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COVID-19 and the Eye: Systemic and Laboratory Risk Factors for Retinopathy and Detection of Tear Film SARS-CoV-2 RNA with a Triplex RT-PCR assay

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Abstract

COVID-19 and the Eye: Systemic and Laboratory Risk Factors for Retinopathy and Detection of Tear Film SARS-CoV-2 RNA with a Triplex RT-PCR assay

By Jessica G. Shantha

Coronavirus-19 (COVID-19) has been associated with ophthalmic manifestations primarily involving the anterior segment, but recent studies have suggested retinal vascular findings may be associated with increased severity of systemic disease. In addition, the relationship between tear film SARS-CoV-2 RNA, timing of illness and eye disease are unknown. We evaluated hospitalized COVID-19 inpatients for retinopathy, tear film viral RNA and their relationship with systemic disease factors, laboratory findings, and outcomes. Sixty hospitalized COVID-19 inpatients were offered enrollment. Full ophthalmic examination and conjunctival swabs were taken for SARS-CoV-2 RT-PCR. Demographic, clinical outcomes and laboratory data were collected to assess the relationship of these parameters to retinopathy. Univariate and multivariate analyses of systemic disease and laboratory risk factors for retinopathy and SARS-CoV-2 RNA detection were assessed. The median age was 59.5 years (Interquartile range, Q1, Q3, 47,69.5 years) and 29 (48%) were female. Retinopathy associated with COVID-19 was observed in 12 of 60 patients (20%). The median age (Q1,Q3) of patients with COVID-19 retinopathy was 51.5 (41,56) compared to 62.5 (48.5,72.5) years in individuals without retinopathy (p=0.01). The median BMI was 34.3 (33.3, 42.8) in patients with retinopathy compared to 30.9 (26.0, 36.5) in those without retinal disease findings (p=0.04). ECMO requirement was observed in 33% of patients with retinopathy compared to 8% in those without retinopathy (p=0.04). Multivariate analyses trended towards increased risk of retinopathy with younger age (aOR 0.95 (95% CI 0.90-1.01, p=0.095) and with increased BMI (aOR. 1.08, 95% CI 1.00-1.18, p=0.056). Fifteen of 60 patients (25%) tested positive in their tear film for SARS-CoV-2 RNA with a trend towards a shorter length of illness and hospitalization in patients who were positive. In this series retinopathy was observed in 20% of patients in this hospitalized cohort of COVID-19 patients. Factors independently associated with retinopathy included younger age and increased BMI. SARS-CoV-2 RNA within tear film was detected in 25% of patients and unrelated to ophthalmic signs/symptoms. The precise relationship of obesity and other potential contributing factors such as age with retinal microvascular disease requires further investigation.

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Table of Contents

Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), leading to the ongoing and evolving coronavirus disease (COVID-19) pandemic, has resulted in nearly 442 million cases and 6 million deaths worldwide.[1] SARS-CoV-2 infection may be asymptomatic or lead to severe respiratory illness with acute respiratory distress syndrome and multi-organ involvement.

A study was performed on lung tissue of patients who have succumbed to COVID-19 secondary to respiratory illness. A few notable findings were described in this study which includes endothelial damage from SARS-CoV-2, vascular thrombosis, and angiogenesis.[2] This suggests multiple pathways involved in the lung manifestations in severely ill patients that includes direct viral damage and thrombotic complications. This study and others describe a similar response in other vascular organ systems in the body. Other pathogenic mechanisms include the overactivation of the immune system both innate and adaptive immunity and coagulopathy.[3]

Ophthalmic manifestations have also been increasingly reported.[2-4] The reported prevalence of ophthalmic manifestations ranges from 1-32%, and these manifestations include both anterior segment (e.g. conjunctival injection, chemosis, subconjunctival hemorrhage)[5-8] and retinal findings. The reported retinal manifestations include retinal hemorrhage and cotton wool spots. Less commonly, retinal vascular occlusive events that include branch retinal artery occlusion, central retinal vein occlusion and central retinal artery occlusion may impact visual acuity.[4,9-17] Our team retrospectively reviewed patients who underwent an ophthalmic examination during their hospital stay for acute COVID-19 infection from March to June of 2020. Thirty-seven patients were evaluated with a mean age of 54 years (Standard deviation: 15) and the majority were female (62%, 23 patients). Indication for consult included retinopathy assessment, optic nerve assessment, fungemia, and ocular symptoms in 32% (12), 24% (9), 24% (9), 19% (7)

