Original paper

Retinal thickness in patients with elevated D-dimer and interleukin-6 levels as a result of SARS-CoV-2 infection

Grubość siatkówki u pacjentów z podwyższonym poziomem D-dimerów i interleukiny 6 w wyniku zakażenia SARS-CoV-2

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Słowa kluczowe: COVID-19, D-dimery, interleukina 6, optyczna koherentna tomografia, grubość siatkówki.

Abstract

Introduction: The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) can lead to various health issues, including severe pneumonia, organ damage, as well as effects on the retina. Researchers have detected SARS-CoV-2 in the retinas of infected patients, and new imaging methods, such as optical coherence tomography (OCT), are being used to investigate its impact on the eye.
Aim of the research: To document changes in retinal thickness (RT) and their association with saturation (SpO2), D-dimers, and interleukin-6 (IL-6) levels in coronavirus disease 2019 (COVID-19) patients hospitalized for bilateral pneumonia.
Material and methods: This prospective study included COVID-19 patients assessed after 2 months (Group 1) and re-evaluated after 8 months from hospital discharge (Group 2). RT was automatically assessed with OCT. Group 2 RT measurements were compared with those of healthy subjects, and D-dimers, IL-6, and SpO2 levels on admission were correlated with RT in group 1.
Results: Group 2 exhibited a significant decrease in RT compared to group 1 in specific macular regions, accompanied by an increase in certain areas. Moreover, Group 2 demonstrated increased RT compared to a control group in specific regions. A positive correlation was observed between $SpO_2 \leq 90\%$ and RT in Group 1 in specific macular regions.
Conclusions: RT in patients after SARS-CoV-2 infection is altered at the 6-month follow-up. Hypoxia, hypercoagulability, and inflammation in COVID-19 can collectively influence RT.

Streszczenie

Wprowadzenie: SARS-CoV-2 może prowadzić do różnych problemów zdrowotnych, w tym ciężkiego zapalenia płuc, uszkodzenia narządów, jak również wpływać na siatkówkę. Badacze wykryli SARS-CoV-2 w siatkówkach zakażonych pacjentów i ustalili, że stosując nowe metody obrazowania, takie jak optyczna koherentna tomografia (OCT), można zbadać jego wpływ na oko.
Cel pracy: Udokumentowanie zmian w grubości siatkówki u pacjentów z COVID-19 hospitalizowanych z powodu obustronnego zapalenia płuc oraz ustalenie, jaki wpływ na grubość siatkówki mają saturacja (SpO_2), poziom D-dimerów i interleukiny 6 (IL-6).
Materiał i metody: Badanie prospektywne obejmowało pacjentów z COVID-19 ocenianych po 2 miesiącach (grupa 1.) i ustalili, że ponownie po 8 miesiącach od wypisu ze szpitala (grupa 2.). Grubość siatkówki była automatycznie mierzona za pomocą OCT. Pomiar grubości siatkówki w grupie 2. porównano z wynikami zdrowych osób, a poziomy D-dimerów, IL-6 i SpO_2 przy przyjęciu do szpitala były korelowane z grubością siatkówki w grupie 1.
Wyniki: W grupie 2. wykazano istotne zmniejszenie grubości siatkówki w porównaniu z grupą 1. w kilku regionach plamki żółtej z jednoczesnym wzrostem w innych obszarach. W grupie 2 stwierdzono większą grubość siatkówki w porównaniu z grupą kontrolną w niektórych regionach. Wykazano dodatnią korelację między $SpO_2 \leq 90\%$ a grubością siatkówki w grupie 1. w określonych regionach plamki żółtej.
Wnioski: Grubość siatkówki mierzona po przebyciu zakażenia SARS-CoV-2 zmienia się po 6 miesiącach od zakażenia. Niedotlenienie, nadkrzepliwość krwi i stan zapalny z powodu COVID-19 mogą wpływać na grubość siatkówki.

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has spread worldwide since the beginning of 2020, causing a variety of systemic manifestations, ranging from mild infections to severe bilateral pneumonia with respiratory failure and damage to multiple organs, including the neurological system, heart, gastrointestinal tract, kidneys, and more [1]. Ophthalmologists have also become interested in the effects of this virus on eye conditions, primarily focusing on the retina. Several researchers have investigated the impact of SARS-CoV-2 infection on the retina and its vascularization [2, 3].

SARS-CoV-2 has a target point in the eye structure, which is the angiotensin-converting enzyme-2 (ACE-2) receptor, which is also detected in many structures of the human eye [1, 4]. The ribonucleic acid of SARS-CoV-2 has been found post-mortem in the retinas of several patients who died from SARS-CoV-2 infection [5].

