Microvascular Research 150 (2023) 104588

Contents lists available at ScienceDirect

Microvascular Research

journal homepage: www.elsevier.com/locate/ymvre

A pilot study: Exploring the influence of COVID-19 on cardiovascular physiology and retinal microcirculation

Adam Saloň [a,h], Ruslan Neshev [a], Kaja Teraž [b,c], Boštjan Šimunič [b], Manca Peskar [b,d], Uroš Marušič [b,e], Saša Pišot [b], Luka Šlosar [b,e], Mladen Gasparini [f], Rado Pišot [b], Patrick De Boever [g], Karin Schmid-Zalaudek [a], Bianca Steuber [a], Per Morten Fredriksen [h], Benedicta Ngwenchi Nkeh-Chungag [i], Harald Sourij [j], Omar Šerý [k,l], Nandu Goswami [a,m,*]

[a] Division of Physiology & Pathophysiology, Otto Loewi Research Center for Vascular Biology, Immunology, and Inflammation, Medical University of Graz, Austria
[b] Science and Research Centre Koper, Institute for Kinesiology Research, Koper, Slovenia
[c] Faculty of Sport, University of Ljubljana, Slovenia
[d] Biological Psychology and Neuroergonomics, Department of Psychology and Ergonomics, Faculty V: Mechanical Engineering and Transport Systems, Technische Universität Berlin, Berlin, Germany
[e] Department of Health Sciences, Alma Mater Europaea – ECM, Maribor, Slovenia
[f] Department of General Surgery, General Hospital Izola, Izola, Slovenia
[g] Centre for Environmental Sciences, Hasselt University, Belgium
[h] Faculty of Health and Social Sciences, Inland Norway University of Applied Sciences, Lillehammer, Norway
[i] Department of Biological and Environmental Sciences, Faculty of Health Sciences, Walter Sisulu University PBX1, 5117 Mthatha, South Africa
[j] Internal Medicine, Division of Endocrinology and Diabetology, Interdisciplinary Metabolic Medicine Trials Unit, Medical University of Graz, Graz, Austria
[k] Laboratory of Neurobiology and Molecular Psychiatry, Department of Biochemistry, Faculty of Science, Masaryk University, Kamenice 753/5, 625 00 Brno, Czech Republic
[l] Laboratory of Neurobiology and Pathological Physiology, Institute of Animal Physiology and Genetics, Czech Academy of Sciences, Veveří 97, 602 00 Brno, Czech Republic
[m] College of Medicine, Mohammed Bin Rashid University of Medicine and Health Sciences, Dubai, United Arab Emirates

ARTICLE INFO

Keywords:
COVID-19
Circulation
Hemodynamics
Pulse wave analysis
Retina
Arterial stiffness
Microcirculation

ABSTRACT

Background: The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) affects the cardiovascular system. The current study investigated changes in heart rate (HR), blood pressure (BP), pulse wave velocity (PWV), and microcirculation in patients recovering from Coronavirus disease 2019 (COVID-19) infection.
Methodology: Out of 43 initially contacted COVID-19 patients, 35 (30 males, 5 females; age: 60 ± 10 years; and body mass index (BMI): 31.8 ± 4.9) participated in this study. Participants were seen on two occasions after hospital discharge; the baseline measurements were collected, either on the day of hospital discharge if a negative PCR test was obtained, or on the 10th day after hospitalization if the PCR test was positive. The second measurements were done 60 days after hospitalization. The vascular measurements were performed using the VICORDER® device and a retinal blood vessel image analysis.
Results: A significant increase in systolic BP (SBP) (from 142 mmHg, SD: 15, to 150 mmHg, SD: 19, $p = 0.041$), reduction in HR (from 76 bpm, SD: 15, to 69 bpm, SD: 11, $p = 0.001$), and narrower central retinal vein equivalent (CRVE) (from 240.94 μm, SD: 16.05, to 198.05 μm, SD: 17.36, $p = 0.013$) were found. Furthermore, the trends of increasing PWV (from 11 m/s, SD: 3, to 12 m/s, SD: 3, $p = 0.095$) and decreasing CRAE (from 138.87 μm, SD: 12.19, to 136.77 μm, SD: 13.19, $p = 0.068$) were recorded.
Conclusion: The present study investigated cardiovascular changes following COVID-19 infection at two-time points after hospital discharge (baseline measurements and 60 days post-hospitalization). Significant changes were found in systolic blood pressure, heart rate, and microvasculature indicating that vascular adaptations may

Abbreviations: SBP, Systolic blood pressure; DBP, Diastolic blood pressure; BP, Blood pressure; HR, Heart rate; PWV, Pulse wave velocity; CRAE, Central retinal artery equivalent; CRVE, Central retinal vein equivalent; AVR, Artery to vein ratio; COVID-19, Coronavirus disease 2019; SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2.

* Corresponding author at: Division of Physiology & Pathophysiology, Otto Loewi Research Center for Vascular Biology, Immunology, and Inflammation, Medical University of Graz, Neue Stiftingtalstraße 6/D.05, A-8010 Graz, Austria
E-mail address: nandu.goswami@medunigraz.at (N. Goswami).

https://doi.org/10.1016/j.mvr.2023.104588
Received 22 May 2023; Received in revised form 12 July 2023; Accepted 16 July 2023
Available online 17 July 2023

be ongoing even weeks after hospitalization from COVID-19 infection. Future studies could involve conducting additional interim assessments during the active infection and post-infection periods.

1. Introduction

Coronavirus disease 2019 (COVID-19) is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus. SARS-CoV-2 enters the cell by binding its spike protein to membrane-bound angiotensin-converting enzyme 2 on the host cells (Chung et al., 2021; Lowenstein and Solomon, 2020; Invernizzi et al., 2020; Aşıkgarip et al., 2021; Aydemir et al., 2021; Invernizzi et al., 2021; Carreno et al., 2022). Post-mortem examinations have revealed that the coronavirus SARS-CoV-2 spreads through the bloodstream and attacks various organs, including the cardiovascular system (Vymazalová et al., 2023; Shankar et al., 2022). We know that the pathophysiological impacts of COVID-19 lead to a proinflammatory phenotype, disruption of oxide-redox metabolism, reduction in nitric oxide bioavailability, and many other factors leading to endothelial dysfunction. Gradually accumulating structural changes in the endothelium, supported by spreading inflammation, can induce a prothrombic phenotype and potentially lead to thrombosis (Chung et al., 2021; Lowenstein and Solomon, 2020; Wadowski et al., 2023; Salton et al., 2022). How the viral infection affects vascular health in peripheral circulation is unclear.