patients respectively. In this cohort 14 (38%) patients displayed clinical features of retinopathy associated with COVID-19. The majority of these patients were admitted to the ICU (95%, 35) indicating a critically ill population. The hospital length of stay was on average 8.5 days greater for patients with retinopathy as compared to those without retinopathy. ICU interventions were common amongst patients which included mechanical ventilation (29 patients, 78%), vasopressor administration (26 patients, 70%), anticoagulation (33 patients, 89%), and corticosteroid administration (28 patients, 76%). Patients that developed retinopathy had a higher proportion of patients that required mechanical ventilation, extracorporeal membrane oxygenation (ECMO), and vasopressors. Laboratory parameters such as D-dimer, C-reactive protein, and fibrinogen were elevated, but only D-dimer was shown to be statistically different between patients with and without retinopathy. Taken together with the laboratory derangements both inflammatory and coagulation as well as the critical illness suggests that the pathogenic mechanisms for COVID-19 associated retinopathy development may be thrombotic microangiopathy.[4]

In addition to ophthalmic manifestations associated with COVID-19, the precise role of the tear film as a transmission route either for pathogen entry or mucosal person-to-person transmission via hand-eye contact is unclear. Studies utilizing conjunctival swabs and Schirmer's strips suggest that the prevalence of SARS-CoV-2 detection in tears and conjunctival secretion through RT-PCR ranges from 0 to 57.1%, and may potentially vary with the presence of conjunctivitis and with concomitant viral RNA positivity within nasopharyngeal mucosa.[18-24] The potential for viral transmission through the tears remains a risk and the current personal protective equipment guidelines (PPE) recommend the use of goggles to healthcare professionals.[25] Other viruses

known to infect ocular surface cells include well-known respiratory viruses such as adenovirus[26] and influenza virus, as well as emerging infectious diseases including SARS-CoV-1 and Zika virus.[27] SARS-CoV-1 and SARS-CoV-2 virus infect cells via the ACE-2 receptor and transmembrane protease serine type 2 receptor, which have been identified in ocular surface epithelia, potentially serving as a conduit for viral transmission.[28-29]

We sought to prospectively assess the prevalence of retinal manifestations associated with COVID-19 inpatients and their relationship to systemic disease severity and laboratory indices within a university-based, tertiary referral care setting. We also sought to systematically assess SARS-CoV-2 viral RNA in the tear film and its relationship to ocular findings, host, and disease characteristics.

Methods

Objective

The first aim of this study was to determine the ophthalmic burden and risk factors for retinal microvascular disease in acute COVID-19 patients. The hypothesis was acute retinopathy will be identified in COVID-19 patients and increased in patients with severe disease and systemic baseline comorbidities. The second aim was to analyze the prevalence of SARS-CoV-2 viral RNA in the tear film of acutely symptomatic patients with SARS-CoV2 infection. SARS-CoV-2 will be identified in the tear film of patients with increased prevalence of positive tear film SARS-CoV-2 RNA during time points closer to symptom onset.

Setting and Overview

This prospective observational, cross-sectional study was conducted at Emory University Hospital between January 1, 2021 and June 1, 2021. Hospitalized COVID-19 patients were offered study inclusion for ophthalmic examination and tear film collection for RT-PCR analysis. The study protocol was approved by the research ethics committee of Emory University (IRB ID STUDY00001071), and in accordance with the Declaration of Helsinki.

Inclusion criteria included adult patients with a confirmed diagnosis of COVID-19 through real-time RT-PCR assay of respiratory specimens, and who were admitted to the hospital ward or Intensive Care Unit (ICU) setting. The COVID-19 ophthalmology research team approached Hospital Medicine and ICU attendings about patients who would potentially be amenable for consideration of a research study, after which the study was discussed with the patient and/or appropriate family member with decision-making capacity. Informed consent was obtained from patients included in the study or their legally authorized representatives for patients who were

incapable of giving informed consent in select circumstances (e.g., intubation and sedation in an intensive care unit setting).

Examination and Sample Collection

Ophthalmic exams were performed at bedside including near visual acuity, pupillary examination, anterior segment exam by penlight, and a dilated posterior segment exam with indirect ophthalmoscopy. Data were collected on a standard case report from (Supplemental appendix 1). Fundus photography was taken with a portable fundus camera, Nidek DS-20 [Nidek, Inc, San Jose, CA]. *COVID-19 Retinopathy* was defined as any one of the following: 1) Retinal hemorrhage, 2) Cotton wool spots, 3) Retinal vein or artery occlusion, *and* no prior history of diabetic retinopathy, hypertensive retinopathy, or findings that were consistent with diabetic or hypertensive retinopathy.

