can be used as a biomarker of retinal disease in patients with systemic disease for microvascular abnormalities and quantitative flow analyses, we found that prior COVID-19 infection was associated with significant changes in retinal microvascular density, foveal avascular zone area, as well as outer retinal and choriocapillaris flows.
- Significant OCTA changes in completely asymptomatic patients could be caused by a variety of pathogenic mechanisms that lead to SARS-CoV-2 infection, such as thrombotic microangiopathy and angiotensin-converting enzyme-2 disruption, or by direct coronavirus infection of the retina tissue or its secondary effects.

RESEARCH PAPER

Retinal microvascular morphology versus COVID-19: What to anticipate?

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ABSTRACT

Background: To investigate retinal microvascular morphological changes in previously COVID-19 infected patients using optical coherence tomography angiography (OCTA), and compare the findings to age- and gender-matched healthy subjects.

Methods: In this cross-sectional study, OCTA findings (6.0x6.0 mm scan size and scan quality index ≥7/10) from previously COVID-19 infected patients (group 1, 32 patients, 64 eyes) with ≥1 month of complete recovery were compared to healthy subjects (group 2, 33 subjects, 66 eyes) with no history of COVID-19 infection. A positive real-time reverse transcription-polymerase chain reaction test on a naso-pharyngeal swab sample confirmed the diagnosis. The AngioVueAnalytics, RTVue-XR 2017.1.0.155 software measured and recorded OCTA parameters.

Results: Group 1 had significantly lower superficial capillary plexus vessel densities in all foveal regions than group 2 ($P<0.05$). Foveal deep capillary plexus vessel density in group 1 was also significantly lower than in group 2 ($P=0.009$); however, no significant differences were found in other regions ($P>0.05$). All foveal avascular zone (FAZ) parameters were higher in group 1 than in group 2, with significant differences in FAZ area ($P=0.019$) and foveal vessel density 300 μm area around FAZ ($P=0.035$), but not FAZ perimeter ($P=0.054$). The outer retina and choriocapillaris flows were significantly lower in group 1 than in group 2 ($P<0.05$).

Conclusions: Prior COVID-19 infection seems to be associated with significant changes in retinal microvascular density, as well as FAZ and flow parameters, which may be attributed to different pathogenic mechanisms that lead to SARS-CoV-2 infection, such as thrombotic microangiopathy and angiotensin-converting enzyme 2 disruption.

Key words

COVID-19, Foveal avascular zone, Microvascular morphology, Optical coherence tomography angiography, Retina, Vessel density.

1. Introduction

Since December 2019, the SARS-CoV-2 pandemic has caused widespread morbidity and mortality globally. Further, since it was officially declared by the World Health Organization on March 11, 2020, this pandemic has had a significant global impact, particularly on healthcare systems **[1]**. There is no effective treatment at the moment; however, several vaccines have been developed and are used solely for prevention. These vaccines are sometimes insufficient due to high mutation capacity of the coronavirus **[2].**

Clinical manifestations and fatal outcomes of Coronavirus Disease 2019 (COVID-19), as well as reports on ocular symptoms are uncommon **[3,4].** This disease may be asymptomatic or affect multiple organs and tissues, including eyes. Many critically ill patients have been found to have an elevated coagulation state which may result in thromboembolic events and disseminated intravascular coagulation **[5,6].** Besides, endothelial damage has recently been reported to be widespread, resulting in ischemic damage to the microcirculatory system and subsequent functional problems in multiple organs **[7,8].** Despite the fact that the ocular effects of COVID-19 have not been thoroughly studied, many recent studies have reported non-specific retinal manifestations such as micro-hemorrhages, vasodilation, cotton wool spots, and flame hemorrhages **[9].** Several studies have recently been conducted, in this context, to determine whether these findings are related to COVID-19 or are simply coincidental **[10].**

Optical coherence tomography angiography (OCTA) is a novel, quick, and reliable non-invasive imaging technique that does not require dye injection. It can provide qualitative and quantitative features of retinochoroidal vascularization, as well as track changes in vascular perfusion in COVID-19 patients **[11,12].** Aside from retinal vascular high-quality images, this technique can also be used to quantify parameters such as foveal avascular zone (FAZ) area and perifoveal capillary vascular density, as well as leak-free retinal ultrastructure **[13].**

The aim of this study was to investigate retinal microvascular morphological changes in previously COVID-19 infected patients using OCTA, and compare the results to age- and gender-matched healthy subjects with no history of infection.