The peripheral vascular system consists of blood vessels (arteries, arterioles, capillaries, venules, and veins) outside the heart. Arteries are vessels under high pressure, responsible for supplying organs with blood and nutrients. The main roles of arterioles are to regulate pressure, flow, and nutrient delivery to the tissue. Their structure which includes elastic tissue and smooth muscles, predisposes them to be primary site for vasomotor regulation of blood pressure. Capillaries branch from arterioles and are followed by venules. Capillaries lack smooth muscle and elastic tissue and are responsible for transferring nutrients and oxygen to tissues. The postcapillary part of microcirculation, venules, has a thin layer of smooth muscle and elastic tissue, making them highly reactive to extravasation and inflammation (Granger and Rodrigues, 2016). When venous endothelial cells recognize the inflammatory phenotype of the blood, they initiate the inflammation process, that intimately leads to the adherence and extravasation of leukocytes into the interstitium through dilated junctions between cells.

COVID-19 can cause endothelitis (Varga et al., 2020), with a microvascular function that is not completely recovered 3 months after the infection (Tehrani and Gille-Johnson, 2021). Additionally, lower vascular function and higher arterial stiffness provide evidence of COVID-19's impact on larger arteries (Ratchford et al., 2021; Jud et al., 2021). The virus can induce cardiovascular conditions such as acute coronary syndrome, myocarditis, arrhythmias, and valvular damage (Ruzzenenti et al., 2021). These effects, along with reduced access to cardiovascular care during the pandemic, have led to increased morbidity and mortality. Further research on the connections between COVID-19 and the cardiovascular system as well as the validation of fast and appropriate diagnostic approaches to prevent the uncontrollable spread of the disease and enable early treatment are warranted.

Pulse wave velocity (PWV) is a widely used non-invasive physiological biomarker to assess changes in arterial stiffness (Vlachopoulos et al., 2010). A PWV instrument measures the transmission of the arterial wall wave velocity between two arterial points, with carotid-femoral PWV accepted as the gold standard (Laurent et al., 2006). The normal mean PWV in the general middle age population (45–65 years) is estimated at 6.0 m/s but increases with the height of the person, age, and blood pressure (van Hout et al., 2021). PWV serves as an independent predictor of cardiovascular risk. Increased arterial PWV has been reported to be a predictor of cardiovascular risk and mortality (Vlachopoulos et al., 2010). Conditions or processes affecting the elasticity of the arterial wall are reflected in PWV changes. One of the most common pathological conditions analysed and evaluated using this approach is atherosclerosis-caused arterial stiffening (Wang et al., 2015).

Retinal image analysis is a methodology to non-invasively and quickly evaluate microvascular health from static photographs of the retinal fundus. It assesses the parameters of retinal arterioles and venules. Changes in these parameters can reflect and predict future changes in larger vessels of the cardiovascular system (Hanssen et al., 2022). Therefore, implementation this method to assess early changes of the cardiovascular system could aid in the diagnosis and treatment of various cardiovascular diseases.

VICORDER® device measuring HR, BP, and PWV and retinal imaging technique to assess microcirculation were used to evaluate the effect of COVID-19 infection on vascular health. To assess the effects of COVID-19 on health, baseline measurements were conducted as soon as possible after discharge from hospitalization; either on the day of hospital discharge (if a PCR test on the day of hospital discharge was negative) or on day 10 (if a PCR test on the day of hospital discharge was positive). The second measurements were carried out 2 months after hospitalization.

The aim of this study is to investigate cardiovascular changes, including both microvascular and macrovascular parameters, in patients who have been discharged from the hospital following COVID-19 infection. Specifically, the study aims to examine these changes at two different time points post-hospital discharge, with the first measurements taken either on day of hospital discharge or on day 10 after hospitalization, while the second set of measurements was conducted 60 days post-hospitalization. By evaluating the cardiovascular changes in the post-hospitalization period, the study provides insights into the cardiovascular changes/adaptations occurring after COVID-19 infection.

2. Material and methods

The longitudinal study was conducted in the general hospital Izola, Slovenia. The data analysis was performed at the Medical University of Graz, Austria. The study was submitted to and approved by the institutional Ethics Committee of general hospital Izola with the application number 1/21 (from 1.2.2021). The clinical trial protocol was registered on ClinicalTrials.gov, with an identifier number NCT04860206. Data collection was performed in accordance with good clinical practices and followed the WMA Declaration of Helsinki (2013). Every participant received detailed information about the study protocol and provided written consent.

2.1. Participants

Forty-three patients were invited for this longitudinal study. Participants were approached by a physician who explained the study protocol. Inclusion criteria were signed informed consent and completed hospital treatment after a positive polymerase chain reaction (PCR) nose swab test on the SARS-CoV-2 virus. The exclusion criteria were a positive PCR test for the SARS-CoV-2 upon discharge from the hospital, major injuries/damage to the musculoskeletal system (disability), and the inability to follow the instructions while performing the tests. However, if only a positive PCR test for the SARS-CoV-2 upon discharge from the hospital, then participants were included and assessed ten days after hospital admission. Out of 43 initially enrolled patients, 35 completed the study. It is highly probable that the ongoing COVID-19 pandemic and the large distance between the hospital and their homes played a significant role in the decision of eight patients to withdraw from the study, as they did not provide specific reasons for their discontinuation. At admission, 25 reported dyspnoea and 33 showed the need for oxygen based on oxygen saturation level below 90 %. No patient received non-invasive mechanical ventilation or was intubated. The patients were hospitalized for 7.0 ± 4.9 days on average ranging between 1 and 30 days. Out of these, two patients spent 8 and 21 days, respectively, in the intensive care unit.