Patient samples were collected including a blood sample and conjunctival tear swab of both eyes, and tear collection via Schirmer's strip from both eyes. All exams and samples were obtained by a fully trained physician wearing full personal protective equipment (PPE) for protection against respiratory transmission of SARS-CoV-2. PPE included a fluid-impervious protective gown, gloves, face shield, and N95 or KN95 mask.

For tear sample collection, a drop of 1% proparacaine was administered 1 minute before sample collection. A conjunctival swab was used to sweep the inferior conjunctival fornix of both eyes with a sterile sampling swab. The tip of the swab was placed in the sample tube containing EMAG lysis buffer solution (bioMérieux, Durham, NC). Samples collected at the time of ophthalmic exam were transported to the laboratory for analysis. Conjunctival swab samples were refrigerated in a 4°C refrigerator for batched RNA analysis.

Data Collection

Deidentified clinical information on all enrolled patients were collected onto a secure data collection platform (DF/Net Research, Seattle, Washington, USA) using an iPad or computer. Prior to each patient visit, demographic characteristics, past medical history, and results of serological tests of each patient was extracted from the electronic medical record by a member of the research team. Demographic information included age, sex, race, and ethnicity. Pertinent past medical history collected included hypertension, diabetes, coronary artery disease, chronic obstructive pulmonary disease, chronic kidney disease and history of immunosuppression. Past ocular history and ophthalmic medications were documented. Hospital documentation and patients were specifically queried as to whether a history of diabetic or hypertensive retinopathy had been observed by prior eye care providers prior to hospital admission.

Medical data related to the patient's hospitalization included ICU vs. hospital ward admission, days of COVID-19 symptoms, respiratory or systemic symptoms, and hospitalization days. Interventions documented included mechanical ventilation, ECMO, history of anticoagulation and corticosteroid use.

Laboratory parameters documented included peak D-dimer, nadir fibrinogen, C-reactive protein, interleukin-6, platelet count peak and nadir, hematocrit nadir, and lymphocyte peak and nadir. COVID-19 disease severity was classified according to the National Institutes of Health COVID-19 Severity Scale (https://www.covid19treatmentguidelines.nih.gov/overview/clinical-spectrum/). Strata for COVID-19 severity included 1) Asymptomatic or presymptomatic infection; 2) Mild illness; 3) Moderate illness; 4) Severe illness; and 5) Critical illness. Asymptomatic or presymptomatic infections included individuals who tested positive for SARS-

CoV-2 but did not have symptoms consistent with COVID-19 (e.g., hospitalized for other reasons besides COVID-19 respiratory illness such as appendicitis).

RT-PCR

Conjunctival swabs placed in lysis buffer and previously stored at 4°C were assessed for SARS-CoV-2 viral RNA. Real-time reverse transcription polymerase chain reaction (rRT-PCR) testing was performed for targets in the SARS-CoV-2 nucleoprotein and envelope E genes as well as RNase P, as previously described.[30] A cycling threshold (Ct) of 40 was used as a cutoff for a positive result for N2, E, and RNase P genes. Conjunctival swab samples were considered RT-PCR positive if RNase P was detected and either N2 or E targets were positive (i.e. $Ct < 40$). Variant testing was performed using a laboratory-developed rRT-PCR targeting specific *spike* mutations, as previously described.[31]

Statistical Analysis

Descriptive and inferential statistics were performed using R and Stata software. Demographic data including age, sex, race and ethnicity are summarized as medians and interquartile range or frequencies with percentages as appropriate. The cohort was stratified into patients with and without COVID-19 retinopathy for analysis.

Patient demographic information, COVID-19 NIH Severity, baseline comorbidities, laboratory data and interventions were assessed as potential risk factors for COVID-19 retinopathy with univariate and multivariate logistic regression models. Unadjusted and adjusted odds ratios and 95% confidence intervals were calculated for each potential contributing patient, disease, or laboratory factor.

Lastly, we assessed for relationships between tear film SARS-CoV-2 RT-PCR positivity, evidence of COVID-19 retinopathy, and systemic disease characteristics including duration of COVID-19 illness/symptoms, duration of hospitalization and NIH COVID-19 Severity Score. Two-sided, unpaired T-tests were utilized for comparison of continuous variables and Fisher's exact test were utilized for proportions. A p-value of 0.05 was considered statistically significant for all analyses.

Results

Sixty patients were enrolled into this study from Emory University Hospital. The median age of patients was 59.5 years (Q1,Q3 47,69.5). Twenty-nine patients (48%) were female and 31 (52%) were male. The majority of patients were black (43, 72%). Other enrollees were white (11, 18%), Asian (1, 2%) or documented as unknown (5, 8%). Four patients were Hispanic ethnicity (7%) and all others (n=56) were non-Hispanic (93%). Patients were stratified by the NIH COVID-19 Severity Scale for analyses. The majority of patients were deemed *critical* (n=45, 75%) but other categories included *severe* (n=2, 3%), *moderate* (n=7, 12%), *mild* (n=1, 2%) and *asymptomatic* (n=5, 8%). Demographic and clinical features are summarized in Table 1.

