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Received: 9 October 2021 | Revised: 15 March 2022 | Accepted: 13 April 2022

DOI: 10.1111/micc.12757

Microcirculation WILEY

ORIGINAL ARTICLE

Coronary microvascular dysfunction is common in patients hospitalized with COVID-19 infection

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Funding information
This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Abstract

Background and Aims: Microvascular disease is considered as one of the main drivers of morbidity and mortality in severe COVID-19, and microvascular dysfunction has been demonstrated in the subcutaneous and sublingual tissues in COVID-19 patients. The presence of coronary microvascular dysfunction (CMD) has also been hypothesized, but direct evidence demonstrating CMD in COVID-19 patients is missing. In the present study, we aimed to investigate CMD in patients hospitalized with COVID-19, and to understand whether there is a relationship between biomarkers of myocardial injury, myocardial strain and inflammation and CMD.

Methods: 39 patients that were hospitalized with COVID-19 and 40 control subjects were included to the present study. Biomarkers for myocardial injury, myocardial strain, inflammation, and fibrin turnover were obtained at admission. A comprehensive echocardiographic examination, including measurement of coronary flow velocity reserve (CFVR), was done after the patient was stabilized.

Results: Patients with COVID-19 infection had a significantly lower hyperemic coronary flow velocity, resulting in a significantly lower CFVR (2.0 ± 0.3 vs. 2.4 ± 0.5, $p < .001$). Patients with severe COVID-19 had a lower CFVR compared to those with moderate COVID-19 (1.8 ± 0.2 vs. 2.2 ± 0.2, $p < .001$) driven by a trend toward higher basal flow velocity. CFVR correlated with troponin ($p = .003$, r: −.470), B-type natriuretic peptide ($p < .001$, r: −.580), C-reactive protein ($p < .001$, r: −.369), interleukin-6 ($p < .001$, r: −.597), and d-dimer ($p < .001$, r: −.561), with the three latter biomarkers having the highest areas-under-curve for predicting CMD.

Conclusions: Coronary microvascular dysfunction is common in patients with COVID-19 and is related to the severity of the infection. CMD may also explain the "cryptic" myocardial injury seen in patients with severe COVID-19 infection.

KEYWORDS
coronary microvascular dysfunction, COVID-19, echocardiography, myocardial injury, SARS-COV-2

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1 | INTRODUCTION

SARS-COV-2 is a novel betacoronavirus that have infected over 177 million individuals and claimed 3.9 million lives globally.[1,2] Cardiac involvement in patients with moderate-to-severe COVID-19 infection ranges from asymptomatic myocardial damage to overt myocarditis and myocardial infarction secondary to epicardial coronary artery disease (CAD).[3–7] Myocardial damage is somewhat common in patients hospitalized for COVID-19. While some cases can be explained with histologically proven myocarditis or epicardial CAD, in most instances the origin of this damage is uncertain.[8] Coronary microvascular dysfunction has been suggested as a possible cause of myocardial injury in COVID-19 patients, as studies have suggested presence of microvascular dysfunction in other vascular beds and there is histologic evidence for SARS-COV-2-associated endotheliitis in specimens obtained from heart, lung, kidney, liver, and other tissues.[9,10] However, this is an indirect assumption as there are no data so far to suggest coronary microvascular dysfunction (CMD) in COVID-19 patients.

Cardiac microvascular dysfunction could be investigated with several invasive or non-invasive methods.[11–14] Coronary flow velocity reserve (CFVR), which can be obtained by comparing velocities obtained before and after administration of a vasodilator agent, is the primary method of assessing CMD with echocardiography. Importantly, echocardiography allows making bedside measurements, which is usually the optimal method for assessing CMD in most patients.

In the present study, we aimed to understand whether patients hospitalized with COVID-19 had echocardiographically demonstrable CMD as compared to healthy individuals, and whether CMD is related to other pathophysiological processes such as myocardial injury, fibrin turnover, or inflammation.

2 | MATERIALS AND METHODS

Present study is a cross-sectional case–control study performed in a single academic center. Patients aged between 20 and 60 years that were diagnosed with COVID-19 infection and hospitalized with this diagnosis were included. Patients that were past or current smokers, those with known coronary artery disease or diabetes, those with a history of heart failure due to any cause and patients on any kind of vasoactive drugs that might affect CFVR measurements were excluded. In addition, patients with a suboptimal image quality on echocardiography or patients with a condition that contraindicates administration of dipyridamole (such as asthma) were excluded. 50 COVID-19 patients were initially screened but 11 patients were excluded after applying these exclusion criteria. 40 age- and gender-matched subjects without a previous history of COVID-19, no active symptoms and had a negative nasopharyngeal swab for COVID-19 were enrolled as controls. Demographic, clinical and laboratory parameters were recorded with direct interviews and with using institutional electronic medical database.

The study was conducted according to the 1975 Helsinki and its subsequent revisions. All patients gave their informed consent, and the study was approved by a local ethics committee.