The first and key site of action of SARS-CoV-2 is the respiratory system, especially the lungs. Damage to the pulmonary system occurs through direct and immune-mediated pathways [6, 7]. Pro-inflammatory cytokines play a pivotal role in this immune stage, known as the "cytokine storm", with interleukin-6 (IL-6) being the most crucial [8]. Coagulopathy complicating the course of pneumonia, characterized by increased levels of D-dimers and fibrin/fibrinogen degradation products, worsens the prognosis for patients. Systemic hypoxia, microvascular damage, blood hypercoagulability, and overproduction of cytokines in severe bilateral pneumonia can lead to diffuse intravascular coagulation (DIC) [9–15].

In the present day, the advancement of novel non-invasive imaging methods, such as optical coherence tomography (OCT), facilitates a comprehensive assessment of individual structures within the eye. This technology allows for detailed evaluation of the posterior segment of the eye in the course of many diseases, including coronavirus disease 2019 (COVID-19) [16].

Aim of the research

This study aimed to evaluate the impact of hypoxia, inflammation, and increased blood clotting following COVID-19 bilateral pneumonia on retinal thickness (RT). In addition, we assessed correlations between the RT parameter obtained by OCT during the initial examination carried out 2 months after post-hospital discharge and baseline oxygen saturation (SpO_2), D-dimer levels, and IL-6 levels received during hospitalization.

Material and methods

Study population

A consecutive, prospective evaluation was conducted on 119 eyes of 62 COVID-19 patients with bilateral pneumonia (Group 1 and Group 2). These patients were admitted to the Department of Infectious Diseases of the Voivodeship Hospital in Kielce (Poland) between March and May 2021. The hospitalizations occurred during the period of dominance of the B.1.1.7 variant of SARS-CoV-2. The infectious disease caused by SARS-CoV-2 was confirmed through a positive polymerase chain reaction (PCR) test, and pneumonia was validated by either a chest X-ray or CT scan.

The control group (group 3) comprised exclusively healthy patients who underwent routine eye examinations at the ophthalmology department. COVID-19 was ruled out through a negative result on a PCR test.

The Bioethics Committee of Collegium Medicum of Jan Kochanowski University in Kielce (Poland) granted approval for the examination (study code 54 was approved on 1 July 2021). This analysis serves as a continuation of a prospective study conducted on COVID-19 patients, 2 months after their hospital discharge, with the previous findings having been published [9, 17].

The inclusion criteria for the ophthalmology study were as follows: patients who were 2 months post-discharge from the hospital following COVID-19 bilateral pneumonia (Group 1) and patients who were 8 months post-discharge from the hospital for COVID-19 bilateral pneumonia, specifically selected from Group 1 (Group 2).

Optical coherence tomography measurements

The OCT examination was conducted using Swept Source DRI-OCT Triton (Topcon Inc., Tokyo, Japan). Swept Source OCT (SS-OCT) represents a modern method of imaging the structures of the posterior segment of the eye. In this method, the light source is characterized by a narrow spectrum, with the wave frequency varying over time within a specified range.

The primary advantage of this method lies in the currently highest scanning speed of 370,000 scans A/s. Advanced technologies such as SS-OCT permit the evaluation of even deeper layers of the eye, including the choroid. Penetration of the light waves extends to the sclera [18, 19].

In SS-OCT, the light wavelength is 1050 nm (infrared), providing an axial resolution of 2.6 µm, a transverse resolution of 14 µm, and a scan width of 12 mm. The utilization of infrared light, which experiences less scattering, enhances the visualization of the posterior vitreous cortex and its structure [20].

The OCT protocol involved the following parameter: 3D Macula 7 × 7 mm scanning protocol. The image quality scale ranges from 0 to 100%. All scans eligible for the study met the criteria of an image quality reaching at least 65%.