2. Materials and methods

2.1. Study design and participants

In this cross-sectional comparative single-centered study, we enrolled previously COVID-19 infected patients (group 1) with ≥1 month of recovery who had received treatment at the Afyonkarahisar Health Science University Hospital Chest Diseases Clinic and were consulted for ocular examination between 2020 and 2021. A

positive real-time reverse transcription-polymerase chain reaction test on a naso-pharyngeal swab sample confirmed the diagnosis.

A control group (group 2) consisted of age- and gender-matched health subjects who visited our Ophthalmology Outpatient Clinic for a routine ophthalmological exam. These subjects had no history of contact with any COVID-19 infected patient or quarantine in the HES (*'Hayat Eve Sığar'*-Life Fits at Home) application developed by the country's health ministry, nor did they show any signs of having had a clinically active disease or complaints related to COVID-19 infection. Furthermore, their body temperatures were all found to be within the normal physiological range.

The study protocol adhered to the Declaration of Helsinki's ethical principles and was fully approved by the Afyonkarahisar Health Sciences University Ethics Committee. All participants gave written informed consent prior to participation.

2.2. Inclusion and exclusion criteria

Patients who had recovered from COVID-19 for ≥1 month, did not have any other ocular or systemic diseases, and did not smoke or drink alcohol on a regular basis, were eligible for the study. Both patients and healthy subjects with; (a) media opacities such as cornea diseases and defects, cataract, vitreous opacities, and other conditions that could impede high-quality imaging or lead to poor OCTA scan quality, (b) history of refractive and/or intraocular surgery, (c) prior ocular travma, particularly affecting the optic axis and posterior vascular system, (d) ocular diseases such as glaucoma, uveitis, and/or clinically significant retinal and optic nerve diseases, (e) any vasculopathy-related posterior ocular segment-involving systemic diseases such as diabetes, hypertension, and so on, (f) migraine, (g) pregnancy and/or breast-feeding, (h) autoimmune disorder, (i) spherical or cylindrical refractive error >±3 diopters (D), and/or an axial length >26.5 mm, (j) any topical or systemic medications, and (k) poor fixation during imaging procedure, were not eligible for the study.

2.3. assessment

All participants had a comprehensive ophthalmologic examination. This included measuring auto-refraction (Canon R-F10m; Canon Inc., Tokyo, Japan), best-corrected visual acuity in logarithm of the minimum angle of resolution (logMAR) and intraocular pressure (Goldmann; Haag-Streit AG, Köniz, Switzerland), as well as slit-lamp biomicroscopy of the anterior and posterior segments before and after pupil dilation with tropicamide 1% and phenylephrine 10%.

2.4. Optical coherence tomography angiography procedure

The same technician performed all OCTA (Optovue, Inc., Fremont, California, USA) procedures using the same device. 304 evenly spaced sections along the x-axis, followed by the y-axis, in the 6.0x6.0 mm retinal area were scanned using the Angio Retina mode during B-scanning procedure. These sections were then stitched together to form 6.0x6.0 mm OCTA images. Using the 'Auto Adjust' mode, the axial length, refraction correction, and image polarization of the patient were all automatically adjusted during the procedure. The device's scan quality index, which is a score (1-10) assigned to OCTA images at the end of each acquisition by combining signal strength, ocular movements, and image focusing, was also used. Images with scan quality index ≤7/10 were omitted from the analysis. The procedure was then repeated several times until the recording quality was satisfactory. The eye tracking mode and motion correction technology significantly reduced ocular motion artifacts. A projection artifact removal system was used to remove any potential artifacts. The signal strength index was set to ≥40 as the cut-off value. All OCTA images were thoroughly assessed and approved by **OE** and **HHG**, as well as other authors **(MD, MA, and MK),** to achieve sufficient image quality and resolution. Images with substantial motion artifacts that hampered the successful quantification of microvascular density were also discarded.