2.1 | Echocardiographic examination

All echocardiographic examinations were performed with an ultrasound platform equipped with a matrix-array transducer (X5-1, Philips Epiq 7, Philips Healthcare). Chamber quantification and other measurements were done according to the relevant international guidelines. For coronary flow measurements, distal part of the left anterior descending artery (LAD) was visualized using high ultrasound beam frequency (5–7 MHz). The color Doppler gain was optimized using conventional techniques, and the Nyquist limit was set to 0.16–0.50 m/s. After visualization of the distal part of the LAD, pulse-wave Doppler cursor was placed to measure coronary flow velocity and measurements were done before and after dipyridamole infusion (0.84 mg/kg for 6 min). Patients were monitored during the procedure, and heart rate and blood pressure data were recorded at baseline, during infusion, and after the procedure. Coronary flow velocity reserve was calculated as the ratio of the hyperemic peak flow velocity to the resting peak flow velocity. Patients with a CFVR <2.0 were accepted as having CMD.[13,15] We have previously reported interobserver and intraobserver variability values for our laboratory.[16,17]

All echocardiographic examinations, including CFVR measurements, were performed immediately after the stabilization of the patient. For patients that needed intubation or intensive unit care due to any cause, echocardiographic examinations were delayed until the patient was transferred to the ward. Theophylline and similar drugs, as well as caffeine-containing beverages, were discontinued for 24 h before the procedure.

2.2 | Definition of moderate and severe COVID-19 infection

All patients included in the present study had: i) A positive nasopharyngeal swab for COVID-19, ii) thoracic CT findings compatible with COVID-19 pneumonia, and iii) hospitalized due to COVID-19 infection. Patients fulfilling one or more of the following criteria were accepted as severe COVID-19 infection: i) A respiratory rate >30 breaths/minute signifying respiratory distress, ii) a resting oxygen saturation 93% or less, iii) ratio of partial arterial oxygen saturation to the fraction of inspired oxygen <300 mmHg, and iv) respiratory failure or a critical life-threatening complication of COVID-19 necessitating admission to intensive care unit. Patients who did not fulfill these criteria were accepted as having a moderate COVID-19 infection. Subjects within the control group had a negative nasopharyngeal swab for COVID-19, with or without a negative CT scan for COVID-19 pneumonia.

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2.3 | Laboratory investigations

Nasopharyngeal swabs were obtained at admission, and COVID-19 infection was diagnosed with real-time reverse-transcription PCR using Coronex COVID-19 rt-qPCR detection kit (Gensutek Inc). For all other tests, blood samples were obtained immediately after the diagnosis of COVID-19 was ascertained with a positive PCR test and a thorax CT scan compatible with COVID-19 pneumonia. Interleukin-6 concentration was determined with electrochemiluminescence immunoassay method, using Elecsys IL-6 biochemical analysis kits and Roche Cobas 6000 analysis device (Roche Diagnostics). Other laboratory analyses were done with conventional methods.

2.4 | Statistical analyses

Data for continuous parameters were given as mean ± SD or median and interquartile range, depending on the distribution of the data. Categorical variables were presented as percentages. For continuous variables, patterns of distribution were analyzed with visual inspection of histograms and with Shapiro–Wilk test. Comparisons between groups were done with *t*-test for independent samples or with Mann–Whitney *U* test as appropriate. For analyses involving three groups, either one-way ANOVA with post hoc Tukey or Games–Howell test or Kruskal–Wallis test with post hoc Dwass–Steel–Critchlow–Fligner analysis. For categorical variables, either chi-square test or Fisher's exact test was used to compare groups. Correlation analyses were done with Pearson test or with Spearman's rho. Linear regression models were built to adjust for the effect of blood O_2 saturation (saO_2) on coronary flow velocities and CFVR in the overall study population and in the subgroup of patients with COVID-19. Nominal variables were encoded as dummy variables for these latter analyses. Finally, a receiver-operator curve was drawn to analyze the accuracy of various biomarkers to predict CMD. For all analyses, *p* value <.05 was accepted as statistically significant. All statistical analyses were done with SPSS 25.0 (IBM Inc.) and Jamovi (The jamovi project [2021]. jamovi version 1.6 for Microsoft Windows).

3 | RESULTS

Demographic, clinical and laboratory characteristics of the study groups were summarized in Table 1. COVID-19 (+) patients had a higher systolic and diastolic blood pressure and a lower oxygen saturation, as well as higher fibrinogen, ferritin, and d-dimer concentrations at baseline. To note, there were no significant differences between patients in terms of age, gender, obesity, or other evaluated risk factors for atherosclerosis. Echocardiographic characteristics and coronary flow measurements of the study groups were summarized in Table 2. Conventional echocardiographic measurements were not different between groups, except for a significantly higher left atrial diameter in the COVID-19 (+) group. Basal diastolic peak flow velocity (DPFV) was similar between groups, but hyperemic DPFV was significantly lower in patients with COVID-19, leading to a statistically significant difference for CFVR between groups. Both basal and hyperemic heart rates were higher in the COVID-19 (+) group, but both findings did not reach statistical significance.