COVID-19 Retinopathy and Ocular Findings

Seventeen of sixty patients were observed to have evidence of retinopathy (28%) including 3 patients with diabetic retinopathy (18%), 1 patient with hypertensive retinopathy (6%), and 2 with other retinopathy (12%, Table 5). Twelve of the 60 patients (20%) who underwent assessment showed evidence of retinopathy that met case definition criteria for COVID-19 associated retinopathy.

Other ocular findings included conjunctival chemosis in 6 (10%), conjunctivitis in 2 (3%), conjunctival injection in 1 patient (2%), and subconjunctival hemorrhage in 1 (2%) patient. Cataract was also documented in 24 patients (40%).

Factors associated with COVID-19 retinopathy

We further assessed whether patient demographic or baseline health factors, COVID-19 NIH severity scale, laboratory parameters or hospital interventions were associated with COVID-19 retinopathy. The median age of patients with COVID-19 retinopathy was 51.5 (Q1,Q3 41,56)

years-old and significantly lower than individuals without retinopathy at 62.5 (Q1,Q3 48.5,72.5) years with an unadjusted odds ratio of 0.75 (95% CI 0.58-0.95, p=0.01). Sex was not statistically associated with a greater risk of retinopathy (p>0.05, Table 1).

The majority of patients with COVID-19 retinopathy were black race, and the odds of COVID-19 retinopathy in Black patients was 2.27 (95%. CI 0.44-11.67, p=0.18) the odds among other patients. Patients with COVID-19 retinopathy also tended to have a higher BMI than those without (median BMI in patients with COVID-19 retinopathy 34.3 (Q1,Q3 33.3, 42,8) versus 30.9 (Q1, Q3 26.0, 36,5) in patients without COVID-19 retinopathy (p=0.04, Figure 3). The odds of having a BMI greater than 30 was 9.2 times higher in patients with COVID-19 retinopathy than in patients without retinopathy (Odds Ratio 1.42 95% CI 1.01-2.00).

The odds of retinopathy was 4.53 (95% CI 0.53-38.48, p=0.19) times higher in patients with critical illness than in patients without critical illness. However, most patients were classified as critical in this cohort according to NIH severity grading given their hospitalization for respiratory failure with or without ICU admission. Other than the association with obesity noted above, none of the other baseline comorbidities was significantly associated with the prevalence of retinopathy in these COVID-19 patients. However, the odds of diabetes was 2.56 (95% CI 0.70-9.28, p=0.19) times higher in patients with COVID-19 retinopathy than in those without.

Laboratory parameters were assessed to determine potential relationships between inflammation and coagulation pathways and retinopathy. Patients with retinopathy showed higher median peak D-dimers of 5703 ng/mL (Q1, Q3 1018,13746) than those patients without retinopathy of 1548.5 ng/mL (Q1, Q3 889,5595) but were not statistically significant (p=0.48, Figure 3). Interleukin-6 was also higher in patients with retinopathy at 2 pg/mL (Q1,Q3 2,25) compared to

6.5 pg/mL (Q1,Q3 3.7, 9.2) and did not reach significance (p=0.75) although the numbers within in each group for comparison were small (n=3 for retinopathy, n=6 for no retinopathy) as IL-6 lab collection was drawn only if deemed clinically indicated by the provider.

Interestingly, the median hematocrit nadir was lower in patients with retinopathy at 29.0% (Q1,Q3 25.7,36.0) than in individuals without retinopathy at 34.3% (Q1, Q3 28.8,38.1). The median peak platelets count was (410.5 x 10^9 cells/L (Q1, Q3 365.5, 499.5)) in patients with COVID-19 retinopathy than in those without retinopathy (336 x 10^9 cells/L (Q1, Q3 280,438.5, p=0.10). No significant differences were observed across other hematologic parameters abstracted included white blood cell counts or lymphocyte counts (i.e., peaks and nadirs recorded for all values).

Interventions assessed included mechanical ventilation, vasopressor requirement, continuous renal replacement therapy, ECMO, clinical trial enrollment, anticoagulation (beyond low molecular weight heparin), and corticosteroid use. Although the numbers of patients requiring extracorporeal membrane oxygenation (ECMO) was limited (n=8), the odds of ECMO were higher among patients with COVID-19 retinopathy than in those without (OR = 5.50, 95% CI 1.14-26.63, p=0.04. The odds of anticoagulation were also higher among patients with COVID-19 retinopathy than those without (OR = 3.27, 95% CI 0.65 – 16.63, p=0.19).