The AngioVueAnalytics, RTVue-XR 2017.1.0.155 software measured and recorded OCTA parameters such as vessel densities in superficial and deep retinal layers (superficial and deep capillary plexi), as well as FAZ area (mm^2), FAZ perimeter (mm), and foveal vessel density 300 μm area around FAZ (FD-300) (%). Microvascular flow parameters from the outer retina and choriocapillaris were also collected and analyzed.

2.5. Data analysis

We used the Statistical Package for the Social Sciences (SPSS Inc., version 23, Chicago, IL, USA) for data analysis. Descriptive statistical methods included number, percentage, mean, standard deviation, median, and interquartile range. Kolmogorov-Smirnov test was used to determine whether the data was normally distributed. Independent sample T-test and Mann-Whitney U test were used to analyze the relationships between variables. The Pearson correlation test was used to determine relationships of the variables. The results were assessed at a 95% confidence interval and a 5% significance level. Statistical significance was defined as $P<0.05$.

3. Results

Group 1 had 32 (64 eyes) patients (female-to-male ratio: 71.90%:28.10%), while group 2 had 33 (66 eyes) health subjects (female-to-male ratio: 57.60%:42.40%). The mean age (P=0.088), best-corrected visual acuity (P=0.076), as well as intraocular pressure (P=0.067) did not differ significantly between the two groups. Five patients were outpatients with mild symptoms, meaning their medical treatment began in the hospital and continued at home, and 27 were hospitalized with moderate symptoms in the Chest Diseases clinic for an average of 5.20±2.20 days but did not experience acute respiratory distress disease. Patients with severe disease (acute respiratory distress admitted to the intensive care unit) were not included in the study. This information is also included elsewhere in the manuscript **(Table 1).**

3.1. Capillary plexus vessel densities and foveal avascular zone parameters

Compared to group 2, group 1 had significantly lower whole (P=0.011), foveal (P=0.014), parafoveal (P=0.011), and perifoveal (P=0.002) superficial capillary plexus vessel densities. Foveal deep capillary plexus vessel density in group 1 were also significantly lower than in group 2 (P=0.009). However, no significant differences were found in whole (P=0.177), parafoveal (P=0.153), and perifoveal (P=0.176) deep capillary plexus vessel densities. All FAZ parameters tended to be higher in group 1 than in group 2, with significant differences in FAZ area (P=0.019) and FD-300 (P=0.035), but not in FAZ perimeter (P=0.054) **(Table 2, Figure 1(a-d) and (a*-d*)).**

3.2. Microvascular flow parameters

Group 1 had significantly lower flow area in both outer retina (P=0.030) and choriocapillaris (P<0.05) than group 2 **(Table 3, Figure 2(a, b) and (a*, b*))**

4. Discussion

We investigated retinal microvascular morphological changes in previously COVID-19 infected patients using OCTA, and compared the findings to age- and gender-matched healthy subjects. There was a fairly benign disease course in these patients, with only a few requiring hospitalization for an average of 5.20±2.20 days prior to getting discharged with full recovery. A post-treatment negative COVID test was confirmed by a PCR test twice in a 24-hour interval in outpatient patients prior to OCTA scanning. In hospitalized patients, after thrombotic and inflammatory markers such as D-Dimer, ferritin, fibrinogen, C-reactive protein, and pre-

calcitonin returned to normal, the PCR test was repeated twice with a 24-hour interval to confirm negativity before discharge.

Overall, prior COVID-19 infection was associated with relatively lower mean retinal microvascular vessel densities. This was especially true for the whole, foveal, parafoveal, and perifoveal superficial capillary plexus vessel densities and foveal deep capillary plexus vessel density. The whole, parafoveal and perifoveal deep capillary plexus vessel densities were also lower in comparison to healthy subjects, despite not being statistically significant. These findings could be attributed to a number of pathogenic mechanisms that lead to SARS-CoV-2 infection, including both thrombotic microangiopathy and angiotensin-converting enzyme 2 disruption **[8,14].**