The individual central retinal parameters assessed by OCT were obtained using the Early Treatment

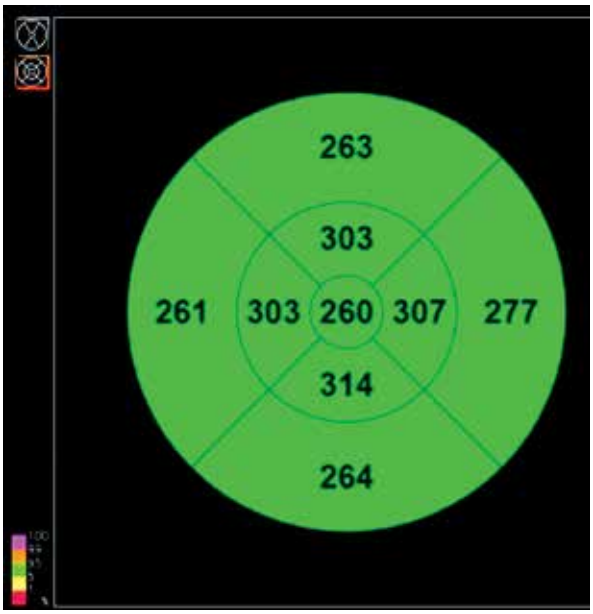

Figure 1. Map of central retinal thickness

Diabetic Retinopathy Study (ETDRS) grid positioned in the fovea by fixation. The central RT was separated automatically with OCT instrument software (IMAGEnet61.34.19388).

According to the nomenclature based on the International Nomenclature for Optical Coherence Tomography within the retina, the following layers can be distinguished starting from the retinal surface: 1 – posterior cortical vitreous (PCV), 2– preretinal space, 3 – nerve fibber layer (NFL), 4 – ganglion cell layer (GCL), 5 – inner plexiform layer (IPL), 6 – inner nuclear layer (INL), 7 – outer plexiform layer (OPL, dendritic), 8a – Henle's fibre layer (Axonal OPL), 8b – outer nuclear layer (ONL), 9 – external limiting membrane (ELM), 10 – myoid zone (MZ), 11 – ellipsoid zone (EZ), 12 – outer segments (OS), 13 – interdigitation zone (IZ), and 14 – retinal pigment epithelium/Bruch's complex (RPE/Bruch's complex) [21]. The retinal thickness measurement we conducted encompassed the entire thickness of the retina covering all the layers mentioned above.

The segmentation of the central retina was reviewed. The errors were assessed, and they were manually corrected, retested, or discarded. The central RT values were measured in micrometres (µm) across all areas of the EDTRS grid using the 3D Macula protocol.

The foveal area (F) had a diameter of 1 mm, the IR had a diameter of 3 mm, and the OR had a diameter of 6 mm.

A comparative analysis of the macular parameters in patients hospitalized due to SARS-CoV-2 infection in IR and OR obtained through OCT examination at 2 months (group 1) and 8 months (group 2) after hospital discharge was also conducted.

A comparative analysis of OCT parameters between the COVID-19 group 8 months after hospital discharge (group 2) and the healthy group (group 3) was also performed.

The image of a central RT map in the right eye was automatically assessed by OCT using the Early Treatment Diabetic Retinopathy Study (ETDRS) grid situated in the fovea by fixation. The automatic map of central RT is divided into 3 areas: the fovea (F = 260 µm), inner ring (IR) consisting of ISR (inner superior ring = 303 µm), INR (inner nasal ring = 307 µm), IIR (inner inferior ring = 314 µm), ITR (inner temporal ring = 303 µm), and outer ring (OR) consisting of OSR (outer superior ring = 263 µm), ONR (outer nasal ring = 277 µm), OIR (outer inferior ring = 264 µm), and OTR (outer temporal ring = 261 µm) (Figure 1).

Laboratory parameters

The following laboratory parameters obtained during hospitalization for COVID-19 bilateral lung inflammation confirmed by computed tomography (CT) scans were analysed: SpO_2, D-dimer levels, and IL-6 levels.

Correlations were assessed between baseline D-dimer levels and IL-6 concentration concerning SpO_2 at hospital admission and RT (group 1) based on OCT.

Statistical analysis

Frequency and descriptive statistics were used to analyse demographic and imaging data.

Using mean (M), standard error of the mean (SEM), median (Me), and quartiles (IQR), quantitative variables were described.

Categorical variables were presented using percentages, and the χ^2 test was used for comparisons between groups. For comparisons between Group 1 (2 months after hospital discharge) and Group 2 (8 months after hospital discharge) and between Group 2 and the control group (Group 3), Student's *t*-test or the Mann-Whitney test was applied depending on the distributions of the compared characteristics.

Spearman rank correlation coefficients were counted to assess the significance, direction, and strength of the relationship between RT in Group 1, based on OCT and levels of D-dimers and IL-6 according to SpO_2 groups: ≤ 90; 90–95; > 95.

J. Guilford's classification was applied in the interpretation of correlation values.

The number of patients (*N*) and the percentage of patients (%) were calculated for qualitative variables such as hypertension, dyslipidaemia, and oxygen therapy. Values were considered statistically significant at $p < 0.05$.

