Research Proposal

Purpose

- **Study rationale:** There is limited and conflicting data exploring the impact of the SARS-CoV-2 virus on the retinal vasculature in patients who have recovered from COVID-19 infection. Recent evidence suggests that long-hauler COVID-19 patients (symptoms persist for at-least 6 weeks) have a propensity to experience neuro-ophthalmic symptoms such as brain fog and blurry vision. In this study, we aim to elucidate the ophthalmic findings in non-hospitalized long-hauler COVID-19 patients experiencing these neurologic symptoms.
- **Hypothesis**: Vascular dysfunction due to increased endothelial permeability and derangement is implicated as the mechanism behind the propensity for SARS-CoV-2 to cause multiorgan failure. We postulate that long-hauler COVID-19 patients experience a higher degree of vascular dysfunction, which in turn leads to persistence of COVID-19 symptoms and neuro-ophthalmological sequelae. We hypothesize that comprehensive eye exam and multi-modal imaging with optical coherence tomography angiography (OCT-A) and fluorescein angiography (FA) on long-hauler COVID-19 patients experiencing neuro-ophthalmic symptoms may demonstrate quantifiable changes in retinal vessel density and vascular permeability when compared to controls.
- **Specific objectives**: To test our hypothesis, we will recruit 3 groups of patients. The first group will be long-hauler COVID-19 patients presenting to Northwestern with neuro-ophthalmic concerns (brain fog and blurry vision). The second group will be long-hauler COVID-19 patients who do not have ophthalmic complaints (blurry vision). Lastly, we will have a third group of age & sex-matched COVID-19-negative controls. A comprehensive eye examination will be performed on all patients along with imaging (OCT, OCT-A, and FA where indicated). The primary endpoints for the study will include OCT-A parameters of retinal vessel density (macular & peripheral), foveal avascular zone (FAZ) size, and retinal perfusion status. Numerous other secondary quantitative parameters will also be included in the analysis (retinal nerve fiber thickness, ganglion cell layer thickness, central macular thickness and best-corrected visual acuity). ANOVA analysis will be utilized to compare each primary and secondary parameter among the 3 aforementioned groups.
- **Clinical relevance:** Presently, there is a dearth of knowledge regarding COVID-19's ophthalmic manifestations, particularly in the emerging population of COVID-19 long-hauler patients. There is an urgent need to understand the mechanism of disease and develop targeted therapies to attenuate the symptoms and sequelae of this debilitating disease. Our study will be the first to establish baseline ophthalmic findings in long-hauler COVID-19 patients experiencing neuro-ophthalmic symptoms versus controls. With this data, physicians could optimize their clinical framework in evaluating and treating long-hauler COVID-19 patients who present to their practice.

Preliminary data

In December 2019, COVID-19, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged as a novel coronavirus and quickly evolved into a pandemic. While respiratory tract infection is the major clinical manifestation of the infection, it also has the propensity to cause multiorgan failure.[1,2] Vascular dysfunction caused by increased endothelial permeability and dysfunction is implicated in the worsening systemic involvement of COVID-19.[2,3] Angiotensin converting enzyme 2 (ACE2), one of the human capillary and venule pericyte entry sites of SARS-CoV-2, is found in the eyes. More specifically, ACE2 has been found in various retinal cell types including ganglion cells, retinal vascular endothelial cells, Muller cells, and photoreceptor cells.[1,2] Therefore, ACE2 mediated infiltration has been a proposed mechanism by which COVID-19 can enter the eye and induce vascular dysfunction. Characteristic retinal findings secondary to COVID-19 such as hemorrhages and maculopathy have been elucidated in multiple case reports and observational studies (see Table 1 below).[4] Additionally, previous retinal biopsies demonstrated SARS-CoV-2 viral RNA in 3 out of 14 COVID deceased patients.[5]

A handful of studies have imaged the retinal vasculature in patients recovered from COVID-19 infection demonstrating reduction in retinal vessel density in the superficial and deep capillary plexus compared to controls. [1,6,7,8] Table 2 shows reduction in retinal vessel density in the superficial, deep, and radial peripapillary plexus in post-COVID-19 pneumonia patients.[8] On the other hand, a study by Savastano et al, with a sample size of 70 COVID-19 patients and 22 controls, did not demonstrate any vessel density alterations in post-COVID patients as measured by OCT-A.[2] Of note, there is also preliminary evidence to suggest significantly thinner ganglion cell layer and thicker retinal nerve fiber layer in post-COVID-19 patients compared to controls.[5]