Multivariate logistic regression modeling

Based on the observed univariate associations, we assessed the potential contribution of age, BMI, hematocrit nadir and anticoagulation. to retinopathy, which included adjusted estimates showed that odds of retinopathy were higher with younger age (aOR 0.95, 95% CI 0.90-1.01,

p=0.095), increased BMI (aOR 1.08, 95% CI 1.00-1.18, p=0.056), anticoagulation (aOR 3.94, 95% CI 0.61-25.6, p=0.15) and hematocrit nadir (aOR 0.91, 95% CI 0.79-1.05, p=0.19).

SARS-CoV-2 RT-PCR

We also assessed the tear film for SARS-CoV-2 RNA by RT-PCR. Nineteen of 120 samples (16%, 95% CI 0.98 – 0.24) tested positive for SARS-CoV-2 RT-PCR with either one or two SARS-CoV-2 targets. Fifteen of 60 patients (25%, 95% CI 0.15 – 0.38) showed positive RT-PCR tests in either eye with 4 of 60 patients (6.7%, 95% 0.019 – 0.16) testing positive in both eyes.

Five eyes of 4 patients were positive with all three targets (N2, E, and RNAse P genes). Ten eyes of 8 patients were positive with the N2 gene detection and only one eye showed E gene detection. No correlation was observed between SARS-CoV-2 RNA detection on the ocular surface and the presence or absence of COVID-19 retinopathy (p>0.05). Of note, alpha variant (lineage B.117) was detected in three eyes of two patients with N2 Ct values varying between 25.9 and 31.8.

We further assessed the relationship between SARS-CoV-2 RNA positivity in the tear film with disease related factors. Patients who were SARS-CoV-2 RT-PCR positive in the tear film had fewer days of COVID-19 symptoms at a mean (SD) of 8.6 days (9.5) compared to 13.6 days (10.9) in patients who were RT-PCR negative (p=0.16). The number of days of hospitalization was also lower at 2.2 days (8.6) in individuals who were SARS-CoV-2 positive compared to 6.1 days (7.9) in patients who tested negative (p=0.16), but these differences were not statistically significant. Further, no differences were observed in the proportion of individuals who were

SARS-CoV-2 RNA positive in tear film when stratified by NIH COVID-19 severity (i.e., critical vs. non-critical illness, $p>0.05$).

Discussion

In this prospectively enrolled, cross-sectional study of hospitalized COVID-19 patients, 20% of patients showed evidence of COVID-19 retinopathy, as defined by acute signs of retinal vascular damage (i.e., cotton wool spots, retinal hemorrhage), not attributable to diabetes, hypertension or other preexisting systemic vascular conditions. The prevalence of retinopathy was associated with younger age, increased BMI, and extracorporeal membrane oxygenation requirement in unadjusted analyses. Other factors of interest, which showed a non-statistically significant association with retinopathy included black race and NIH COVID-19 Severity. The observed associations between retinopathy and young age and increasing BMI remained after adjusting for other potential predictors.

The reported prevalence of retinopathy in COVID-19 patients has varied from 22% to 38% with higher prevalence of retinopathy in COVID-19 patients with worse severity.[4,9-17] Factors previously associated with retinopathy include higher sequential organ failure assessment scores, respiratory failure requiring mechanical ventilation, hypotension requiring vasopressors and elevated D-dimers.[4] While D-dimers did not differ between COVID-19 patients with and without retinopathy in this COVID-19 population, D-dimers were increased in individuals with COVID-19 retinopathy in our cohort. Anticoagulation, defined by medication requirements in addition to low molecular weight heparin, was also increased in patients with COVID-19 retinopathy and these relationships related to coagulation diathesis, which are potentially involved in the pathogenesis of retinal microvascular disease, may warrant further study.

BMI was one particular risk factor of note that differed significantly between patients with and without retinopathy. Specifically, while the median BMI of 32.9 in the entire cohort met criteria

for obesity, those with retinopathy showed a BMI of 34.3 compared with 30.9 in individuals without retinopathy with 42% increased odds of retinopathy, and the odds of obesity were 9 times higher in patients with retinopathy than in those without. While BMI and obesity are associated with diabetes and hypertension that may also be associated with retinopathy independent of COVID-19, the relationship of BMI and retinopathy in the setting of COVID-19 raises the question of whether components of metabolic syndrome (e.g., obesity, dyslipidemia) may contribute to endothelial dysfunction and inflammation[32-33], which may also contribute to retinopathy. Investigations studying the precise relationships between obesity and microvascular disease are currently underway, particularly related to cardiac and thromboembolic disease.[34]