3.1 | Patients with moderate and severe COVID-19 infection

Patients with severe COVID-19 infection were more likely to have a higher respiratory rate, lower oxygen saturation, and higher fibrinogen concentration as compared to patients with moderate COVID-19 infection and controls (Table 3), and both BNP and troponin concentrations were higher in patients with severe COVID-19 infection as compared to those with moderate disease (Table S1). Despite these differences, conventional echocardiographic parameters of left ventricular structure or systolic/diastolic functions were not different between groups (Table 4). Patients with severe COVID-19 had a significantly lower CFVR as compared to both moderate COVID-19 group and controls. As compared to the controls, patients with severe COVID-19 had a significantly lower hyperemic DPFV (Table 4). In contrast, post hoc comparisons between patients with moderate and severe COVID-19 did not show a statistically significant difference for either basal or hyperemic DPFV, although there was a trend toward higher basal DPFV in the latter subgroup ($p = .07$ in the pairwise comparison). While there was also a trend toward lower hyperemic DPFV in patients with moderate COVID-19 as compared to the control group, this finding did not reach statistical significance ($p = .15$ in the pairwise comparison).

3.2 | Relationships between CFVR and biomarkers

Coronary flow velocity reserve showed a significant negative correlation with proinflammatory biomarkers, as well as with B-type natriuretic peptide (BNP), d-dimer, and troponin. Of those, CFVR had a weak to moderate correlation with C-reactive protein ($p < .001$, r: −.369), troponin ($p = .003$, r: −.470), and white blood cell count ($p = .043$, r: −.326), while it had a moderate to well correlation with BNP ($p < .001$, r: −.580), interleukin-6 ($p < .001$, r: −.597), and d-dimer ($p < .001$, r: −.561). Figure 1 summarizes correlations between CFVR and various biomarkers.

Of all biomarkers tested, fibrin-turnover marker d-dimer (AUC: 0.87 [0.73–1.00], $p = .001$) had the highest accuracy for predicting CMD. For a cutoff value of 0.25, d-dimer had a sensitivity of 90% and specificity of 70% to predict CMD. Inflammatory biomarkers C-reactive protein (AUC: 0.81 [0.65–0.98], $p = .004$) and interleukin-6 (AUC: 0.80 [0.62–0.97], $p = .008$) also offered good predictive accuracies, with the former having a sensitivity of 95% and specificity of 60% for a cut-off value of 6.5 mg/dl and the latter had a sensitivity of 82% and specificity of 80% for a cut-off value of 13.9 pg/ml. Other biomarkers, including white blood cell count, troponin, and BNP, had

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TABLE 1 Demographic, clinical and laboratory characteristics of subjects with and without COVID-19 infection

Characteristic	COVID-19 (+) ($n = 39$)	COVID-19 (-) ($n = 40$)	p
Age, years	42.5 ± 7.8	41.1 ± 4.8	.337
Male, n (%)	24 (61)	22 (55)	.556
BMI, kg/m^2	28.1 ± 4.3	27.0 ± 3.2	.223
Hypertension, n (%)	3 (7)	4 (10)	.718
SAP, (mmHg)	125.7 ± 9.2	118.6 ± 10.1	.002
DAP, (mmHg)	82.4 ± 7.8	75.3 ± 5.8	<.001
RR (/min)	21.2 ± 3.8	17.5 ± 2.6	<.001
saO_2 (%)	90.1 ± 5.7	97.7 ± 1.9	<.001
Albumin (g/dl)	3.4 ± 0.4	3.7 ± 0.4	.005
AST (IU/L)	31 (24–52)	28 (21–32)	.043
ALT (IU/L)	27 (18–41)	22 (18–32)	.035
CRP (mg/dl)	19 (9–60)	3 (0–7)	<.001
Glucose (mg/dl)	124.5 ± 45.1	95.4 ± 23.4	.001
Creatinine (mg/dl)	0.8 ± 0.2	0.8 ± 0.2	.690
GFR (ml/min/1.73 m^2)	98.6 ± 18.9	100.5 ± 18.6	.671
Uric acid (mg/dl)	4.0 ± 1.4	4.1 ± 1.4	.857
WBC count (10^3/μl)	7.1 ± 3.2	6.7 ± 1.7	.552
Hemoglobin (g/dl)	13.3 ± 1.8	12.8 ± 1.2	.203
Platelet count (10^3/μl)	226.2 ± 69.4	217.6 ± 68.1	.580
TC (mg/dl)	156.1.0 ± 32.0	191.5 ± 31.4	<.001
LDL (mg/dl)	87.9 ± 24.1	118.8 ± 26.9	<.001
HDL (mg/dl)	39.1 ± 9.8	44.0 ± 8.0	.017
Triglycerides (mg/dl)	135.9 ± 57.3	136.4 ± 50.4	.967
Fibrinogen (mg/dl)	610.5 ± 156.2	277.3 ± 48.3	<.001
Ferritin (ng/ml)	486 (83–994)	83 (55–286)	<.001
D-dimer (μg/ml)	1.0 (0.7–1.5)	0.5 (0.2–0.6)	<.001

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CRP, C-reactive protein; DAP, diastolic arterial pressure; GFR, glomerular filtration rate; HDL, high-density lipoprotein; HT, hypertension; LDL, low-density lipoprotein; RR, respiratory rate; saO_2, blood oxygen saturation; SAP, systolic arterial pressure; TC, total cholesterol; WBC, white blood cell.