After the virus fuses with upper respiratory epithelial cells, replication typically begins. Following this, the virus spreads and migrates down the respiratory tract, eliciting an innate immune reaction. The primary receptor for SARS-CoV2 has been described as angiotensin-converting enzyme type 2 **[15].** The receptors for this enzyme are observed in cell membranes of type II alveolar cells in lung and enterocytes in small intestine, as well as arterial and venous endothelial cells and arterial smooth muscle cells in almost every organ **[16].** Aside from retinal vascular endothelial and photoreceptor cells, angiotensin-converting enzyme and angiotensin-converting enzyme type 2 have also been found in choroid and various retinal cells **[17].** Moreover, immunohistochemistry revealed significant levels of angiotensin-converting enzyme type 2 receptors in the ciliary body, choroid, retina, and retinal pigment epithelium of the COVID-19-infected patient eye **[18],** leading to a suggestion that the SARS-CoV-2 infection could subsequently be associated with retinochoroidal microvascular damages **[19].**

Even though severe respiratory disorder is the most prominent clinical feature of the COVID-19, this disease also has a wide range of thromboembolic events, with an inevitable development of multi-organ dysfunction **[20].** Thrombotic microangiopathy is thought to be one of the major contributors to the COVID-19-related microvascular damage **[21],** with complement activation being critical in its pathophysiology, determining platelet activation, leukocyte recruitment, endothelial cell dysfunction, and coagulation **[22,23].** The complement cascade is activated in response to endothelial damage caused by a disruption in the local renin-angiotensin system **[20].** The angiotensin-converting enzyme type 2 receptors, which SARS-CoV-2 uses to enter disrupted cells, are abundant in endothelial cells **[24].** Endothelial cell damage and the consequent thrombotic microangiopathy cause microvascular occlusion, which may lead to hypercoagulation and multi-organ failure. The COVID-19-related thrombotic microangiopathy may cause vascular perfusion damage in superficial and deep capillary plexi, interfering with axoplasmic flow and resulting in retinal ultrastructural alterations.

Anastomosis between superficial and deep capillary plexi could explain the possible relationship between these plexi and retinal nerve fiber thickness.

The novel OCTA technique can be used as a biomarker of retinal disease in patients with systemic disease as microvascular abnormalities and quantitative flow analysis by this technique are associated with clinical stage of retinopathy **[25,26].** Likewise, using the same technique, we revealed that COVID-19 infection was associated with pathological changes in microvascular density, FAZ, as well as outer retinal and choriocapillaris flows, just as it was in patients with diabetes **[25]**, even if they were asymptomatic.

Four COVID-19 patients have been recently reported to have cotton-wool spots and microhemorrhages suggestive of an inner retinal ischemia **[27].** While the authors observed abnormalities on OCT imaging, others speculated that the findings could be due to normal vascular signs **[28].** Soft exudates, retinal dot-blot hemorrhages, central retinal artery occlusion, and sectorial retinal pallor have all been reported as indications of retinal vascular occlusion following thromboembolic complications in COVID-19 patients **[29,30].** Also, decreased perfusion density of the radial peripapillary capillary plexus has been reported in COVID-19 patients one month after infection when compared to healthy controls **[31].**

In our study, we performed a systematic analysis in previously COVID-19-infected patient population, comparing changes in retinal microvascular density to age- and gender-matched healthy subjects, and revealed, among other things, that COVID-19 infection was associated with sıgnıficant enlargement of FAZ area and FD-300. The reasons for changes in retinal microvascular morphology discovered in our study are unclear. While direct coronavirus infection of the retina tissue is possible, secondary effects of infection cannot be ruled out either. Aggravation of underlying systemic diseases is also unimaginable, considering the relatively young age of the study patients and the absence of pre-existing pathological conditions.

In addition to changes in FAZ and microvascular flow parameters, we found that patients who had previously been infected with COVID-19 had lower microvascular density at least one month after full recovery. The presence of virus-infected cells in lung and endothelial cells several weeks after COVID-19 diagnosis could also describe retinal microvascular changes as long-term consequences of SARS-CoV-2 infection **[32].** Moreover, there was no decrease in best-corrected visual acuity in infected patients, and an extraocular examination was also unremarkable. We presume, consequently, that demonstrating the presence of retinal microvascular morphological changes in completely healthy asymptomatic eyes using OCTA is clinically valuable. It would also be interesting to know if these changes are related to retinal electrophysiological changes. Most importantly, a longer period of observation may be necessary to determine if these subclinical

microvascular morphological changes are to blame for the development of ischemic diseases and/or choroidal neovascularization.