The statistical package Statistica 13.3, Polish version (STATSOFT, Cracow, Poland) was applied for statistical analysis.

Results

Study population

COVID-19 Group 1 consisted of 62 patients (n = 62; eyes = 119) and included 42 (67.7%) men and 20 women. The M (SEM) age in the COVID-19 group was 51.33 (1.45). Hypertension was found in 20 (32.2%) patients, while dyslipidaemia was found in 3 (4.76%) patients. M(SEM) of spherical equivalent (D – Diopters) was 0.13 (0.13), and the axial length was 23.55 (0.08). Two eyes were excluded due to a history of ocular trauma, one eye after uveitis, one eye with hyperopia > 3 dioptres, and one eye with myopia > 3 dioptres (Table 1). In Group 1 22 patients were differentiated into 3 categories: with SpO_2 > 95%, 29 with $Sp0_2$ between 91 and 95%, and 11 patients with $SpO_2 \leq 90\%$.

Six months later (8 months after discharge), we contacted these 62 patients and invited them for a second eye examination. Forty-nine patients (n = 49; eyes = 83) (Group 2) agreed to participate in the second eye examination. The remaining eyes were excluded due to refractive errors that did not meet the inclusion criteria, a history of cataract surgery, and eye trauma. The final study group included 31 men (63.3%) and 18 women with a history of bilateral COVID-19-related pneumonia.

The control group (Group 3) consisted of 43 subjects (n = 43; eyes = 83) with a M (SEM) age of 47.76 (1.38).

The following ocular and general disorders were excluded in all patients: myopia > –3 dioptres, hyperopia > +3 dioptres, age-related macular degenerations, glaucoma, previous eye surgery including cataract surgery and trabeculectomy, uveitis, eye injuries, opaque media affecting SS-OCT scan or image quality, and diabetes mellitus.

All patients including COVID-19 subjects (Group 1 and Group 2) and healthy subjects (Group 3) signed consent for ophthalmic examination.

Table 1. Demographic, systemic, and ocular characteristics of the study group and healthy group

Variables	COVID-19 patients n = 62	Control group n = 49	P-value
Gender, n (%):			
Men	42 (67.7)	28 (62.22)	0.515
Women	20 (32.3)	17 (37.78)	
Age [years]:			0.087[A]
M (SEM)	51.33 (1.45)	47.76 (1.38)	
Me (IQR)	51.00 (18.00)	47.00 (10.00)	
BMI [kg/m^2]:			0.047[A]
M (SEM)	28.41 (0.51)	26.77 (0.64)	
Me (IQR)	28.00 (6.00)	26.50 (6.00)	
LogMar Visual acuity:			–
M (SEM)	0.0	0.0	
Me (IQR)	0.0	0.0	
LogMar Reading vision:			–
M (SEM)	0.3	0.3	
Me (IQR)	0.03	0.0	
Spherical equivalent (D):			< 0.001[B]
M (SEM)	0.13 (0.13)	–0.67 (0.13)	
Me (IQR))	0.0 (2.25)	0.63 (1.37)	
Axial length [mm]:			0.111[A]
M (SEM)	23.55 (0.08)	23.35 (0.10)	
Me (IQR)	23.45 (1.05)	23.45 (1.22)	
Hypertension, n (%)	20 (32.2)		
Dyslipidaemia, n (%)	3 (4.76)		
Oxygen therapy, n (%)	23 (37.09)		

[A]Student's t-test, [B]Mann-Whitney test. M – mean, Me – median, SEM – standard error of the mean, IQR – quartiles, BMI – body mass index, D – dioptres.

Ophthalmic examination

The M (SEM) LogMar Visual Acuity was 0.0 (0.0) and LogMar Reading Vision was 0.3 (0.0) in both Group 1 and Group 2 during the 2 consecutive eye examinations.

In the 2 examinations conducted among Groups 1 and 2, no significant changes were observed for the following variables: Visual acuity LogMAR, Reading Vision LogMAR, and intraocular pressure in the patients hospitalized due to SARS-CoV-2 infection. Additionally, COVID-19 patients reported no ocular complaints during the 2 consecutive eye examinations.

Laboratory and imaging tests at admission

On hospital admission, the M (SEM) SpO_2 was 93.3 (0.5)%, the M (SEM) of IL-6 was 35.79 (3.91) pg/ml (references values 1.22 ±0.706 pg/ml), and the M (SEM) of D-dimers was 1095.29 (415.43) µg/l (references values < 500 µg/l).