AUCs ranging between 0.70 and 0.72, thus offering lower accuracies to predict CMD (Figure 2).

3.3 | Relationships between blood oxygen saturation, coronary flow velocities, and CFVR

In the overall study population, saO_2 correlated with hyperemic DPFV ($r = .295$, $p = .01$) and CFVR ($r = .532$, $p < .001$) but not with basal DPFV (Figure 3). However, after adjustment for the presence of COVID-19, neither hyperemic DPFV nor CFVR had a statistically significant correlation with saO_2 ($p = .47$ and $p = .06$, respectively). In contrast, COVID-19 positivity remained as a statistically significant predictor of CFVR after adjusting for saO_2 ($p = .002$ and $p < .001$, respectively).

In the subgroup of patients with COVID-19, saO2 correlated with both basal DPFV ($r = -.398$, $p = .012$) and CFVR ($r = .69$, $p < .001$), but not with hyperemic DPFV (Figure 4). However, after adjusting for the severity of the COVID-19, both correlations lost their statistical significance ($p = .81$ for basal DPFV and $p = .94$ for CFVR). Similar to the previous analysis, the association between the severity of COVID-19 infection and basal DPFV/CFVR remained significant after adjusting for saO_2 ($p = .04$ and $p < .001$, respectively).

4 | DISCUSSION

It has been suggested that COVID-19 is a disorder of the microvasculature. Given that microvascular dysfunction is seen in the subcutaneous and retinal vasculature in COVID-19 patients, several investigators have speculated that the same should also be true for the coronary microvasculature, but direct evidence was missing so far.[18–21] Present study supports the validity of this latter hypothesis, since our findings indicate that CFVR, which is a measure of CMD, is lower in COVID-19 patients. Moreover, these findings also indicate a relationship between several biomarkers (including troponin) and

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TABLE 2 Echocardiographic characteristics of subjects with and without COVID-19 infection

Characteristic	COVID-19 (+) ($n = 39$)	COVID-19 (-) ($n = 40$)	p
LVEF (%)	61.5 ± 7.4	64.0 ± 2.5	.057
LVDD (mm)	46.0 ± 3.3	45.3 ± 3.2	.325
LVSD (mm)	28.1 ± 3.1	27.8 ± 2.8	.633
IVS (cm)	0.9 ± 0.1	0.9 ± 0.1	.235
PW (cm)	0.9 ± 0.1	0.9 ± 0.1	.851
AoD (cm)	3.0 ± 0.4	3.2 ± 0.3	.031
LA (cm)	3.5 ± 0.3	3.0 ± 0.4	<.001
Mitral E wave (m/s)	0.8 ± 0.1	0.8 ± 0.1	.303
Mitral A wave (m/s)	0.7 ± 0.1	0.6 ± 0.1	.230
Mitral IVRT (ms)	88.6 ± 16.5	86.0 ± 16.8	.485
Septal E wave (cm/s)	9.8 ± 1.8	10.5 ± 2.4	.183
Lateral E wave (cm/s)	14.4 ± 3.0	14.8 ± 2.7	.504
Tricuspid S wave (cm/s)	12.8 ± 2.3	13.2 ± 2.8	.55
TAPSE (cm)	2.3 ± 0.3	2.4 ± 0.3	.050
Basal DPFV (m/s)	28.4 ± 6.0	27.9 ± 5.7	.658
Hyperemic DPFV (m/s)	56.8 ± 12.1	66.9 ± 15.5	.002
CFVR	2.0 ± 0.3	2.4 ± 0.5	<.001
Basal HR (bpm)	75.3 ± 8.5	72.4 ± 7.9	.118
Hyperemic HR (bpm)	99.7 ± 10.0	95.6 ± 11.3	.089

Abbreviations: AoD, aortic diameter; CFVR, coronary flow velocity reserve; DPFV, diastolic peak flow velocity; HR, heart rate; IVRT, interventricular relaxation time; IVS, interventricular septum; LA, left atrium; LVDD, left ventricular diastolic diameter; LVEF, left ventricular ejection fraction; LVSD, left ventricular systolic diameter; PW, posterior wall; TAPSE, tricuspid annular plane systolic excursion.

the degree of reduction in CFVR, thus providing a possible explanation for the "cryptic" myocardial injury in severe COVID-19 patients.