Our study has some limitations. There were no data available for changes in microvascular density during the acute phase of COVID-19 infection. We measured microvascular densities of the foveal, parafoveal, and perifoveal regions. Measurements taken outside the fovea, however, were referred to as the outer retina, and were not divided into superior, inferior, nasal, and temporal quadrants. Fluorescein angiography was not used to investigate integrity of the retinal microvasculature, which appears to be one of the study limitations: however, as previously stated, we planned to investigate changes in microvascular density using a novel OCTA technique, which could also reveal retinochoroidal microvascular morphological changes without using contrast materials. While all patients' ocular environments were clean, ocular surface irregularities may have hampered scan quality, and asymptomatic dry eye has been linked to COVID-19 **[33,34].** Additional validation of our findings may be needed to verify that decreased microvascular density in previously COVID-19-infected patients resulted from actual anatomical changes rather than artifacts. Moreover, since COVID-19 is a novel disease entity, longer follow-ups are clearly necessary. However, our study was a cross-sectional study, and OCTA measurements were only taken once for those who completely recovered from the disease. Thus, more long-term large scale clinical studies may be of merit to assess the relationship between OCTA parameters and the onset and duration of COVID-19 infection. Furthermore, longitudinal screening with re-imaging at predetermined intervals could provide important knowledge on the short- and long-term consequences of COVID-19 on the retinal microvasculature.

One of the advantages of our study is that all patients were free of COVID-19 infection and had no signs of ocular involvement, although some of them reported visual discomfort during active infection. In addition, compared to prior studies, more participants were evaluated. Measurement of FAZ parameters and microvascular flows in the outer retina and choriocapillaris may be another advantage of this study. The scan quality may have an effect on OCTA microvascular density analysis **[35].** In this context, all participants included in the final analysis were subjected to a strict quality screening threshold ≥7/10.

5. Conclusions

Prior COVID-19 infection was associated with significant changes in retinal microvascular density, as well as FAZ and flow parameters. Despite the fact that we found significant OCTA changes in completely asymptomatic patients compared to healthy subjects, clinical significance of the visual symptoms observed

during active infection remained uncertain. Potential effects of COVID-19 on the retina necessitate larger-scale studies that may better represent the ever-growing global population of COVID-19 infected patients.

Financial interest

All authors certify that they have no association or participation with any organization or individual with any financial interest or non-financial interest in the subject matter or materials discussed in this article.

Ethics approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Consent to participate

Informed consent was obtained from all participants included in the study.

Availability of data and materials

The manuscript contains all data. The datasets used and/or analyzed during the current study, however, are available upon reasonable request from the corresponding author.

Conflicts of Interest

The authors declare that they have received no public or private financial support or involvement in the products, methods, or materials mentioned in this manuscript, and there is no conflict of interest to disclose.

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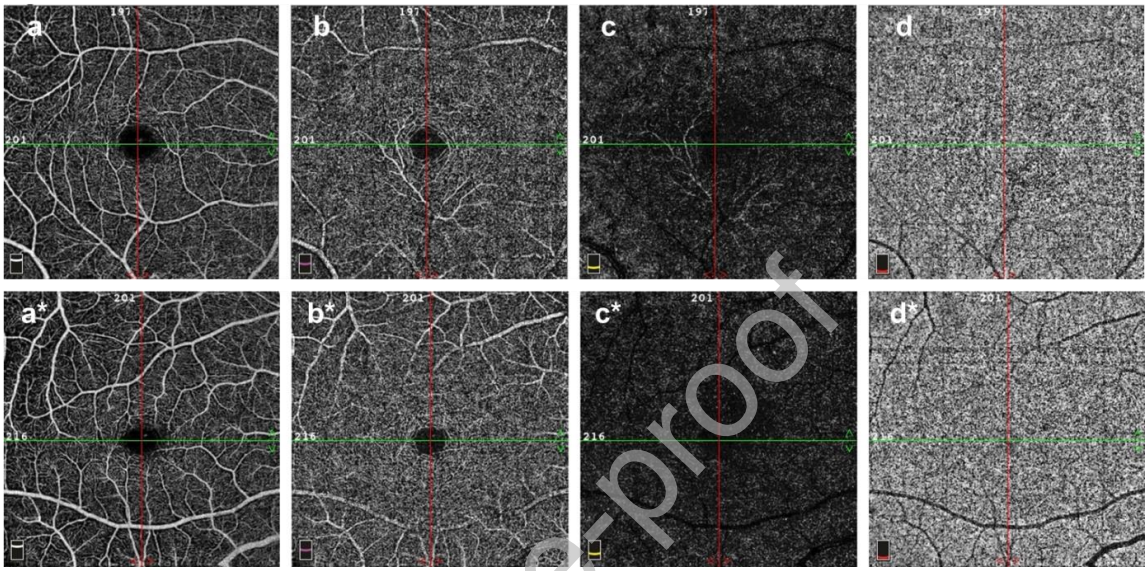