2.2. Study protocol

Participants were tested on two points in time after discharge from hospitalization (Fig. 1). The baseline measurements (post-COVID-19, collected between the 7th of January and to 18th of March 2021) were taken on the day of hospital discharge if a negative PCR test was obtained (PCR test, done in General Hospital Isola); otherwise, they were conducted on the 10th day after hospitalization. The second measurement was done 60 days after hospitalization (collected between 11th of March and the 19th of May 2021). The aim of this study was to investigate the changes in the cardiovascular health of post-COVID-19 patients during an encouraged but not supervised recovery period. After the first measurements, participants received a brochure titled "Stay active" which described appropriate motor and cognitive exercises along with nutritional recommendations (Pišot et al., 2020). All measurements were performed in the same room in the General Hospital Isola during morning hours. Physiological measurements using the Vicorder and retinal imaging techniques were used to assess cardiovascular health. This study was part of a larger project in which other measures, such as physical performance tests, pulmonary function, muscle properties and cognitive abilities were addressed.

2.3. Cardiovascular measurements

All measurements were performed using the VICORDER® (SMT medical GmbH & Co. KG, Würzburg, Germany). The non-fasting participants were in a supine position in a quiet room, with the head raised to approximately 15° so that the skin and muscles over the carotid were relaxed. Pulse wave velocity was measured by a cuff placed over the right carotid and the right thigh. The length between the carotid and femoral arteries was measured between the suprasternal notch and the mid-point of the thigh cuff (Stoner et al., 2012). Measurements were taken until pressure waveforms over the carotid and thigh area were clear and reproducible. During the second measurement, the same length was assured. To ensure accurate pulse wave velocity measurements, blood pressure readings were obtained using the VICORDER® device with a cuff placed on the left upper arm while the person was seated. Only one measurement per person, per session was taken in accordance with the ESH/ESC guidelines.

2.4. Microvascular measurements

The optic disc-focused retinal images (resolution of 1536 × 1536) from patients were collected in two runs, as mentioned in the study protocol. The retinal images of the right eye were taken by a trained person using a hand-held, portable 30° field-of-view digital retinal camera Optomed Aurora (Optomed Oy, Oulu, Finland). The retinal images were analysed by a trained grader without any previous knowledge about the details of the study. Image processing was done using MONA REVA software (VITO, Mol, Belgium; (Khan et al., 2022)). Details about the software and its use are reported in Khan and coworkers (Khan et al., 2022). The diameters of the 6 largest arterioles and 6 largest venules were used in the revised Parr-Hubbard-Knudtson formula (Knudtson et al., 2003) for calculating the central retinal arteriolar equivalent (CRAE) and central retinal venular equivalent (CRVE). The CRAE and CRVE were expressed in micrometres (μm).

2.5. Statistical analysis

All collected data were checked for normal distribution by the Shapiro-Wilk test. The pulse pressure index (PPI) was not normally distributed and thus transformed by a decadic logarithm, and analysed separately by a non-parametric Wilcoxon test. To compare the effect of post-COVID-19 changes, paired samples *t*-test and analysis of covariance (to check the covariates: age, height, weight, and body mass index) were applied. Since there are only 2-time points, Mauchly's test of sphericity assumes an ε of 1 for all parameters. All analyses were performed by SPSS (Version 27.0, SPSS Inc., USA). Significant results ($p \leq 0.05$) were boldened.

3. Results

Thirty-five participants (30 males, 5 females; age: 60 ± 10 years; height: 174.6 ± 8.8 cm; weight: 97.1 ± 16.7 kg; and BMI: 31.9 ± 4.9) completed this study. Over time, a decrease in heart rate (HR) ($p = 0.001$) and CRVE ($p = 0.013$) and a decrease in systolic blood pressure (SBP) ($p = 0.004$) were observed. PWV was higher ($p = 0.095$) and central retinal arteriolar equivalent (CRAE) lower ($p = 0.068$), respectively. Table 1 summarizes the basic characteristics of the study

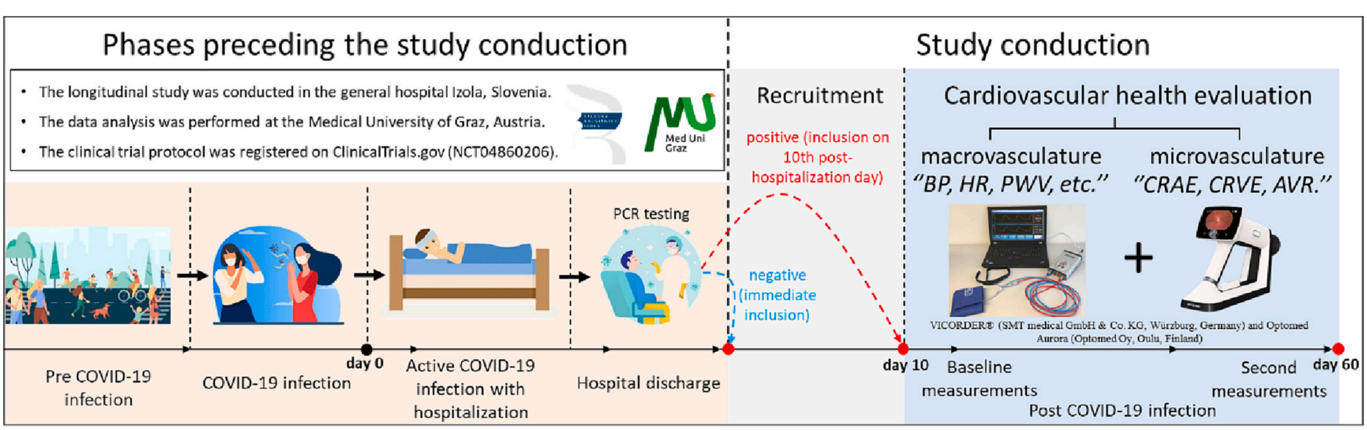

Fig. 1. Simplified overview of the study flow including phases preceding the study conduction. The vertical line with the arrows toward the right poses the time flow of the study. The black dot highlights day 0 (the first hospitalization day). The red dots highlight the points of the baseline and second measurements. The baseline measurements were conducted after discharge from hospitalization; either on the day of hospital discharge (if a PCR test on the day of hospital discharge was negative) or on day 10 (if a PCR test on the day of hospital discharge was positive). The second measurements were carried out 2 months (60 days)after hospitalization. Indicated are also the devices used to measure the parameters of (micro-) macrovasculature and measured parameters. Blood Pressure (BP), Heart Rate (HR), Pulse Wave Velocity (PWV), Central Retinal Arteriolar Equivalent (CRAE) and Central Retinal Venular Equivalent (CRVE), Artery to Vein Ratio (AVR).