Several explanations have been offered to explain microvascular dysfunction in SARS-COV-2 infection. SARS-COV-2 gains access to cells by binding to the ACE2 receptor, which is expressed ubiquitously is many tissues, including perivascular tissue.[22,23] There is histopathologic evidence to suggest SARS-COV-2-induced lymphocytic endothelial infection (endotheliitis) in a variety of tissues, including kidney, lung, and heart.[10,24,25] In an autopsy report, direct coronary microvascular involvement and endotheliitis was demonstrated in a SARS-COV-2-positive young patient died due to hemodynamic instability and ventricular fibrillation.[9] In another small autopsy study, Maccio and associates had shown that six patients died of COVID-19 had a prominent lymphocytic-monocytic endotheliitis in the epicardial capillaries without involvement of the main epicardial coronaries.[26] Given that endotheliitis and perivascular inflammation directly injures endothelial cells, these findings may explain why CMD is widespread in COVID-19 patients. Moreover, endothelial disruption can activate a prothrombotic cascade by liberating P-selectin and von Willebrand Factor that are stored in the endothelial cells, and there is evidence to suggest increased circulating P-selectin and vWF in patients with COVID-19 infection.[27] This mechanism is widely though as the cause of hypercoagulability that is typically seen in COVID-19.[18,27] It may also underlie CMD in COVID-19 given that microvascular thrombosis and obstruction reduce recruitable capillaries, which in turn leads to microvascular dysfunction.[27,28] Finally, overactivation of inflammatory pathways with accompanying "cytokine storm," which is somewhat common in patients with severe COVID-19, can exacerbate endothelial dysfunction by either worsening endothelial inflammation or by activating prothrombotic cascades.[18,29] Probably not a single pathway is responsible for the development of CMD and all pathophysiological pathways are interwoven into each other, ultimately leading to endothelial dysfunction and CMD. Present findings suggest a close association between CFVR and biomarkers of fibrin turnover and inflammation, supporting a role for the aforementioned pathophysiological mechanisms for the initiation and propagation of CMD in COVID-19. Theoretically, treatments aimed to disrupt the pathways of microvascular dysfunction, such as anticoagulants or anti-inflammatory agents, should improve microvascular dysfunction in those with moderate or severe COVID-19 infection, but this assumption needs further studies.

Lower blood oxygen saturation increases basal coronary flow and thus reduces coronary flow reserve in response to pharmacologic agents, although this effect is less pronounced unless the oxygen saturation drops below 60%.[30] Given that patients with COVID-19 had lower saO2 as compared to controls, lower oxygen saturation might have contributed to the lower CFVR in these patients, especially in those with severe disease in whom basal coronary flow was higher than the controls. That said, the effect of saO2 on the present results should be minimal, if any, since a reduced hyperemic DPFV

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TABLE 3 Demographic, clinical and laboratory characteristics of patients with moderate and severe COVID-19 infection as compared to the control group

Characteristic	Severe COVID-19 ($n = 24$)	Moderate COVID-19 ($n = 15$)	COVID-19 (-) ($n = 40$)	p
Age, years	42.9 ± 8.1	41.9 ± 7.5	41.1 ± 4.8	.53
Male, *n* (%)	17 (70)	7 (46)	22 (55)	.28
BMI, kg/m^2	27.7 ± 4.5	28.6 ± 4.1	27.0 ± 3.2	.70
SAP, (mmHg)	124.6 ± 9.2	127.5 ± 9.3**	118.6 ± 10.1	.005
DAP, (mmHg)	81.5 ± 8.4*	83.8 ± 6.8***	75.3 ± 5.8	.001
RR	23.0 ± 3.8^{***,ℙ}	18.3 ± 1.5	17.5 ± 2.6	<.001
saO_2	86.8 ± 4.8^{***,ℙ}	95.5 ± 1.7**	97.7 ± 1.9	<.001
Albumin (g/dl)	3.3 ± 0.3***	3.6 ± 0.3	3.7 ± 0.4	<.001
AST (IU/L)	31 (27–50)	30 (20–65)	28 (21–32)	.10
ALT (IU/L)	25 (18–44)	27 (18–41)	22 (18–32)	.20
CRP (mg/dl)	37 (10–88)***,†	11 (5–30)***	3 (0–7)	<.001
Glucose (mg/dl)	139.5 ± 49.6***,‡	100.6 ± 22.0	95.4 ± 23.4	<.001
Creatinine (mg/dl)	0.8 ± 0.1	0.8 ± 0.2	0.8 ± 0.2	.83
GFR (ml/dk/1.73 m^2)	99.9 ± 14.9	96.7 ± 24.6	100.5 ± 18.6	.87
Uric acid (mg/dl)	3.9 ± 1.2	4.2 ± 1.6	4.1 ± 1.4	.83
WBC count (10^3/μl)	7.7 ± 3.8	6.1 ± 1.7	6.7 ± 1.7	.37
Hemoglobin (g/dl)	13.4 ± 2.0	13.1 ± 1.3	12.8 ± 1.2	.09
Platelet count (10^3/μl)	241.7 ± 73.4	201.4 ± 56.3	217.6 ± 68.1	.27
TC (mg/dl)	151.4 ± 24.3***	163.7 ± 41.4	191.5 ± 31.4	<.001
LDL (mg/dl)	84.9 ± 21.0***	92.8 ± 28.4*	118.8 ± 26.9	<.001
HDL (mg/dl)	39.2 ± 11.3	38.8 ± 7.4	44.0 ± 8.0	.11
Triglycerides (mg/dl)	136.0 ± 56.9	135.7 ± 60.1	136.4 ± 50.4	.90
Fibrinogen (mg/dl)	657.5 ± 133.5***	535.2 ± 164.6***	277.3 ± 48.3	<.001
Ferritin (ng/ml)	614 (132–994)***	210 (66–914)	83 (55–286)	.001
D-dimer (μg/ml)	1.2 (0.7–1.7)***	1.0 (0.7–1.4)***	0.5 (0.2–0.6)	<.001