Figure 1. Right eye representation of the Angio Retina Quickview (6.0x6.0 mm scan size with scan quality index=9/10) segmented at the level of the superficial capillary plexus (a, a*), deep capillary plexus (b, b*), outer retina (c, c*), and choriocapillaris (d, d*) from a previously COVID-19 infected patient (*letters without asterixis*) and a healthy subject (*letters with asterixis*), respectively. Note the lower vessel densities in both superficial and deep capillary plexi in previously COVID-19 infected patients (a, b) compared to healthy controls (a*, b*). Previously COVID-19 infected patients appear to have a larger foveal avascular zone when compared to healthy subjects (a, b versus a*, b*).

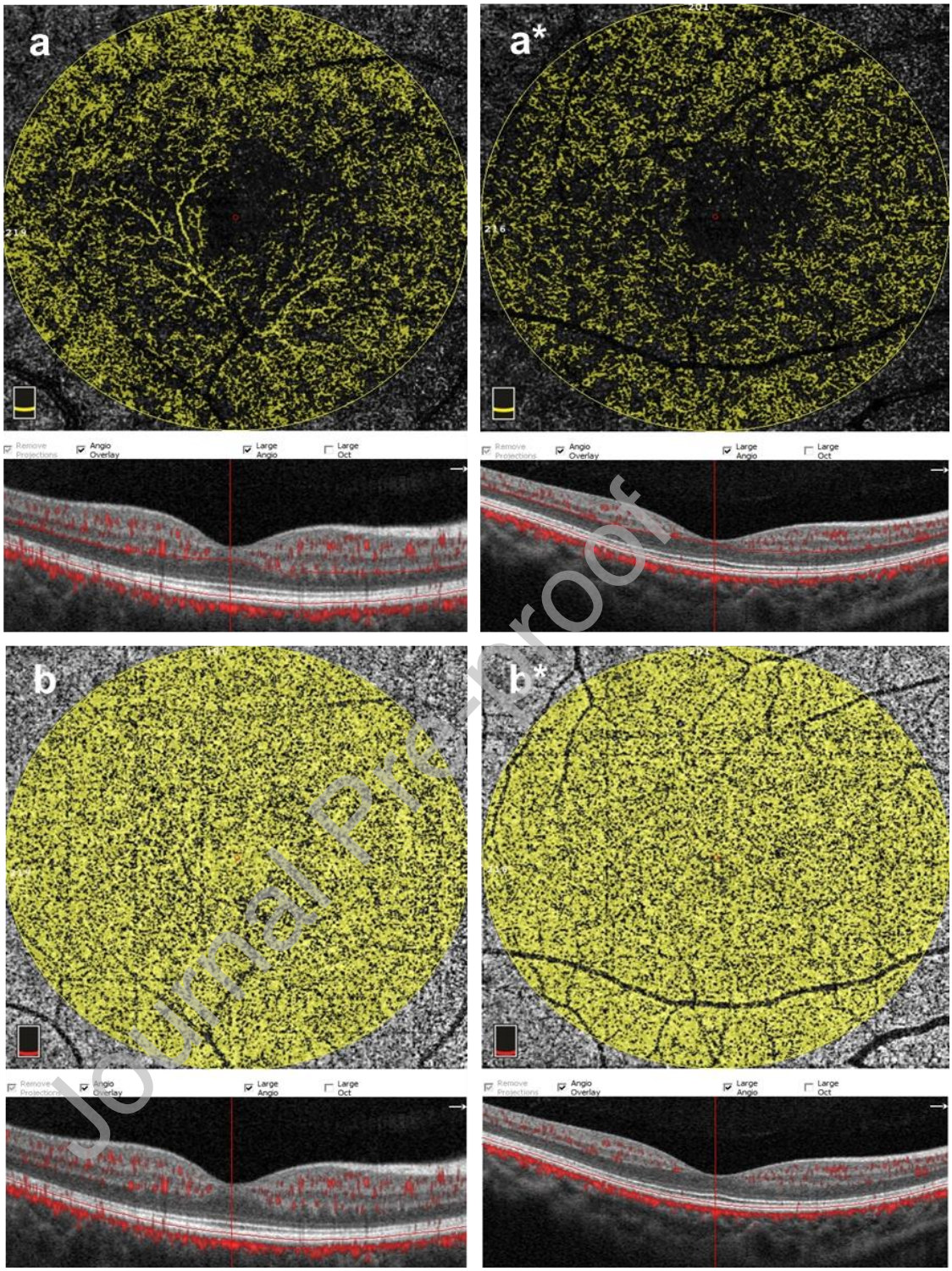

Figure 2. En-face optical coherence tomography angiograms (6.0x6.0 mm scan size and 20.749 mm^2 flow area with scan quality index=9/10) displaying relative flow deficits in the outer retina (P=0.030) (a/a*) and choriocapillaris (P<0.05) (b/b*) segments of the right eyes of previously COVID-19 infected patients (*letters without asterixis*) when compared to healthy subjects (*letters with asterixis*). The corresponding OCT cross-sections through the foveal centralis of both the patient and the health subject are shown beneath each figure part.

Table 1. Demographic and ocular characteristics

Parameters	**Group 1 ($\bar{x}$±s)**	**Group 2 ($\bar{x}$±s)**	**P-value**
Age (Years)	49.60±13.50	48.40±12.40	0.088
Best-corrected visual acuity (logMAR)	0.06±0.06	0.05±0.05	0.076
Intraocular pressure (mmHg)	12.50±3.20	12.70±3.30	0.067