In addition to our analyses of risk factors related to retinopathy, we also assessed SARS-CoV-2 RNA with RT-PCR of conjunctival tear film swabs and showed that approximately 25% of patients had positive results with a validated assay for SARS-CoV-2 detection. Interestingly, alpha variant was also identifiable via a conjunctival swab, indicating that these samples can also be used for typing SARS-CoV-2 variants. Whether other specific variants may be detected on the ocular surface, including the more recent delta and omicron variants, remains unknown. Patients in whom SARS-CoV-2 was detected in the tear film showed a shorter duration of COVID-19 symptoms of 9-10 days and were hospitalized for a shorter time period of 2-3 days compared to patients in whom SARS-CoV-2 was not detected. Earlier assessments could potentially increase the rate of tear film viral RNA detection and the prevalence of tear film SARS-CoV-2 detection has been estimated as high as 57% in prior series.[24]

Moreover, while no clear relationships were identified between either anterior or posterior segment ocular findings and surface and tear film SARS-CoV-2 RNA, we were able to detect

SARS-CoV-2 on the ocular surface of patients without conjunctival signs indicating the potential for viral RNA in the absence of ocular symptoms (i.e., asymptomatic ocular surface carrier). Detection of SARS-CoV-2 RNA may represent aerosolized virus from respiratory secretions or contiguous spread of SARS-CoV-2 within the respiratory / ocular surface mucosa. The Centers for Disease Control and Prevention currently recommends eye protection during the evaluation of patients with confirmed or suspected COVID-19, as well as in high transmission areas, and infection prevention and control practices to prevent disease transmission to health care providers via the ocular surface mucosa remains paramount.[25] Whether the ocular surface is an efficient route of disease transmission is unknown, but recent studies have described ACE2 receptor and serine protease TMPRSS2 within both corneal and conjunctival epithelial cells.[28-29]

Limitations of this study include the relatively sample size and potential for selection bias given the tertiary referral center-based recruitment, which may be biased towards greater disease severity and a higher prevalence of retinopathy than other COVID-19 patient populations. Interestingly, we previously described a nearly 40% prevalence of retinopathy in a retrospective study of COVID-19 patients who were hospitalized primarily in the intensive care unit setting.[4] It is plausible that increasing microvascular disease including retinopathy may be observed with higher degrees of systemic morbidity in COVID-19 patients.

In addition, we found in this prospectively enrolled, cross-sectional study that age and body mass index were associated with a higher rate of retinopathy. Whether these factors may contribute to microvascular disease pathogenesis via inflammation, endothelial dysfunction or other mechanisms is unknown. Moreover, whether retinopathy may serve as a biomarker for concurrent systemic disease morbidity, coagulation diathesis, inflammation or hypoxemia

requires further understanding. Given the ongoing COVID-19 disease pandemic and imperative to understand SARS-CoV-2 mucosal transmission dynamics, ocular surface immunity, and clinical disease findings, further investigations in these areas are needed and ongoing.

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Tables 1: Demographic and Baseline Characteristics

Patient Characteristics	**All (n=60 patients)**	**COVID Retinopathy (n=12 patients)**	**No COVID Retinopathy (n=48 patients)**	**Odds Ratio (95% CI)**
Age, Years (Median, Q1, Q3)	59.5 (47, 69.5)	51.5 (41, 56)	62.5 (48.5, 72.5)	0.75 (0.58, 0.95)
Sex				
Female	29 (48%)	6 (50%)	23 (48%)	1.09 (0.31, 3.85)
Male	31 (52%)	6 (50%)	25 (52%)	
Race				
Black	43 (72%)	10 (83%)	33 (69%)	2.27 (0.44, 11.67)
White	11 (18%)	0 (0%)	11 (23%)	
Asian	1 (2%)	0 (0%)	1 (2%)	
Unknown	5 (8%)	2 (17%)	3 (6%)	
Hispanic Ethnicity	4 (7%)	1 (8%)	3 (7%)	1.30 (0.12, 13.78)
NIH COVID-19 severity				
Asymptomatic	5 (8%)	0 (0%)	5 (10%)	
Mild	1 (2%)	0 (0%)	1 (2%)	
Moderate	7 (12%)	1 (8%)	6 (13%)	
Severe	2 (3%)	0 (0%)	2 (4%)	
Critical	45 (75%)	11 (92%)	34 (71%)	4.53 (0.53, 38.48)[1]
BMI (Median, Q1, Q3)	32.9 (26.4, 36.5)	34.3 (33.3, 42.8)	30.9 (26.0, 36.5)	1.42 (1.01, 2.00)
ICU admission	21 (36%)	6 (50%)	15 (32%)	2.13 (0.59, 7.73)
Days of COVID illness (Median, Q1, Q3)	6.5 (3, 12.5)	11.0 (4.5, 17.5)	6 (2.5, 10.5)	1.04 (0.99, 1.11)
Days of hospitalization (Mean, SD)	2.9 (8.58)	6.1 (7.9)	2.2 (8.6)	1.04 (0.98, 1.12)
Symptoms				
Shortness of breath	44 (73%)	10 (83%)	34 (71%)	2.06 (0.40, 10.62)
Cough	31 (52%)	7 (58%)	24 (50%)	1.40 (0.39, 5.03)
Fatigue	24 (40%)	4 (33%)	20 (43%)	0.68 (0.18, 2.56)