The emergence of a growing population of COVID-19 patients whose symptoms persist beyond 6 weeks (COVID long-haulers) requires a separate, but similar analysis of retinal findings considering one study in an outpatient setting showed 32% of 669 patients continued having at least 1 symptom at 30-45 days from symptom onset.[9] There is currently a dearth of data on the ophthalmologic findings in COVID long-haulers.

A recent study at Northwestern by Graham et al. characterized the neurologic manifestations of non-hospitalized COVID-19 long-haulers.[10] In the study, 85% of participants reported experiencing at least four neurologic symptoms with the most common being brain fog (85%) and headache (81%). Among the 100 patients, 30% described blurry vision.[6] (Table 3) . Given the frequency of patients experiencing neurologic complications with a significant subset complaining of blurry vision, further study is needed to examine ophthalmologic findings in these patients.

Authors (year)	Type of study/SARS-CoV-2 detection methods	Retinal findings
Casagrande et al. [1]	Human retinal biopsies of 14 deceased patients with positive PCR nasopharyngeal swabs	SARS-CoV-2 RNA detection in 21%
Marinho et al. [2]	Prospective study of 12 patients. Nine with positive PCR from nasal and oral swabs, and two with positive serology for SARS-CoV-2	Subtle cotton wool spots and microhemorrhages in retinal surface (33%). Hyper-reflective lesions in the GC and IP layers (100%)
Virgo and Mohamed [3]	Case report of a 37-year-old female with positive SARS-CoV-2 IgG (PCR was not performed) Case report of a 32-year-old male with positive PCR from nasopharyngeal swab	Paracentral acute middle maculopathy (hyper-reflective lesion in the IP and OP layers with IN layer volume loss) Acute macular neuroretinopathy (hyper-reflective lesion in OP layer and disruption of the interdigitation zone)
Bettach et al. [4]	Case report of a 54-year-old female with negative SARS-CoV-2 PCR from nasopharyngeal swab and positive SARS-CoV-2 IgG	Small focal intraretinal hemorrhage in the left fovea associated with bilateral anterior uveitis

GC: ganglion cell; IP: inner plexiform; IN: inner nuclear; OP: outer plexiform; PCR: polymerase chain reaction; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2

Table 1: Retinal findings in COVID-19 patients. Table 1, adapted from De Figueiredo et al, summarizes the limited data available as of July 2020 linking SARS-CoV-2 to the retina.[4]

SCP, %			
Whole image	48.86 ± 4.32	50.94 ± 4.49	.038
Parafovea	52.34 ± 5.29	52.59 ± 6.72	.858
Fovea	25.21 ± 5.28	25.30 ± 4.22	.929
DCP, %			
Whole image	52.42 ± 7.18	55.79 ± 6.35	.029
Parafovea	56.27 ± 6.31	59.72 ± 6.20	.016
Fovea	44.08 ± 7.16	47.80 ± 7.57	.027
RPC, %			
Whole image	46.43 ± 4.01	50.44 ± 4.67	<.001
Inside disc	52.40 ± 3.42	53.61 ± 4.34	.171

Table 2: Comparison of vessel densities between post-COVID-19 pneumonia and healthy control patients, as measured by optical coherence tomography. Table 2, adapted from Cennamo et al, shows vessel density alterations in patients status post COVID-19.[8] SCP = superficial capillary plexus; DCP = deep capillary plexus; RPC= radial peripapillary capillary plexus. Student *t*-test was used for independent samples. Statistical significance *P* value <.05. Data are expressed as mean ± SD.