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; BNP, brain natriuretic peptide; CRP, C-reactive protein; DAP, diastolic arterial pressure; DM, diabetes mellitus; GFR, glomerular filtration rate; HDL, high-density lipoprotein; HT, hypertension; LDL, low-density lipoprotein; RR, respiratory rate; saO_2, blood oxygen saturation; SAP, systolic arterial pressure; TC, total cholesterol; WBC, white blood cell.

$^{*}p < .05$ as compared to COVID-19 (-) group.

$^{**}p < .01$ as compared to COVID-19 (-) group.

$^{***}p < .001$ as compared to COVID-19 (-) group.

$^{†}p < .05$ as compared to the moderate COVID-19 group.

$^{‡}p < .01$ as compared to the moderate COVID-19 group.

$^{ℙ}p < .001$ as compared to the moderate COVID-19 group.

was the primary reason for the diminished CFVR in COVID-19 patients and the association between SaO2 and CFVR was lost after adjusting for the presence and severity of COVID-19. As such, low saO2 should be considered as a contributing factor rather than the primary mechanism of CMD in patients with COVID-19.

Present observations are in line with the available evidence showing an abnormal microvascular function and/or density in COVID-19 patients. Previous studies have suggested that COVID-19 patients have reduced flow reserve in the forearm skin, as well as reduced microcirculatory flow index and perfused vessel density in the sublingual circulation.[31–33] Interestingly, in one of these studies, Sabioni et al. have observed that peak hyperemic flow was impaired in either moderate or severe COVID-19 patients, but basal flow velocity was only affected in patients with severe COVID-19.[33] These findings were strikingly similar to the present results, despite different methods that were used to measure flow reserve. Hyperemic flow is primarily determined by functional and/or structural abnormalities in the microvasculature, and reduction of hyperemic flow is suggestive of a "true" microvascular dysfunction in COVID-19.[34] In contrast, further reduction of the flow reserve was caused by an increased basal flow in severe COVID-19, which suggests partial recruitment of the flow reserve in severe COVID-19 patients to compensate for increased baseline metabolic needs rather than a further deterioration of the microvascular structure and/or function.

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TABLE 4 Echocardiographic characteristics of patients with moderate and severe COVID-19 infection as compared to the control group

Characteristic	Severe COVID-19 ($n = 24$)	Moderate COVID-19 ($n = 15$)	COVID-19 (-) ($n = 40$)	p
LVEF (%)	61.4 ± 7.4	61.6 ± 7.7	64.0 ± 2.5	.29
LVDD (mm)	46.5 ± 3.1	45.2 ± 3.6	45.3 ± 3.2	.24
LVSD (mm)	28.8 ± 2.8	27.0 ± 3.2	27.8 ± 2.8	.10
IVS (cm)	0.9 ± 0.1	0.8 ± 0.1	0.9 ± 0.1	.33
PW (cm)	0.8 ± 0.0	0.8 ± 0.1	0.9 ± 0.1	.13
AoD (cm)	3.0 ± 0.4	2.9 ± 0.3	3.2 ± 0.3	.10
LA (cm)	3.5 ± 0.3***	3.4 ± 0.3**	3.0 ± 0.4	<.001
Mitral E wave (m/s)	0.8 ± 0.1	0.7 ± 0.1	0.8 ± 0.1	.45
Mitral A wave (cm/s)	0.6 ± 0.1	0.7 ± 0.2	0.6 ± 0.1	.22
Mitral IVRT (ms)	87.1 ± 17.9	91.2 ± 14.2	86.0 ± 16.8	.48
Septal E wave (cm/s)	9.8 ± 1.7	9.8 ± 2.0	10.5 ± 2.4	.48
Lateral E wave (cm/s)	14.0 ± 2.9	14.9 ± 3.2	14.8 ± 2.7	.70
Tricuspid S wave (cm/s)	13.3 ± 2.3	12.0 ± 2.2	13.2 ± 2.8	.43
TAPSE (cm)	2.3 ± 0.3	2.2 ± 0.3	2.4 ± 0.3	.17
Basal DPFV (m/s)	30.0 ± 6.2	26.0 ± 4.8	27.9 ± 5.7	.08
Hyperemic DPFV (m/s)	55.2 ± 12.5***	59.3 ± 11.5	66.9 ± 15.5	<.001
CFVR	1.8 ± 0.2***,ℙ	2.2 ± 0.2	2.4 ± 0.5	<.001
Basal HR (bpm)	77.2 ± 9.2	72.2 ± 6.3	72.4 ± 7.9	.11
Hyperemic HR (bpm)	101.2 ± 9.5	97.4 ± 10.5	95.6 ± 11.3	.14