Table 1
Characteristics of study participants population. The data are presented as mean (standard deviation), unless otherwise mentioned.

Parameter	Basic characteristics of the study participants
N (males/females)	35 (30/5)
Age (years)	60 (10)
Height (cm)	174.6 (8.8)
Weight (kg)	97.1 (16.7)
Body mass index (kg/m^2)	31.9 (4.9)
Dyspnea (n)	25
O2 saturation < 90 % (n)	33
Patients in intensive care unit (n)	2
Hospitalization time (days)	7 (4.9)

participants.

3.1. Cardiovascular measurements

Comparing macrocirculation parameters between two measurements after discharge from hospitalization (Table 2), two significant changes were noted. An increase in SBP ($\bar{X}_1 = 142$; $SD_1 = 15$; $\bar{X}_2 = 150$; $SD_2 = 19$; $F_{(1,32)} = 4.555$; $p = 0.041$) and a decrease in HR ($\bar{X}_1 = 76$; $SD_1 = 15$; $\bar{X}_2 = 69$; $SD_2 = 11$; $F_{(1,34)} = 14.268$; $p = 0.001$) were observed. Additionally, an increase in PWV ($\bar{X}_1 = 11$; $SD_1 = 3$; $\bar{X}_2 = 12$; $SD_2 = 3$; $F_{(1,32)} = 2.966$; $p = 0.095$) was observed.

*$\bar{X}_1$ (the mean value of the baseline measurement); $\bar{X}_2$ (the mean value of the second measurement); SD_1 (the standard deviation of the baseline measurement); SD_2 (the standard deviation of the second measurement).

3.2. Retinal microcirculation

Comparing microcirculation parameters between two measurements after discharge from hospitalization (Table 2), the present study observed a significant decrease in CRVE ($\bar{X}_1 = 204.94$; $SD_1 = 16.05$; $\bar{X}_2 = 198.05$; $SD_2 = 17.36$; $F_{(1,17)} = 7.681$; $p = 0.013$). Additionally, a reduction in CRAE ($\bar{X}1 = 138.87$; $SD1 = 12.19$; $\bar{X}2 = 136.77$; $SD2 = 13.19$; $F_{(1,17)} = 3.810$; $p = 0.068$) was observed.

*$\bar{X}_1$ (the mean value of the baseline measurements); $\bar{X}_2$ (the mean value of the second measurement); SD_1 (the standard deviation of the baseline measurement); SD_2 (the standard deviation of the second measurements).

Table 2
Comparison of the macro- and microcirculation parameters between two measuring time points after hospital discharge. Baseline measurements were conducted on the day of hospital discharge (if a PCR test on the day of hospital discharge was negative) or on day 10 (if a PCR test on the day of hospital discharge was positive) and second measurements were carried out 2 months after hospitalization. The *P*-values in bold highlight significant results. The data is presented as mean (standard deviation). SPB (systolic blood pressure), DPB (diastolic blood pressure), L (length), HR (heart rate), PWV (pulse wave velocity), PPI (pulse pressure index), CRAE (central retinal artery equivalent), CRVE (central retinal vein equivalent), AVR (artery to vein ratio).

Parameter	Baseline measurements	Two months after hospitalization	p-Value
Macrocirculation			
SBP (mmHg)	142 (15)	150 (19)	**p = 0.041**
DBP (mmHg)	76 (13)	79 (12)	*p* = 0.105
L (cm)	90 (5)	90 (4)	*p* = 0.747
HR (bpm)	76 (15)	69 (11)	**p = 0.001**
PWV (m/s)	11 (3)	12 (3)	p = 0.095
PPI	1.1 (0.3)	1.1 (0.4)	*p* = 0.880
Microcirculation			
CRAE (μm)	138.87 (12.19)	136.77 (13.19)	p = 0.068
CRVE (μm)	204.94 (16.05)	198.05 (17.36)	**p = 0.013**
AVR	0.679 (0.055)	0.693 (0.069)	*p* = 0.188

4. Discussion

The present study investigating the effect of COVID-19 infection on vascular health compared macro and microvascular parameters of the study participants at two-time points after hospital discharge. Baseline measurements were taken either on day 0 (if a PCR test on the day of hospital discharge was negative) or on day 10 (if a PCR test on the day of hospital discharge was positive), and the second measurement occurred 2 months after hospitalization. A significant increase in SBP, a lower HR, and a narrower CRVE were found (Table 2). Furthermore, there were recorded trends of increasing PWV and decreasing CRAE (Table 2).

Some studies did not find significant changes in BP between COVID-19 patients and non-infected individuals (Zanoli et al., 2022; Heckel et al., 2022; Lambadiari et al., 2021). On the other hand, the preliminary results of the LOCHINVAR study showed that COVID-19 patients have an 8.6 mmHg increase in their average 24 h SBP, compared to healthy controls (Lip et al., 2022). However, compared to the present study group, they showed elevation of SBP in actual COVID-19 patients, while the present study found an elevated SBP post-infection.

In another study, significantly higher SBP and diastolic blood pressure (DBP) in non-COVID individuals compared to COVID-19 patients were observed (Schnaubelt et al., 2021). Because the first measurements of the present study were done immediately after hospital discharge, when the effect of COVID-19 on the body is still distinct, these measurements could be considered as COVID-19 patients and the second measurements as healthy individuals. It would suggest, that the findings in the present study are in partial agreement with their findings.

There are two studies that observed long-term effect on BP post COVID-19 disease (Akpek, 2022; Szeghy et al., 2022). One includes 154 COVID-19 patients and confirmed significantly higher SBP as well as DBP post COVID-19 (31.6 ± 5.0 days after baseline) than on admission (baseline) (Akpek, 2022). Even though the duration of their study was shorter than the present study, the results of both studies are in partial agreement.