	Overall	SARS-CoV-2$^+$	SARS-CoV-2$^-$	*p*
Subjective impression of recovery compared to pre-Covid-19 baseline (mean % (1 SD))	63.9 (20.7)	67.8 (18.8)	60.3 (21.9)	0.09
Number of neurologic symptoms attributed to Covid-19 (median [IQR])	5 [4-7]	5 [4-6]	5.5 [4-7]	0.74
Neurologic symptom n (%)				
≥4	85 (85)	43 (86)	42 (84)	1
Brain fog	81 (81)	41 (82)	40 (80)	1
Headache	68 (68)	32 (64)	36 (72)	0.52
Numbness/tingling	60 (60)	29 (58)	31 (62)	0.84
Dysgeusia	59 (59)	32 (64)	27 (54)	0.42
Anosmia	55 (55)	37 (74)	18 (36)	<0.001
Myalgia	55 (55)	30 (60)	25 (50)	0.42
Dizziness	47 (47)	20 (40)	27 (54)	0.23
Pain other than chest	43 (43)	20 (40)	23 (46)	0.69
Blurred vision	30 (30)	9 (18)	21 (42)	0.02

Table 3: Neurologic symptoms and signs attributed to Covid-19. Table 3, adapted from Graham et al, shows notable neurologic findings observed in long-hauler COVID-19 patients, stratified by patients who are laboratory positive (SARS-CoV-2+) vs. negative.[9] Notable neuro-ophthalmic findings include blurred vision and brain fog, as highlighted in the table.

Research plan

Study design & methods

This study will be conducted with approval from the IRB at Northwestern University (in submission). Patients will be recruited from Northwestern Medicine's Neurology COVID-19 Clinic or Northwestern Medicine's Ophthalmology Clinic based on the following inclusion and exclusion criteria:

INCLUSION CRITERIA:

- HEALTHY CONTROLS
    a. Absence of symptoms suggestive of SARS-CoV-2 infection during the previous months
- COVID LONG-HAULERS WITH NEUROLOGIC SYMPTOMS AND NO OCULAR COMPLAINTS
    a. Patients who tested positive for SARS-CoV-2 and had neurologic symptoms persisting at least 6 weeks from symptom onset
- COVID LONG-HAULERS WITH NEUROLOGIC SYMPTOMS AND OCULAR COMPLAINTS
    a. Patients who tested positive for SARS-CoV-2 and had neurologic symptoms persisting at least 6 weeks from symptom onset
    b. Participants who have at least one ocular complaint since diagnosis of SARS-CoV-2

GLOBAL EXCLUSION CRITERIA:

- Participants with history of media opacities such as cataract and vitreous hemorrhage
- Any evidence of glaucoma, high myopia, retinal occlusive diseases, choroidal atrophy, choroidal neovascularization, central serous chorioretinopathy, infectious choroiditis, fovea plana, and age-related macular degeneration
- Lack of capacity to provide informed consent
- Unable to participate in a clinical eye examination setting
- Patients who are younger than 18 years old

GROUP SPECIFIC EXCLUSION CRITERIA:

- LONG-HAULERS WITH NEUROLOGIC SYMPTOMS AND NO OCULAR COMPLAINTS
    - Participants who have any new ocular complaints since COVID diagnosis
    - Hospitalization for COVID-19 complications
- COVID LONG-HAULERS WITH NEUROLOGIC SYMPTOMS AND OCULAR COMPLAINTS
    - Hospitalization for COVID-19 complications

Those who meet the inclusion & exclusion criteria and are interested in participating will be recruited as follows:

HEALTHY CONTROLS:

Patients will be recruited from the ophthalmology clinic at Northwestern Medicine at their standard visits. Individuals will be age and sex matched to COVID-long hauler patients. Patients will be consented during their standard-of-care visit and additional study data will be collected thereafter.

LONG-HAULER COVID PATIENTS:

Patients will be recruited through the Neurology COVID-19 clinic. These patients will then be called by study personnel and the details of the study will be outlined for the patient. Patients who agree to participating in the study will be scheduled for a study visit at Northwestern Medicine eye clinic, where they will be consented and examined.