Abbreviations: AoD, aortic diameter; CFVR, coronary flow velocity reserve; DPFV, diastolic peak flow velocity; HR, heart rate; IVRT, interventricular relaxation time; IVS, interventricular septum; LA, left atrium; LVDD, left ventricular diastolic diameter; LVEF, left ventricular ejection fraction; LVSD, left ventricular systolic diameter; PW, posterior wall; TAPSE, tricuspid annular plane systolic excursion.

*$p < .05$ as compared to COVID-19 (-) group.

**$p < .01$ as compared to COVID-19 (-) group.

***$p < .001$ as compared to COVID-19 (-) group.

†$p < .05$ as compared to the moderate COVID-19 group.

‡$p < .01$ as compared to the moderate COVID-19 group.

ℙ$p < .001$ as compared to the moderate COVID-19 group.

To note, while present results demonstrate a clear reduction in peak hyperemic flow and CFVR in those with severe COVID-19 infection as compared to the controls, it remains ambiguous whether CMD is also present in patients with moderate COVID-19. While there was also a trend toward lower hyperemic DPFV in this latter subgroup, neither peak hyperemic flow nor CFVR differed significantly as compared to the controls. It remains unclear whether this finding simply represents a limitation of this subgroup analysis (there were only 15 cases with moderate COVID-19) or whether CMD is relatively rare in patients with moderate COVID-19, thus underlining the need for further data on this topic.

Troponin elevation is a common observation and a marker of worse prognosis in COVID-19 patients. Myocarditis was initially regarded as the most plausible cause of the myocardial injury in the early stages of the pandemic, but histopathologic proof is scarce and is unlikely to explain troponin elevation in most cases.[35] Other explanations, such as overt myocardial infarction or stress cardiomyopathy, have other electrocardiographic and imaging findings that were not found in most cases with COVID-19 infection.[5,6,36] Although direct evidence was lacking, it has been long speculated that CMD could explain myocardial injury seen in severe COVID-19 patients.[15,21] Present findings indicate that the degree of CMD correlates with both troponin and B-type natriuretic peptide concentration, suggesting a relationship between CMD, myocardial injury, and an increased myocardial fiber strain. To note, correlation should not be interpreted as causality and all of these findings might simply represent the severity of the underlying disease rather than a causal association between CMD and myocardial damage. Present findings are nonetheless intriguing and warrant further search for a causal association between CMD and myocardial injury.

A strength of the present analysis is that patients with known conditions that could affect CFVR have been excluded. While not all confounders could be safely excluded and some patients might have unknown CAD or other conditions, exclusion of known confounders nonetheless strengthens the association between COVID-19 and CMD. Also, echocardiographic examinations were done as soon as the patients were stabilized, and the flow measurements reflect the status of the coronary microvasculature during the acute or subacute phases of COVID-19. Ideally, an echocardiographic examination should be done at the time of admission but exposing a critically ill patient to a drug that could deteriorate her condition would be