[1] Critical v. non-critical

Fever	16 (27%)	4 (33%)	12 (25%)	1.50 (0.38, 5.89)
Diarrhea	16 (27%)	5 (42%)	11 (23%)	2.40 (0.64, 9.09)
Baseline comorbidities				
None	12 (20%)	2 (17%)	10 (21%)	
Hypertension	40 (67%)	9 (75%)	31 (65%)	1.65 (0.39, 6.90)
Diabetes	24 (40%)	7 (58%)	17 (35%)	2.56 (0.70, 9.28)
Asthma	6 (10%)	2 (17%)	4 (9%)	2.15 (0.34, 13.42)
COPD	7 (12%)	0 (0%)	7 (15%)	1.68 (0.28, 9.95)
Obesity (BMI =>30)	34 (57%)	10 (91%)	24 (52%)	9.2 (1.1, 77.6)
Other immunosuppression	11 (18%)	1 (8%)	10 (21%)	0.35 (0.04, 3.00)

Table 2. Ocular Findings in COVID-19 Subjects by Patients and Eyes

Ocular Findings	**Patients (%) (n=60)**	**Eyes (%) (n=120)**
Conjunctival injection	1 (2%)	2 (2%)
Conjunctivitis	2 (3%)	4 (3%)
Conjunctival chemosis	6 (10%)	11 (9%)
Subconjunctival hemorrhage	1 (2%)	1 (1%)
Cataract	24 (40%)	48 (40%)

Table 3: Laboratory findings in COVID-19 subjects

Laboratory Values	All (n=60 patients) (Median, Q1, Q3)	COVID Retinopathy (n=12 patients) (Median, Q1, Q3)	No COVID Retinopathy (n=48 patients) (Median, Q1, Q3)	Odds Ratio
D-dimer, Peak, mg/mL	1583 (906, 7032)	5703 (1018, 13746)	1548.5 (889, 5595)	1.03 (0.96, 1.10)[2]
Fibrinogen, Nadir, mg/dL	363 (211, 488) (n=25)	488 (175, 589)	353 (266, 488)	1.07 (0.66, 1.74)[3]
C-reactive protein, mg/L	111.4 (57.1, 162.9) (n=57)	121.7 (80.1, 165.1)	97.6 (53.7, 162.9)	1.04 (0.74, 1.46)[4]
Interleukin-6, pg/mL	5.8 (2.3, 9.2) (n=9)	2 (2, 25)	6.5 (3.7, 9.2)	n/a
Platelets, Peak, x10^9 cells/L	348 (292, 457.5)	410.5 (365.5, 499.5)	336 (280, 438.5)	1.72 (0.99, 2.99)[3]
Platelets, Nadir, x10^9 cells/L	211 (150.5, 248.5)	218 (151.5, 259)	210 (150.5, 244.5)	1.19 (0.51, 2.81)[3]
Hematocrit, Nadir, %	33.4 (27.6, 37.5)	29.0 (25.7, 36.0)	34.3 (28.8, 38.1)	0.66 (0.39, 1.14)[5]
WBC, Peak, x10^9 cells/L	13.3 (7.4, 16.9)	14.8 (11.8, 18.3)	12 (7.15, 16.1)	1.23 (0.82, 1.85)[5]
WBC, Nadir, x10^9 cells/L	6.1 (4.2, 8.0)	5.9 (4.1, 8.4)	6.2 (4.4, 8.0)	0.84 (0.26, 2.74)[5]
Lymphocytes, Peak, x10^9 cells/L	16 (8, 30), (n=51)	16 (9, 30)	16 (9, 30)	1.02 (0.79, 1.33)[5]
Lymphocytes, Nadir, x10^9 cells/L	7 (3, 13), (n=47)	7 (3, 14)	7 (3, 14)	0.92 (0.60, 1.43)[5]