Following recruitment and consent, the patients will undergo standard care ophthalmic examination, which include:

- Visual acuity measurement: Snellen (BCVA
- Pupillary assessment
- Visual field testing

- Slit-lamp examination
- Tonometry
- Dilated-Indirect fundus examination

Patients will also undergo Diagnostic testing consisting of:

- Fundus angiography (FA) at discretion of the principal investigator based on clinical findings suspicious for vascular abnormalities, ischemia or inflammation
- Color fundus photography posterior pole+periphery (Topcon montage, or ultra-widefield Optos 200Tx, Optos PLC, Dunfermline, Scotland, United Kingdom)
- SD-OCT (Spectralis HRA2; Heidelberg Engineering, Heidelberg, Germany), 30 frames, dense macular cube centered to the fovea (High resolution line and Raster scan to get CMT)

OCTA: Optovue or Zeiss Plex-elite 3x3, 6x6, 9x9 or 12x12 cube and HD scans centered on the fovea.

PRIMARY STUDY ENDPOINTS:

1. Quantification of vessel density and vessel length density for the superficial capillary plexus (SCP) and deep capillary plexus (DCP) for the central 3/6-mm circle (*para-fovea*) on OCTA.
2. Correlation between vessel density on OCTA and foveal thickness on 3/6 mm ETDRS map on OCT (given automatically by Heidelberg software)
3. Calculation of FAZ area based on measurements taken on 3 x 3-mm scans.
4. Enface and cross-sectional b-scan imaging of the choriocapillaris

Statistical plan

We plan to enroll 75 patients. We anticipate a low drop-out rate (~5%) as study participants will be consented on the same day they have their research visit. We will have 3 groups of patients: 25 long-haulers with ocular symptoms, 25 long-haulers without ocular symptoms, and 25 healthy age and sex-matched controls. Our study will be consistent with prior ophthalmic imaging studies analyzing COVID-19 patients and anticipate our sample size will provide us with an appropriate power for this study.

This study will have numerous endpoints. We will classify our endpoints into 2 categories: categorical parameters and continuous parameters.

The categorical parameters that will be collected in this study include: presence of afferent pupillary defect, visual field deficits, cataracts, inflammatory cells, flare, microaneurysms, hemorrhages, exudates, edema, epiretinal membrane, posterior vitreous detachment.

The continuous parameters that will be measured in his study include: logMAR best corrected visual acuity, intraocular pressure, cup to disc ratio, retinal nerve fiber thickness, ganglion cell layer thickness, central macular thickness, macular vessel density, peripheral vessel density, foveal avascular zone size.

For all categorical parameters, a chi-square test of homogeneity will be utilized to compare each variable across all 3 groups (long-haulers with ocular symptoms, long-haulers without ocular symptoms, and healthy controls). The test will be conducted using the open-source software R.

For all continuous parameters, ANOVA analysis with Tukey HSD post-hoc analysis will be utilized to compare each variable across all 3 groups (long-haulers with ocular symptoms, long-haulers without ocular symptoms, and healthy controls). The test will also be conducted using the open-source software R.

Baseline demographic data (i.e., age, sex, race, history, smoking status) for the study participants will be collected. ANOVA or chi-square analysis will similarly be used to discern inter-group differences in continuous and categorical variables, respectively. An alpha level of 0.05 will be utilized for all statistical calculations in this study to determine significance.

Anticipated results

As described in the "preliminary data" section, there is evidence to suggest that SARS-CoV-2 has some degree of tropism to the eye and can mediate systemic endothelial damage. Although the data is conflicting, prior studies have exhibited reductions in vessel density in COVID-19 patients as detected by OCT-A. Our study is unique in that we are focusing on COVID-19 long-haulers with ocular complaints; therefore, we expect the retinal vasculature to be more significantly impacted than has been described in COVID-19 recovered populations. We may expect to find characteristic quantifiable changes in retinal vessel densities on OCT-A and changes in vascular perfusion on fluorescein angiography in long hauler patients exhibiting ocular symptoms. Furthermore, as some preliminary data suggests nerve fiber and ganglion cell layer thinning in post-COVID-19 patients, we expect to find similar findings in our long-hauler cohort. We may also expect to find signs of ocular inflammation such as inflammatory cells, flare, and edema on clinical exam on long-hauler patients with ocular symptoms compared to controls.