A longitudinal study, observing post-COVID-19 effect on BP showed significant reduction in SPB between 1 month and 6 months after infection (Szeghy et al., 2022). Although this study was closest to the present study in terms of duration, the present study observed the opposite effect on SBP two months after hospitalization. One hypothesis is that COVID-19 caused alterations in sympathetic activity or endothelial function leading to disturbances in blood pressure regulations (Szeghy et al., 2022). High blood pressure increases the chance to a longer and more difficult COVID-19 course with increasing hospitalization rate and deaths (Guan et al., 2020; Roncon et al., 2020).

COVID-19 may cause irregularities in HR. Ongoing inflammation and fever in the body affect the heart, causing it to pump more blood to overcome the infection. The effect can be reinforced by dehydration, anxiety, or medications, common conditions during ongoing disease. The present study showed a significant reduction in HR after COVID-19 infection (Table 2). Other studies did not disclose any significant change in HR between COVID-19 patients and the control group (Zanoli et al., 2022; Heckel et al., 2022; Lambadiari et al., 2021). Maloberti et al. found higher HR in COVID-19 patients at admission (90 ± 18 bpm) than at discharge (mean decrease of 10 bpm) (Maloberti et al., 2021). In another study where COVID-19 patients and control group were compared, the tendency in higher HR in COVID-19 patients was observed (Schnaubelt et al., 2021). This result is partially in agreement with the present study in which a reduction in HR after two months of recovery was observed.

Natarajan and colleagues presented 3-phases course of HR during

COVID-19 (1. the elevation during onset of symptoms (maximal peak), 2. decreasing to minimum around 13 days after symptoms onset, 3. increase around 28 days after symptoms onset) (Natarajan et al., 2022). They noted that HR returned to baseline levels around 112 days after symptoms onset. Because the present study did not measure HR at the onset of symptoms but only after hospital discharge, it is possible that the baseline measurements of the present study have been around the 28th day after symptoms onset. The second measurements of the present study would, in that case, reflect the results of Natarajan and colleagues when they showed a permanent course of decreasing HR from the 28th day after symptoms onset.

It is unclear why people experience changes to their HR after COVID-19. It can be due to the immune response during the COVID-19 infection, affecting the autonomic nervous system. However, other factors should be considered as COVID-19 disease affects the everyday lifestyle of sick people, lockdowns, prolonged inactivity, and spending weeks in bedrest for recovery can have a long-term impact on the cardiovascular system.

Vessel stiffening and worse PWV in COVID-19 patients has been observed (Ratchford et al., 2021; Jud et al., 2021; Lambadiari et al., 2021; Schnaubelt et al., 2021). The level of deterioration depends on the severity of the disease (Kumar et al., 2021). The present study recorded an increasing trend in PWV two months after hospitalization compared to the baseline measurements.

The study examining vascular alterations among young adults with and without SARS-CoV-19 revealed increased PWV in COVID-19 individuals (Ratchford et al., 2021). The worsening in PWV was confirmed even 4 months after overcoming COVID-19 (Lambadiari et al., 2021), which could partially agree with present results. Additionally, Schnaubelt et al. showed that COVID-19 is related not only to an enhanced PWV, but it correlated with the length of hospital stay (Schnaubelt et al., 2021). On the other hand, a study observing 24-hour hemodynamic load did not find any changes in PWV between adults with and without a history of COVID-19 (Heckel et al., 2022). Furthermore, opposite to the findings in present study, a case report of a 24-year-old woman showed improvement in PWV 6 weeks after overcoming Covid-19 (Jud et al., 2021). This is supported by the longitudinal study of Zanoli et al. showed a trend toward improvement in PWV in follow-up measurements (Zanoli et al., 2022). However, this improvement was still not at the level of healthy controls. These results were partially confirmed by another study that observed a significant reduction in PWV between 1 and 6 months post COVID-19 (Szeghy et al., 2022).

Current research confirms the deterioration of PWV reflecting arterial stiffening in COVID-19 patients as well as its improvement after recovery. The present study observed the opposite effect, thereby deterioration of PWV in the course of recovery. The results from the present study suggest that adaptive responses of the vascular system are ongoing even two months after hospitalization from the COVID-19 infection.

The first study investigating the effect of COVID-19 on retinal microcirculation, the SERPICO-19 study found that CRAE and CRVE values are higher in COVID-19 patients compared to unexposed subjects (Invernizzi et al., 2020). The present study partially confirmed these findings with a widening in retinal venules.

One study investigated 25 COVID-19 patients and 25 healthy controls and compared characteristics of retinal arteries and veins at baseline and at 4 months after remission. They confirmed findings in the SERPICO-19 study when they observed an increase in the diameters of retinal arteries and veins in COVID-19 patients during the disease (Aşıkgarip et al., 2021).

Similarly, the prospective case-control study from Turkey revealed dilatation not only in venules as observed in the present study but also in arteries as the effect of COVID-19 (Aydemir et al., 2021). Another study confirmed previous ones when they observed larger diameters of retinal arteries and venules in COVID-19 patients compare to unexposed subjects. Six months later, a favourable decrease in diameters in COVID-19 patients was shown (Invernizzi et al., 2021). However, the diameters of the vessels were still significantly narrower in severe COVID-19 patients compared to unexposed subjects (Invernizzi et al., 2021).

A study performed in Spain did not find a correlation between retinal vessel diameters and clinical outcome (Carreno et al., 2022). This study used a different setup with no control group or follow-up measurements, and the clinical assessment scale was different.

In addition to retinal fundus imaging, a recent review examines the microvascular changes in COVID-19 using nailfold capillaroscopy (Mondini et al., 2023). While both retinal imaging and nailfold video capillaroscopy provide valuable insights into microcirculation, they differ in terms of the anatomical location and specific vascular beds being examined. Retinal imaging focuses on the blood vessels within the retina, which receive their blood supply from the ophthalmic artery, the first branch of the internal carotid artery. As a result, retinal imaging is more likely to reflect changes in larger vessels of the cardiovascular system. Additionally, retinal imaging allows for the separate analysis of arterioles and venules. On the other hand, nailfold video capillaroscopy primarily assesses the capillaries in the nailfold region and is commonly used in diagnosing connective tissue diseases, particularly to identify the scleroderma-like pattern (Smith et al., 2020; Ruaro et al., 2019).