[2] Per 1000 unit increase

[3] Per 100 unit increase

[4] Per 50 unit increase

[5] Per 5 unit increase

Table 4. Interventions in COVID-19 subjects with and without retinopathy

Interventions	**All (n=60)**	**COVID Retinopathy**	**No COVID Retinopathy**	**Odds Ratio (95% CI)**
Mechanical Vent	17 (28%)	5 (71%)	12 (43%)	3.33 (0.55, 20.22)
Vasopressors	4 (7%)	2 (17%)	2 (4%)	4.60 (0.58, 36.67)
Continuous renal replacement therapy	2 (3%)	1 (8%)	1 (2%)	4.27 (0.25, 73.75)
Extracorporeal Membrane O2 (ECMO)	8 (13%)	4 (33%)	4 (8%)	5.50 (1.14, 26.63)
Trial enrollment	15 (25%)	3 (27%)	12 (25%)	1.09 (0.25, 4.81)
Lovonox	27 (45%)	3 (25%)	24 (50%)	0.33 (0.08, 1.38)
Anticoagulation beyond Lovonox	39 (65%)	10 (83%)	29 (60%)	3.27 (0.65, 16.63)
Corticosteroid	25 (42%)	6 (50%)	19 (40%)	1.52 (0.43, 5.44)

Table 5. Multivariable Model Results

	Adjusted Odds Ratio (95% CI)	**p-value**
Model 1		
Age, Years	0.95 (0.90, 1.01)	0.0947
Body mass index	1.08 (1.00, 1.18)	0.0563
Anticoagulation	3.94 (0.61, 25.60)	0.1517
Hematocrit, Nadir, %	0.91 (0.79, 1.05)	0.1890

Table 6. Retinopathy Classification in Enrolled Patients

	Patients	Bilateral Patients	Unilateral Patients	Eyes
Retinopathy	17	6	11	23
COVID Retinopathy	12	2	10[6]	14
Diabetic Retinopathy	3	1	2	4
Hypertensive Retinopathy	1	1	0	2
Other Retinopathy	2	1	1	3

Note: One patient is counted in both COVID retinopathy and diabetic retinopathy categories. Bilateral patient has one eye with COVID retinopathy and one eye with diabetic retinopathy.

[6] One person had bilateral retinopathy, but unilateral covid retinopathy (OS COVID retinopathy, OD diabetic retinopathy)

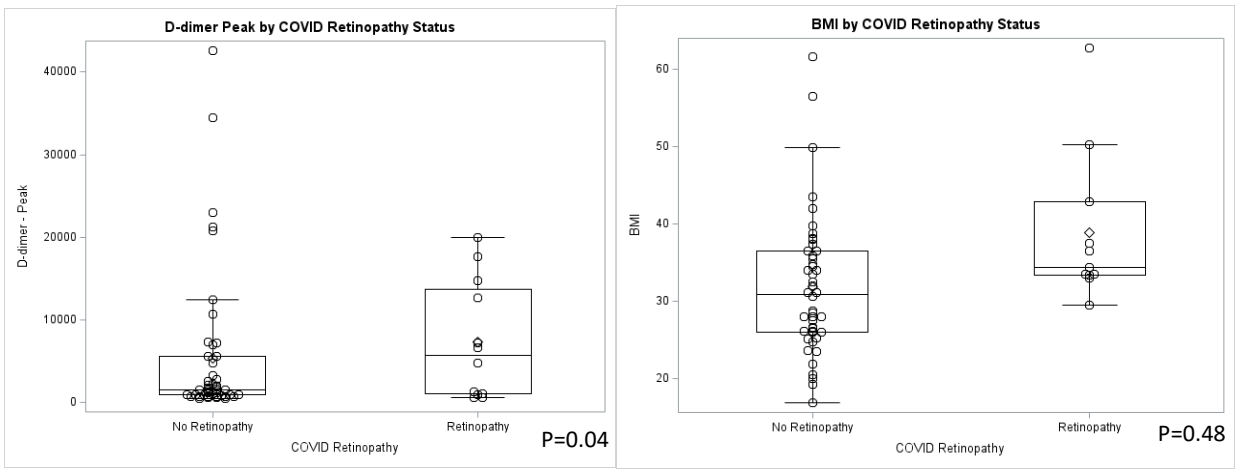

Figure 1. Box-and-whisker plots compare factors associated with retinopathy. (A) Median body mass index was greater in individuals with retinopathy than those without retinopathy (p=0.04). (B) Peak D-dimers were numerically increased in COVID-19 retinopathy patients compared to those without retinopathy, but this comparison was not statistically significant (p=0.48).