$\bar{x}$=Mean, s=Standard deviation, logMAR=logarithm of the minimum angle of resolution, mmHg=millimeter of Mercury, Group 1=Previously COVID-19 infected patients with ≥1 month of recovery, Group 2=Health subjects

Table 2. Analysis of optical coherence tomography angiography parameters

Parameters	**Group 1 ($\bar{x}$±s)**	**Group 2 ($\bar{x}$±s)**	**P-value**
Superficial capillary plexus vessel densities (%)			
Whole	50.74±4.13	52.36±2.88	0.011
Foveal	16.75±6.55	21.65±12.58	0.014
Parafoveal	53.45±5.05	55.20±4.05	0.011
Perifoveal	51.30±3.87	53.22±2.81	0.002
Deep capillary plexus vessel densities (%)			
Whole	53.65±10.28	55.50±7.60	0.177
Foveal	36.73±5.93	39.44±5.72	0.009
Parafoveal	56.20±8.65	57.00±6.58	0.153
Perifoveal	55.90±10.50	57.80±7.43	0.176
Foveal avascular zone parameters			
FAZ area (mm^2)	0.31±0.11	0.28± 0.07	0.019
FAZ perimeter (mm)	2.17±0.51	2.10±0.33	0.054
FD-300 (%)	56.85±8.93	53.10±2.46	0.035

$\bar{x}$=Mean, s=Standard deviation, Group 1=Previously COVID-19 infected patients with ≥1 month of recovery, Group 2=Health subjects, FAZ=Foveal avascular zone, FD-300=Foveal VD 300 μm area around the FAZ, mm^2=millimeter square, mm=millimeter, %=percentage

Table 3. Analysis of microvascular flow parameters

	Group 1 ($\bar{x}$±s)	**Group 2 ($\bar{x}$±s)**	**P-value**
Outer retinal flow area **(**mm^2)	8.310±2.87	9.40±2.55	0.030
Choriocapillaris flow area **(**mm^2)	19.59±1.39	20.61±1.03	0.000

$\bar{x}$=Mean, s=Standard deviation, Group 1=Previously COVID-19 infected patients with ≥1 month of recovery, Group 2=Health subjects, mm^2=millimeter square