Hormonal response during inflammatory reactions leads to release of vasodilatation mediators such as histamine, bradykinin, and prostaglandins. A cytokine storm, which is a very typical consequence of COVID-19 accompanies these mediators and multiplies the pro-inflammatory effect (Chung et al., 2021; Lowenstein and Solomon, 2020; Wadowski et al., 2023; Salton et al., 2022). The vasodilatation and the increase in flow and pressure increase intravascular hydrostatic pressure and therefore extravasation through postcapillary venules into tissue. Furthermore, the virus causes disruption of the delicate balance between platelets and the vessel wall predominantly via the interplay between the factors such as activation of von Willebrand factor, coagulation cascade, and extensive neutrophil extracellular traps (NETs) formation, with histones as their components. These actions promote inflammation and play significant roles in the development of microthrombi, thrombosis, and tissue ischemia (Lowenstein and Solomon, 2020; Wadowski et al., 2023). In this study, significantly narrower retinal venules were observed two months after hospitalization from COVID-19 infection. This suggests ongoing vascular adaptations even two months after hospitalization from COVID-19 infection.

5. Limitations

Due to the limited number of enrolled subjects, the present pilot study did not include stratification based on the severity of infection, such as ICU hospitalization, thereby preventing the incorporation of clinical severity parameters into the model. Assessments before and during the infection were not performed and this situation hampers the correlation of the study data with the infection status. The inclusion of the participants in the study was confirmed by negative COVID-19 tests (in case PCR test on day 0 was positive, baseline measurements have been collected on day 10), thereby, the present study investigated the process of recovery and not the infection itself. The present study investigated cardiovascular physiology parameters after hospital discharge; measures prior to and during infection would have made the interpretation stronger. Considering the complexity experienced with COVID-19 patient interactions and difficulties with setting up studies during the pandemic period, such intense research protocols were almost impossible to set up. The study has been performed with reliable and well-accepted methods for assessing macrocirculation and microcirculation. Therefore, we are of the opinion that the study brings useful information about the possible impact of COVID-19 on the cardiovascular system. The present study does not have matched healthy control group to compare with. However, the confirmed previous COVID-19 infection and inclusion to the study as soon as possible after hospital discharge strongly suggest that the results of the baseline measurements are caused by the effect of COVID-19 infection, as well as that results from the second measurements reflect an ongoing recovery from the infection. Even though only one grader was engaged in the evaluation of retinal images thereby the inter-rater variability factor could affect the results, we do not believe this is the case since the grader was specifically trained by the staff of MONA Reva. Due to the limited ability to perform repeated measurements on the same study participants caused by the ongoing pandemic COVID-19, only one image per session per participant was taken and thereby intra-rater variability factor could have an impact on reached results.

6. Conclusions and future directions

The evaluation of changes in cardiovascular health parameters using the Vicorder and retinal imaging technique was performed. Measurements were carried out at two time points: 1.) the baseline measurements were taken on day 0 (hospital discharge) if a negative PCR test was obtained; otherwise, they were conducted on the 10th day after hospitalization; 2.) the second measurement was done 60 days after hospitalization. Significant increase in SBP, decrease in HR, and CRVE and the trends of increasing PWV and decreasing CRAE were found. The present findings have important clinical implications, suggesting that vascular adaptations may be ongoing even weeks after the COVID-19 infection, which can contribute to improved patient management and follow-up care, as well as the development of targeted interventions to support cardiovascular health in post-COVID-19 patients. Future could involve conducting additional interim assessments during the active infection and post-infection periods, and emphasize the adequately balanced study group selection.

Funding disclosure

K.T., B.Š., M.P., U.M., S.P., L.S., R.P. acknowledge financial support by the Slovenian Research Agency (research programme funding no. P5-0381) M.P. and U.M. also acknowledge financial support from the European Union's Horizon 2020 Research and Innovation Program under grant agreement No 952401 (TwinBrain – TWINning the BRAIN with machine learning for neuromuscular efficiency).

CRediT authorship contribution statement

Conceptualization, B.Š., U.M., and R.P.; methodology, K.T., M.P., S.P., and N.G.; contributed to data collection, K.T., M.P. and S.P.; validation, P.D.B., B.Š., and N.G.; assessment and data processing, P.D.B., N.G., B.Š., P.M.F., B.N·N, M.G., S.P., K.T., M.P., L.S., U.M., K.S.-Z. and B.S.; formal analysis, A.S., B.Š., and R.N.; data curation, R.N., B.Š., and A.S.; writing - original draft preparation, A.S.; writing - review and editing, P.D.B., B.S., B.Š., P.M.F., B.N.N., M.G., S.P., K.T., M.P., L.S., U.M., K.S.-Z., O.Š., H.S., and N.G. All authors have read and agreed to the published version of the manuscript.

Declaration of competing interest

None.

Data availability

Data will be made available on request.

References

Akpek, M., 2022. Does COVID-19 cause hypertension? Angiology [Internet] 73 (7), 682–687. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9260192/. Aug [cited 2022 Dec 5].

Aşıkgarip, N., Temel, E., Hızmalı, L., Örnek, K., Sezgin, F.M., 2021. Retinal vessel diameter changes in COVID-19 infected patients. Ocul. Immunol. Inflamm. 29 (4), 645–651. May 19.

Aydemir, E., Bayat, A.H., Ören, B., Atesoglu, H.I., Şakir Göker, Y., Özçelik, K.Ç., 2021. Retinal vascular findings in patients with COVID-19. Ther. Adv. Ophthalmol. [Internet] 13, 25158414211030420. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8280837/. Jul 13 [cited 2022 Nov 9].

Carreno, E., Estébanez, N., Liew, G., Nguyen, H., Rodriguez-Alonso, B., Avila, P.D., et al., 2022. Retinal findings and vessel caliber measurements in admitted patients with COVID-19. Invest. Ophthalmol. Vis. Sci. 63 (7), 1731–F0191. Jun 1.