Pitfalls

Long-hauler COVID-19 patients will be recruited from Dr. Igor Koralnik's neuro-COVID clinic, where approximately 20 long-haulers are seen per week. Recruitment of long-haulers with ocular symptoms will be slower given that only 30% of long-haulers presenting to Dr. Koralnik exhibit ocular symptoms.

Alternative strategies

Expand recruitment of patients from other neurology providers in the neuro-COVID long hauler clinic.

Timeline

Recruitment of study participants will begin following IRB approval in the summer of 2021 (anticipate early June). We expect to recruit 2-3 patients per week, which would result in a data collection phase of 25 weeks. We anticipate that recruitment and scheduling may be delayed in the first few weeks as study visit flow is established; therefore, we expect the total data collection phase to be completed in 30 weeks. Consequently, all data should be obtained by January 2022.

While data from recruited patients is being collected, our study team will begin the process of manuscript preparation. Data analysis will occur as the data is being collected by the study team. Key procedures will include organizing and analyzing imaging data to determine key parameters such as retinal vessel density and foveal avascular zone size, that are not calculated automatically by built in software. A working de-identified spreadsheet will be developed during the data collection phase, in which all relevant demographic and endpoint data will be recorded. By the end of the data collection phase in January 2022, our data will be ready for statistical analysis. We plan to submit our preliminary findings to the Association for Research in Vision and Ophthalmology's annual meeting and prepare a final manuscript for publication by the spring of 2022. During this process, we will meet all deadlines as per ISPB grant guidelines.

Cited References

1. Abrishami M, Emamverdian Z, Shoeibi N, et al. Optical coherence tomography angiography analysis of the retina in patients recovered from COVID-19: a case-control study. *Canadian Journal of Ophthalmology*. 2021;56(1):24-30. doi: 10.1016/j.jcjo.2020.11.006
2. Savastano MC, Gambini G, Cozzupoli GM, et al. Retinal capillary involvement in early post-COVID-19 patients: a healthy controlled study. *Graefe's Archive for Clinical and Experimental Ophthalmology*. 2021. doi:10.1007/s00417-020-05070-3

3. Roberts KA, Colley L, Agbaedeng TA, Ellison-Hughes GM, Ross MD. Vascular Manifestations of COVID-19 – Thromboembolism and Microvascular Dysfunction. *Frontiers in Cardiovascular Medicine*. 2020;7. doi:10.3389/fcvm.2020.598400
4. De Figueiredo, Camila Saggioro, Ícaro Raony, and Elizabeth Giestal-de-Araujo. "SARS-CoV-2 Targeting the Retina: Host–virus Interaction and Possible Mechanisms of Viral Tropism." *Ocular immunology and inflammation* 28.8 (2020): 1301-1304. Doi: 10.1080/09273948.2020.1799037
5. Casagrande M, Fitzek A, Püschel K, et al. Detection of SARS-CoV-2 in Human Retinal Biopsies of Deceased COVID-19 Patients. *Ocular Immunology and Inflammation*. 2020;28(5):721-725. doi:10.1080/09273948.2020.1770301
6. Savastano, Alfonso, et al. "Peripapillary retinal vascular involvement in early post-COVID-19 patients." *Journal of Clinical Medicine* 9.9 (2020): 2895. Doi: 10.3390/jcm9092895
7. González-Zamora J, Bilbao-Malavé V, Gándara E, et al. Retinal Microvascular Impairment in COVID-19 Bilateral Pneumonia Assessed by Optical Coherence Tomography Angiography. *Biomedicines*. 2021;9(3):247. doi:10.3390/biomedicines9030247
8. Cennamo, Gilda, et al. "Optical coherence tomography angiography features in post COVID-19 pneumonia patients: a pilot study." *American Journal of Ophthalmology* (2021). doi:10.1016/j.ajo.2021.03.015
9. Nehme M, Braillard O, Alcoba G, et al. COVID-19 Symptoms: Longitudinal Evolution and Persistence in Outpatient Settings. *Annals of Internal Medicine*. 2020. doi:10.7326/m20-5926
10. Graham EL, Clark JR, Orban ZS, et al. Persistent neurologic symptoms and cognitive dysfunction in non-hospitalized Covid-19 “long haulers.” *Annals of Clinical and Translational Neurology*. 2021. doi:10.1002/acn3.51350