Treatment of COVID-19 patients during hospitalization

Fifty-nine COVID-19 patients received a prophylactic dose of the low-molecular-weight heparin according to the label for an average of 9 (6.25–12) days. Twenty-two patients were treated with dexamethasone at a daily dose of 4 mg orally or 8 mg intravenously for 7–10 days, and 3 patients were treated intravenously with tocilizumab in a single dose of 600–800 mg depending on the patient's weight in accordance with labels and national recommendations.

Twenty-six patients received intravenous treatment with remdesivir, starting with an initial dose of 200 mg on the first day followed by 100 mg for the subsequent 4 days [22–24]. Additionally, 23 patients underwent oxygen therapy for an average duration of 5 (4–10) days.

Structural OCT outcomes in COVID-19 patients 2 months and 8 months after discharge

A statistically significant decreased M (SEM) of retinal thickness (RT) was found in patients hospitalized due to SARS-CoV-2 infection 8 months after discharge (Group 2), compared to 2 months after discharge (Group 1) in the inner inferior ring (IIR) (314.39 ±2.05 vs. 316.83 ±1.96, $p = 0.004$), outer superior ring (OSR) (275.23 ±1.47 vs. 279.76 ±1.84, $p < 0.001$), outer nasal ring (ONR) (290.09 ±1.88 vs. 292.88 ±1.92, $p < 0.001$), and outer inferior ring (OIR) (264.44 ±1.57 vs. 267.72 ±1.54, $p < 0.001$) and a statistically significant increase of RT in the inner temporal ring (ITR) (305.39 ±27.00 vs. 304.56 ±2.74, $p < 0.001$) and in the outer temporal ring (OTR) (260.72 ±2.02 vs. 259.64 ±2.04, $p < 0.001$) (Table 2). The above parameters were obtained by OCT examination.

Comparison of OCT parameters between COVID-19 patients 8 months after discharge and the control group

A statistically significant increase M(SEM) of RT was found in patients hospitalized due to SARS-CoV-2 infection 8 months after discharge (Group 2), compared to the healthy group (Group 3) in the in-

Table 2. Comparison of parameters of retinal thickness (foveal (F), inner superior ring (ISR), inner nasal ring (INR), inner inferior ring (IIR), inner temporal ring (ITR), outer superior ring (OSR), outer nasal ring (ONR), outer inferior ring (OIR), outer temporal ring (OTR)) between 2 months and 8 months after discharge in COVID-19 patients

Retinal thickness (RT) [µm]	First visit (n = 62)		Second visit (n = 49)		P-value
	M (SEM)	Me (IQR)	M (SEM)	Me (IQR)	
F	243.45 (3.22)	249.00 (30.00)	246.88 (2.27)	250.00 (26.00)	0.105[B]
ISR	319.59 (1.93)	319.00 (28.00)	316.43 (2.25)	315.00 (26.00)	0.077[A]
INR	318.92 (2.20)	320.00 (29.00)	318.19 (1.92)	317.00 (24.00)	0.482[A]
IIR	316.83 (1.96)	317.50 (25.00)	314.39 (2.05)	313.00 (25.00)	0.004[A]
ITR	304.56 (2.74)	308.00 (29.00)	305.39 (27.00)	305.00 (27.00)	< 0.001[B]
OSR	279.76 (1.84)	278.00 (17.00)	275.23 (1.47)	273.00 (18.00)	< 0.001[B]
ONR	292.88 (1.92)	291.00 (21.00)	290.09 (1.88)	288.00 (18.00)	< 0.001[B]
OIR	267.72 (1.54)	266.00 (19.00)	264.44 (1.57)	261.00 (20.00)	< 0.001[A]
OTR	259.64 (2.04)	266.00 (19.00)	260.72 (2.02)	258.00 (18.00)	< 0.001[B]

Mean ± SEM (standard error of the mean) structural OCT values. Values were statistically significant at p < 0.05. [A]Student's t-test, [B]Wilcoxon rank test, RT – retinal thickness, M – mean, Me – median, SEM – standard error of the mean, IQR – quartiles, F – foveal, ISR – inner superior ring, INR – inner nasal ring, IIR – inner inferior ring, ITR – inner temporal ring, OSR – outer superior ring, ONR – outer nasal ring, OIR – outer inferior ring, OTR – outer temporal ring.