Chung, M.K., Zidar, D.A., Bristow, M.R., Cameron, S.J., Chan, T., Harding, C.V., et al., 2021. COVID-19 and cardiovascular disease. Circ. Res. [Internet] 128 (8), 1214–1236. Available from: https://www.ahajournals.org/doi/10.1161/CIRCRESAHA.121.317997. Apr 16 [cited 2022 Nov 9].

Granger, D.N., Rodrigues, S.F., 2016. Microvascular responses to inflammation. In: Parnham, M.J. (Ed.), Compendium of Inflammatory Diseases [Internet]. Springer, Basel, pp. 942–948. Available from: https://doi.org/10.1007/978-3-7643-8550-7_178 [cited 2022 Nov 9].

Guan, W.J., Liang, W.H., Zhao, Y., Liang, H.R., Chen, Z.S., Li, Y.M., et al., 2020. Comorbidity and its impact on 1590 patients with COVID-19 in China: a nationwide analysis. Eur. Respir. J. 55 (5), 2000547. May.

Hanssen, H., Streese, L., Vilser, W., 2022. Retinal vessel diameters and function in cardiovascular risk and disease. Prog. Retin. Eye Res. [Internet] 91, 101095. Available from: https://www.sciencedirect.com/science/article/pii/S1350946222000556. Nov 1 [cited 2023 Mar 29].

Heckel, A.R., Arcidiacono, D.M., Coonan, K.A., Glasgow, A.C., DeBlois, J.P., Gump, B.B., et al., 2022. 24-Hour central hemodynamic load in adults with and without a history of COVID-19. Am. J. Hypertens. [Internet] 35 (11), 948–954 hpac100. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9452129/. Aug 25 [cited 2022 Dec 5].

Invernizzi, A., Torre, A., Parrulli, S., Zicarelli, F., Schiuma, M., Colombo, V., et al., 2020. Retinal findings in patients with COVID-19: Results from the SERPICO-19 study. EClinicalMedicine [Internet] 27, 100550. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7502280/. Sep 20 [cited 2022 Nov 9].

Invernizzi, A., Schiuma, M., Parrulli, S., Torre, A., Zicarelli, F., Colombo, V., et al., 2021. Retinal vessels modifications in acute and post-COVID-19. Sci. Rep. [Internet] 11, 19373. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8481283/. Sep 29 [cited 2022 Nov 9].

Jud, P., Kessler, H.H., Brodmann, M., 2021. Case report: changes of vascular reactivity and arterial stiffness in a patient with Covid-19 infection. Front. Cardiovasc. Med. [Internet] 8, 671669. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8149731/. May 12 [cited 2022 Dec 5].

Khan, A., Boever, P.D., Gerrits, N., Akhtar, N., Saqqur, M., Ponirakis, G., et al., 2022. Retinal vessel multifractals predict pial collateral status in patients with acute ischemic stroke. PLoS ONE [Internet] 17 (5), e0267837. Available from: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0267837, 5 [cited 2022 Dec 21].

Knudtson, M.D., Lee, K.E., Hubbard, L.D., Wong, T.Y., Klein, R., Klein, B.E.K., 2003. Revised formulas for summarizing retinal vessel diameters. Curr. Eye Res. [Internet] 27 (3), 143–149. Available from: https://doi.org/10.1076/ceyr.27.3.143.16049. Jan 1 [cited 2022 Jan 20].

Kumar, N., Kumar, S., Kumar, A., Bhushan, D., Kumar, A., Kumar, A., et al., 2021. The COSEVAST study outcome: evidence of COVID-19 severity proportionate to surge in arterial stiffness. Indian J. Crit. Care Med. [Internet] 25 (10), 1113–1119. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8645816/. Oct [cited 2022 Dec 5].

Lambadiari, V., Mitrakou, A., Kountouri, A., Thymis, J., Katogiannis, K., Korakas, E., et al., 2021. Association of COVID-19 with impaired endothelial glycocalyx, vascular function and myocardial deformation 4 months after infection. Eur. J. Heart Fail. [Internet] 23 (11), 1916–1926. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8426810/. Nov [cited 2022 Dec 5].

Laurent, S., Cockcroft, J., Van Bortel, L., Boutouyrie, P., Giannattasio, C., Hayoz, D., et al., 2006. Expert consensus document on arterial stiffness: methodological issues and clinical applications. Eur. Heart J. 27 (21), 2588–2605. Nov.

Lip, S., Mccallum, L., Delles, C., McClure, J.D., Guzik, T., Berry, C., et al., 2022. Rationale and design for the LOnger-term effects of SARS-CoV-2 INfection on blood Vessels And blood pRessure (LOCHINVAR): an observational phenotyping study. Open Heart [Internet] 9 (1), e002057. Available from: https://openheart.bmj.com/content/9/1/e002057. Jun 1 [cited 2022 Dec 5].

Lowenstein, C.J., Solomon, S.D., 2020. Severe COVID-19 is a microvascular disease. Circulation [Internet] 142 (17), 1609–1611. Available from: https://www.ahajournals.org/doi/10.1161/CIRCULATIONAHA.120.050354. Oct 27 [cited 2022 Nov 9].

Maloberti, A., Ughi, N., Bernasconi, D.P., Rebora, P., Cartella, I., Grasso, E., et al., 2021. Heart rate in patients with SARS-CoV-2 infection: prevalence of high values at discharge and relationship with disease severity. J. Clin. Med. [Internet] 10 (23), 5590. Available from: https://www.mdpi.com/2077-0383/10/23/5590. Jan [cited 2022 Dec 5].

Mondini, L., Confalonieri, P., Pozzan, R., Ruggero, L., Trotta, L., Lerda, S., et al., 2023. Microvascular alteration in COVID-19 documented by nailfold capillaroscopy. Diagnostics 13 (11), 1905. Jan.

Natarajan, A., Su, H.W., Heneghan, C., 2022. Occurrence of relative bradycardia and relative tachycardia in individuals diagnosed with COVID-19. Front. Physiol. [Internet] 13. Available from: https://www.frontiersin.org/articles/10.3389/fphys.2022.898251 [cited 2022 Dec 5].