Table 3. Comparison of parameters of retinal thickness (foveal (F), inner superior ring (ISR), inner nasal ring (INR), inner inferior ring (IIR), inner temporal ring (ITR), outer superior ring (OSR), outer nasal ring (ONR), outer inferior ring (OIR), outer temporal ring (OTR)) between 8 months after discharge in COVID-19 patients and the control group

Retinal thickness (RT) [µm]	Second visit (n = 49)		Control group (n = 49)		P-value
	M (SEM)	Me (IQR)	M (SEM)	Me (IQR)	
F	246.86 (2.22)	250.00 (26.00)	244.02 (2.12)	243.00 (22.00)	0.348[A]
ISR	318.62 (2.30)	318.50 (21.50)	313.30 (2.02)	314.00 (20.00)	0.046[A]
INR	318.03 (1.50)	317.00 (24,00)	315.10 (1.81)	315.00 (24.00)	0.235[B]
IIR	315.48 (1.63)	315.50 (20.00)	311.93 (1.77)	312.00 (20.00)	0.124[B]
ITR	304.74 (1.47)	307.50 (21.00)	302.46 (1.77)	300.00 (20.00)	0.073[A]
OSR	277.86 (1.89)	275.00 (17.50)	273.05 (1.57)	273.00 (17.00)	0.056[A]
ONR	290.88 (1.40)	288.00 (20.50)	288.25 (1.86)	288.00 (21.00)	0.397[A]
OIR	266.26 (1.50)	264.50 (18.00)	262.23 (1.59)	263.00 (20.00)	0.042[B]
OTR	259.89 (1.21)	260.50 (19.00)	258.11 (1.58)	258.00 (17.00)	0.245[A]

Mean ± SEM (standard error of the mean) structural OCT values. Values were statistically significant at $p < 0.05$. [A]Student's t-test, [B]Mann-Whitney test, RT –retinal thickness, M – mean, Me – median, SEM – standard error of the mean, IQR – quartiles, F – foveal, ISR – inner superior ring, INR – inner nasal ring, IIR – inner inferior ring, ITR – inner temporal ring, OSR – outer superior ring, ONR – outer nasal ring, OIR – outer inferior ring, OTR – outer temporal ring.

ner superior ring (ISR) (318.62 ±2.30 vs. 313.30 ±2.02, $p = 0.046$) and in the outer inferior ring (OIR) (266.26 ±1.47 vs. 262.23 ±1.59, $p = 0.042$) (Table 3). The above parameters were obtained by OCT examination.

Structural OCT outcomes obtained 2 months (group 1) after hospital discharge depending on SpO_2, D-dimers, and IL-6

A statistically significant positive correlation was identified between SpO_2 equal to or lower than 90% and RT in the following areas: inner superior ring (ISR) ($r = 0.50$, $p = 0.029$), inner temporal ring (ITR) ($r = 0.49$, $p = 0.034$), outer superior ring (OSR) ($r = 0.56$, $p = 0.012$), and outer temporal ring (OTR) ($r = 0.62$, $p = 0.004$).

Moreover, a statistically significant negative correlation was found between the RT and D-dimers but only in patients with the SpO_2 equal to or lower than 90% in the following areas: outer nasal ring (ONR) ($r = -0.66$, $p = 0.002$), outer inferior ring (OIR) ($r = -0.63$, $p = 0.004$), and outer temporal ring (OTR) ($r = -0.61$, $p = 0.006$).

A statistically significant positive correlation was found between the RT and IL-6 but only in patients with the SpO_2 equal to or lower than 90% in the following areas: inner temporal ring (ITR) ($r = 0.59$, $p = 0.009$) and outer temporal ring (OTR) ($r = 0.62$, $p = 0.004$) (Table 4).

Discussion

Cytokine storm, endothelial dysfunction, oxidative stress, and cell death are the processes responsible for COVID-19 pathogenesis and organ damage in the severe form of the disease, including ocular lesions [25, 26].

In the current study, we documented a statistically significant decrease of the RT in several areas of the macula in the COVID-19 group studied 8 months after hospital discharge compared to assessment at 2 months after discharge on the basis of IIR, OSR, ONR, and OIR, and a statistically significant increase of RT in ITR and OTR. The criteria for the interval between ophthalmic examinations with OCT were based on those used by other researchers in a study assessing the effect of COVID-19 on ocular parameters examined with different OCT devices [27].

We also observed a significant increase in RT in 2 areas of the macula: the ISR and the OIR, in COVID-19 patients 8 months after hospital discharge, compared to the healthy group. Bilbao-Malave *et al.* compared central retinal RT on OCT in patients after SARS-CoV-2 infection 14 days and 6 months after discharge from the hospital. They found a statistically significant increase in central RT in the second follow-up compared with the first ($p < 0.001$), which is partially consistent with our observations [27]. Furashova and Matthe reported that the thickening of the central retina based on OCT examination as a result of acute ischaemia is due to the thickening of the inner and middle layers of the retina, including the RNFL and GCL [28].

Similarly to our previous study, we divided Group 1, hospitalized due to SARS-CoV-2 infection related to SpO_2 on hospital admission, to demonstrate the effect of the degree of systemic hypoxia on ocular param-

Table 4. Correlations between the retinal thickness (Group 1) in the following areas: foveal (F), inner superior ring (ISR), inner nasal ring (INR), inner inferior ring (IIR), inner temporal ring (ITR), outer superior ring (OSR), outer nasal ring (ONR), outer inferior ring (OIR), and outer temporal ring (OTR) and oxygen saturation (SpO_2), interleukin-6 (IL-6), and D-dimers

Retinal thickness	SpO_2	D-dimers	IL-6
F	$r = -0.07$, $p = 0.474^{Sp}$	$r = -0.04$, $p = 0.646^{Sp}$	$r = 0.12$, $p = 0.186^{Sp}$
ISR	$r = -0.06$, $p = 0.537^{Sp}$ for $SpO_2 \leq 90\%$ $r = 0.50$, $p = 0.029^{Sp}$	$r = -0.13$, $p = 0.155^{Sp}$	$r = 0.15$, $p = 0.105^{Sp}$
INR	$r = 0.06$, $p = 0.505^{Sp}$	$r = -0.07$, $p = 0.469^{Sp}$	$r = 0.16$, $p = 0.077^{Sp}$
IIR	$r = 0.04$, $p = 0.680^{Sp}$	$r = -0.13$, $p = 0.148^{Sp}$	$r = 0.14$, $p = 0.131^{Sp}$
ITR	$r = -0.09$, $p = 0.309^{Sp}$ for $SpO_2 \leq 90\%$ $r = 0.49$, $p = 0.034^{Sp}$	$r = -0.04$, $p = 0.685^{Sp}$	$r = 0.21$, $p = 0.025^{Sp}$ for $SpO_2 \leq 90\%$ $r = 0.59$, $p = 0.009^{Sp}$
OSR	$r = -0.05$, $p = 0.579^{Sp}$ for $SpO_2 \leq 90\%$ $r = 0.56$, $p = 0.012^{Sp}$	$r = -0.13$, $p = 0.164^{Sp}$	$r = 0.07$, $p = 0.459^{Sp}$
ONR	$r = -0.07$, $p = 0.457^{Sp}$	$r = -0.21$, $p = 0.021^{Sp}$ for $SpO_2 \leq 90\%$ $r = -0.66$, $p = 0.002$	$r = 0.06$, $p = 0.500^{Sp}$
OIR	$r = -0.02$, $p = 0.816^{Sp}$	$r = -0.20$, $p = 0.032^{Sp}$ for $SpO_2 \leq 90\%$ $r = -0.63$, $p = 0.004^{Sp}$	$r = 0.09$, $p = 0.309^{Sp}$
OTR	$r = -0.07$, $p = 0.446^{Sp}$ for $SpO_2 \leq 90\%$ $r = 0.62$, $p = 0.004^{Sp}$	$r = -0.04$, $p = 0.675^{Sp}$ for $SpO_2 \leq 90\%$ $r = -0.61$, $p = 0.006^{Sp}$	$r = 0.18$, $p = 0.051^{Sp}$ for $SpO_2 \leq 90\%$ $r = 0.62$, $p = 0.004^{Sp}$

Values were statistically significant at $p < 0.05$. p for Sp – Spearman rank correlation. F – foveal, ISR – inner superior ring, INR – inner nasal ring, IIR – inner inferior ring, ITR – inner temporal ring, OSR – outer superior ring, ONR – outer nasal ring, OIR – outer inferior ring, OTR – outer temporal ring.

eters based on OCT. We found a statistically significant positive correlation between SpO_2 equal to or less than 90% and RT in several macular areas: ISR, ITR, OSR, and OTR. Most of the macular areas became thinner 8 months after discharge; perhaps further OCT studies and further observations are needed to assess the continuity of this trend. It is reasonable to conclude that retinal thinning may result from systemic hypoxia and local retinal ischaemia as an expression of inflammatory microvascular damage and increased blood clotting [9].

The retina is a highly oxygen-consuming structure, making it particularly sensitive to systemic hypoxia. It is more robust against hypoxia than the central nervous system because of its double blood supply. Many papers have shown that ischaemia leads to a stress response and results in the loss of retinal ganglion cells [29–31].