Pišot, R., Kleva, M., Teraž, K., Paravlić, A., Marušič, U., Pišot, S., et al., 2020. Stay active. Ann. Kinesiol. [Internet] 11 (2), 145–148. Available from: http://ojs.zrs-kp.si/index.php/AK/article/view/272 [cited 2023 Jan 10].

Ratchford, S.M., Stickford, J.L., Province, V.M., Stute, N., Augenreich, M.A., Koontz, L.K., et al., 2021. Vascular alterations among young adults with SARS-CoV-2. Am. J. Physiol. Heart Circ. Physiol. 320 (1), H404–H410. Jan 1.

Roncon, L., Zuin, M., Zuliani, G., Rigatelli, G., 2020. Patients with arterial hypertension and COVID-19 are at higher risk of ICU admission. Br. J. Anaesth. [Internet] 125 (2), e254–e255. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7183951/. Aug [cited 2022 Dec 5].

Ruaro, B., Smith, V., Sulli, A., Pizzorni, C., Tardito, S., Patané, M., et al., 2019. Innovations in the assessment of primary and secondary Raynaud's phenomenon. Front. Pharmacol. [Internet] 10. Available from: https://www.frontiersin.org/articles/10.3389/fphar.2019.00360 [cited 2023 Jul 10].

Ruzzenenti, G., Maloberti, A., Giani, V., Biolcati, M., Leidi, F., Monticelli, M., et al., 2021. Covid and cardiovascular diseases: direct and indirect damages and future perspective. High Blood Press. Cardiovasc. Prev. 28 (5), 439–445. Sep 1.

Salton, F., Confalonieri, P., Campisciano, G., Cifaldi, R., Rizzardi, C., Generali, D., et al., 2022. Cytokine profiles as potential prognostic and therapeutic markers in SARS-CoV-2-induced ARDS. J. Clin. Med. 11 (11), 2951. Jan.

Schnaubelt, S., Oppenauer, J., Tihanyi, D., Mueller, M., Maldonado-Gonzalez, E., Zejnilovic, S., et al., 2021. Arterial stiffness in acute COVID-19 and potential associations with clinical outcome. J. Intern. Med. [Internet] 290 (2), 437–443. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8013324/. Aug [cited 2022 Dec 5].

Shankar, P., Singh, J., Joshi, A., Malhotra, A.G., Shrivas, A., Goel, G., et al., 2022. Organ involvement in COVID-19: a molecular investigation of autopsied patients. Microorganisms [Internet] 10 (7), 1333. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9318581/. Jul 1 [cited 2023 Mar 27].

Smith, V., Herrick, A.L., Ingegnoli, F., Damjanov, N., De Angelis, R., Denton, C.P., et al., 2020. Standardisation of nailfold capillaroscopy for the assessment of patients with Raynaud's phenomenon and systemic sclerosis. Autoimmun. Rev. 19 (3), 102458. Mar 1.

Stoner, L., Young, J.M., Fryer, S., 2012. Assessments of arterial stiffness and endothelial function using pulse wave analysis. Int. J. Vasc. Med. [Internet] 2012, 903107. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3361177/ [cited 2022 Dec 5].

Szeghy, R.E., Stute, N.L., Province, V.M., Augenreich, M.A., Stickford, J.L., Stickford, A.S. L., et al., 2022. Six-month longitudinal tracking of arterial stiffness and blood pressure in young adults following SARS-CoV-2 infection. J. Appl. Physiol. [Internet] 132 (5), 1297–1309. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9126215/. May 1 [cited 2022 Dec 5].

Tehrani, S., Gille-Johnson, P., 2021. Microvascular dysfunction in patients with critical Covid-19, a pilot study. Shock 56 (6), 964–968. Dec 1.

van Hout, M.J., Dekkers, I.A., Westenberg, J.J., Schalij, M.J., Widya, R.L., de Mutsert, R., et al., 2021. Normal and reference values for cardiovascular magnetic resonance-based pulse wave velocity in the middle-aged general population. J. Cardiovasc. Magn. Reson. [Internet] 23 (1), 46. Available from: https://doi.org/10.1186/s12968-021-00739-y. Apr 19 [cited 2022 Dec 5].

Varga, Z., Flammer, A.J., Steiger, P., Haberecker, M., Andermatt, R., Zinkernagel, A.S., et al., 2020. Endothelial cell infection and endotheliitis in COVID-19. Lancet 395 (10234), 1417–1418. May 2.

Vlachopoulos, C., Aznaouridis, K., Stefanadis, C., 2010. Prediction of cardiovascular events and all-cause mortality with arterial stiffness: a systematic review and meta-analysis. J. Am. Coll. Cardiol. [Internet] 55 (13), 1318–1327. Available from: https://www.sciencedirect.com/science/article/pii/S0735109710002809. Mar 30 [cited 2022 Dec 5].

Vymazalová, K., Šerý, O., Králík, P., Dziedzinská, R., Musilová, Z., Frišhons, J., et al., 2023. Substantial decrease in SARS-CoV-2 RNA after fixation of cadavers intended for anatomical dissection. Anat. Sci. Int. 4, 1–7. Mar.

Wadowski, P.P., Panzer, B., Józkowicz, A., Kopp, C.W., Gremmel, T., Panzer, S., et al., 2023. Microvascular thrombosis as a critical factor in severe COVID-19. Int. J. Mol. Sci. 24 (3), 2492. Jan.

Wang, Z., Yang, Y., Yuan, L. jun, Liu, J., Duan, Y. you, Cao, T. sheng, 2015. Noninvasive method for measuring local pulse wave velocity by dual pulse wave Doppler: in vitro and in vivo studies. PLoS One [Internet] 10 (3), e0120482. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4364771/. Mar 18 [cited 2022 Dec 5].

Zanoli, L., Gaudio, A., Mikhailidis, D.P., Katsiki, N., Castellino, N., Lo Cicero, L., et al., 2022. Vascular dysfunction of COVID-19 is partially reverted in the long-term. Circ. Res. [Internet] 130 (9), 1276–1285. Available from: https://www.ahajournals.org/doi/full/10.1161/CIRCRESAHA.121.320460. Apr 29 [cited 2022 Dec 5].