An important element of our current study was to identify correlations among SpO_2, D-dimer levels, IL-6 concentration, and ocular parameters obtained 2 months after hospital discharge on OCT in patients after SARS-CoV-2 infection. This study aimed to clarify whether factors such as hypoxia, blood hypercoagulability, and increased immune response to SARS-CoV-2 virus infection affect RT [27, 28]. We assessed a statistically important negative correlation between RT and D-dimers, but only in test subjects in group 1 with SpO_2 equal to or lower than 90% in several areas of the central retina in ONR ($p = 0.002$), OIR ($p = 0.004$), and OTR ($p = 0.006$). To the best of our knowledge, the available literature does not currently report correlations between D-dimer levels and central RT, so we cannot relate them to other papers.

Fibrin is degraded into D-dimers, which are released into the circulation when the clot is dissolved by fibrinolysis [32]. The D-dimer consists of two D fragments of fibrin protein that are attached by cross-linking. It serves as a crucial blood parameter whose elevated levels are indicative of a hypercoagulable state in diseases such as COVID-19. Thromboembolic events in the retinal vessels of post-viral infection patients, which is the subject of our study, result in an elevated embolic risk in these patients [33]. Guemes-Villahoz *et al.* assessed that patients with a D-dimer score equal to or greater than 500 ng/ml during COVID-19 disease had reduced perfusion (19.6 (SD 9.3) vs. 14.7 (SD 8.0), $p = 0.018$) and VD in SCP

(8.8 (SD 4.0) vs. 6.6 (SD 3.6), $p = 0.013$) after the acute phase of viral infection caused by SARS-CoV-2 virus. They explained the above correlation by the fact that SARS-CoV-2 can lead to subclinical changes at the level of the retinal microcirculation, probably secondary to the increased blood clotting induced by the above infection [34].

We found a statistically significant positive correlation between RT and IL-6 concentration, but only in subjects with SpO_2 equal to or lower than 90% in consecutive areas in ITR ($p = 0.009$) and OTR ($p = 0.004$). Haemostasis and coagulation parameters can be severely disrupted as a secondary effect of the severe inflammation that can be observed during COVID-19 [35–37].

There are no data in the available literature on the correlation between IL-6 levels and RT in patients hospitalized due to SARS-CoV-2 infection. We compared our results to the correlation between these 2 parameters in oedema within the central retina during retinal vein occlusion (BRVO), which also leads to central retinal ischaemia. As shown in other studies, IL-6 levels in the vitreous body in subjects after thrombus of the central retinal vein were significantly elevated in addition to increased levels of vascular growth factors. Changes in the retina caused by BRVO lead to localized inflammatory processes. The closure of the retinal vein leads to increased rolling and adhesion of leukocytes to the vein walls, causing stagnation of blood flow, so inflammatory processes play an essential role in BRVO [38].

Our research has limitations of which we are aware. Among them, there is a relatively small number of saturation-dependent groups. In addition, the initial ophthalmoscopy was performed only 2 months after hospitalization due to COVID-19. Patients were not examined during hospitalization or immediately after discharge, due to the epidemiological risk. Patients with COVID-19 bilateral pneumonia did not undergo an OCT examination before becoming infected, so it was not possible to compare the results of this examination with the results before hospitalization. We could not assess the impact of general diseases in the study group, such as hypertension or dyslipidaemia, on retinal thickness due to the small number of patients with these disorders. This is the first study reported in the literature to assess correlations between RT and D-dimer and IL-6 levels in COVID-19 patients. A notable strength of our study is the inclusion of relatively young patients without significant comorbidities, which allowed us to find the real impact of SARS-CoV-2 infection on RT. Further examination with OCT may help to estimate progression or improvement in RT.

Conclusions

In our study, we found changes in RT at 6-month follow-up based on OCT in patients after hospitalization for COVID-19 bilateral pneumonia.

Fluctuations in the RT parameter analysed by OCT may result from systemic hypoxia as well as local ischaemia resulting from the overproduction of inflammatory factors and increased blood coagulation in these patients.

In these patients, parameters obtained during hospitalization, such as SpO_2, D-dimer levels, and IL-6 levels, should be analysed. In patients with decreased SpO_2 and elevated D-dimer and IL-6 levels, thinning of the RT may be expected on OCT examination, which will be performed a long time after pneumonia caused by SARS-CoV-2 infection.

During the ophthalmological examination, a history of bilateral pneumonia during COVID-19 should be considered in the differential diagnosis of macular disease based on OCT.

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Conflict of interest

The authors declare no conflict of interest.

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