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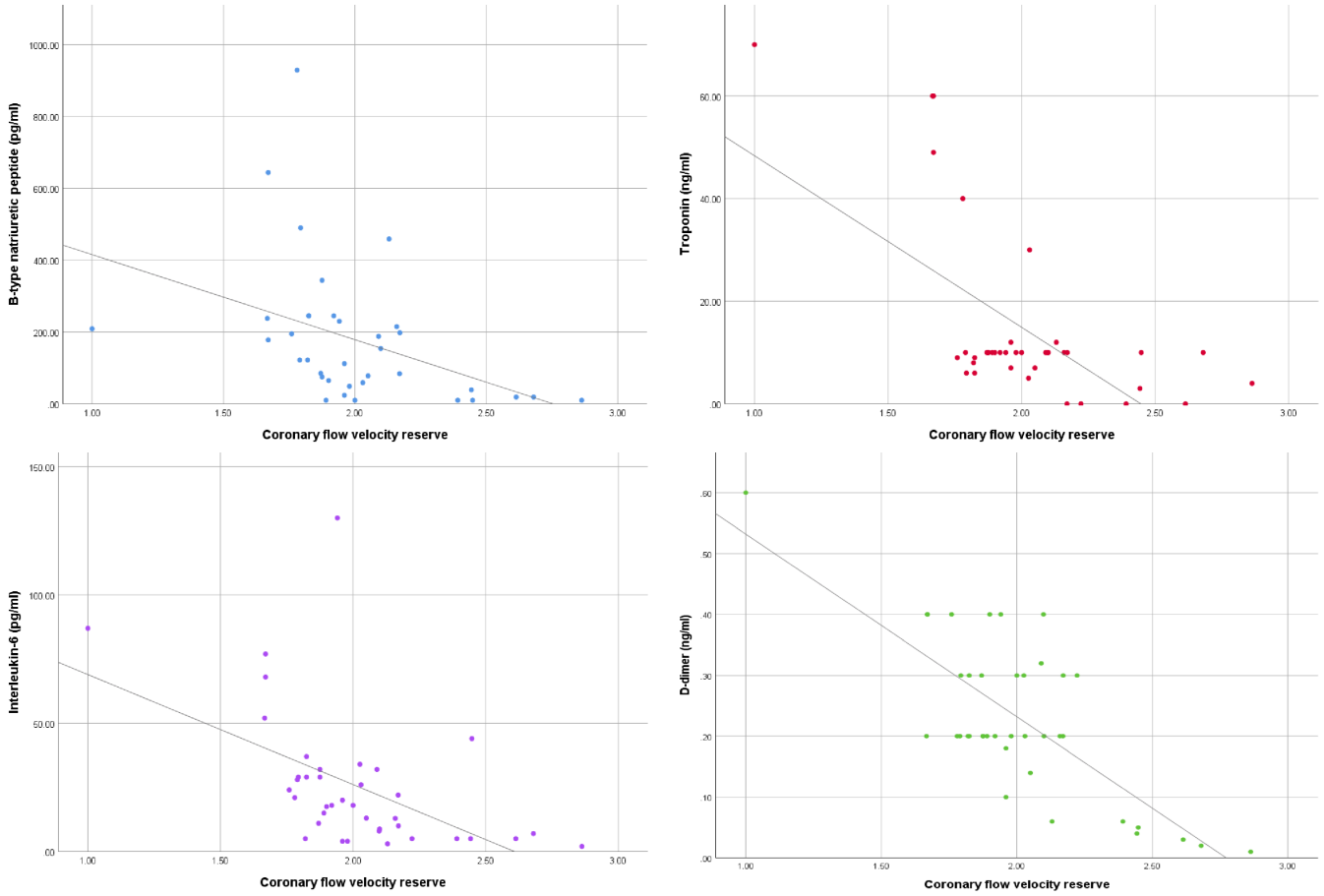


FIGURE 1 Scatter plots showing correlations between coronary flow velocity reserve and B-type natriuretic peptide (top left), troponin (top right), d-dimer (bottom right), and interleukin-6 (bottom left)

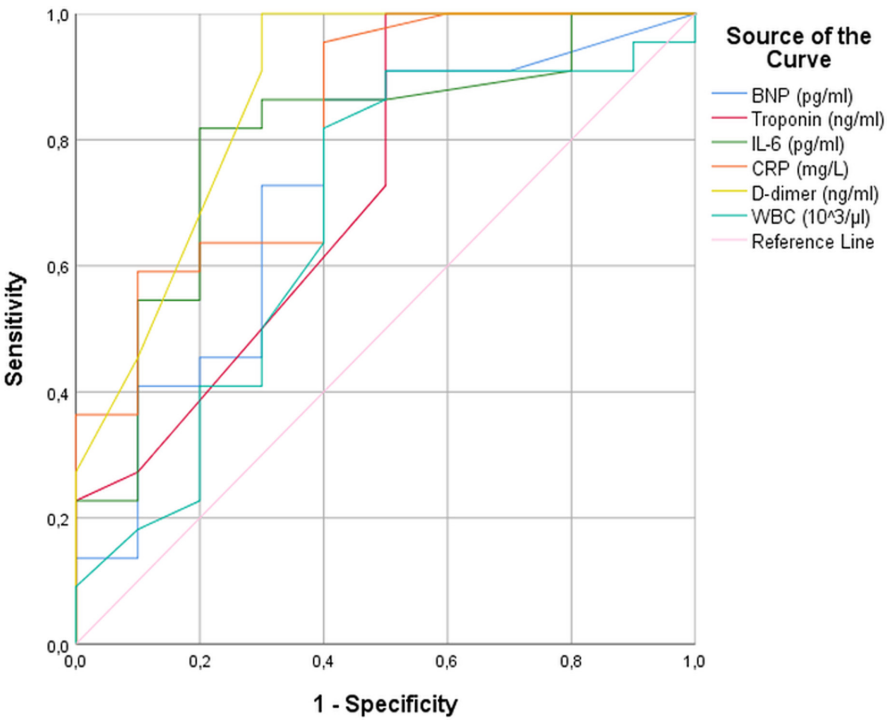


FIGURE 2 Receiver-operator curves showing accuracy for various biomarkers to predict coronary microvascular dysfunction. Lines were color-coded, and references for lines were provided on the top right. Diagonal line shows reference. BNP: B-type natriuretic peptide, IL-6: interleukin-6, CRP, C-reactive protein, and WBC, white blood cell count

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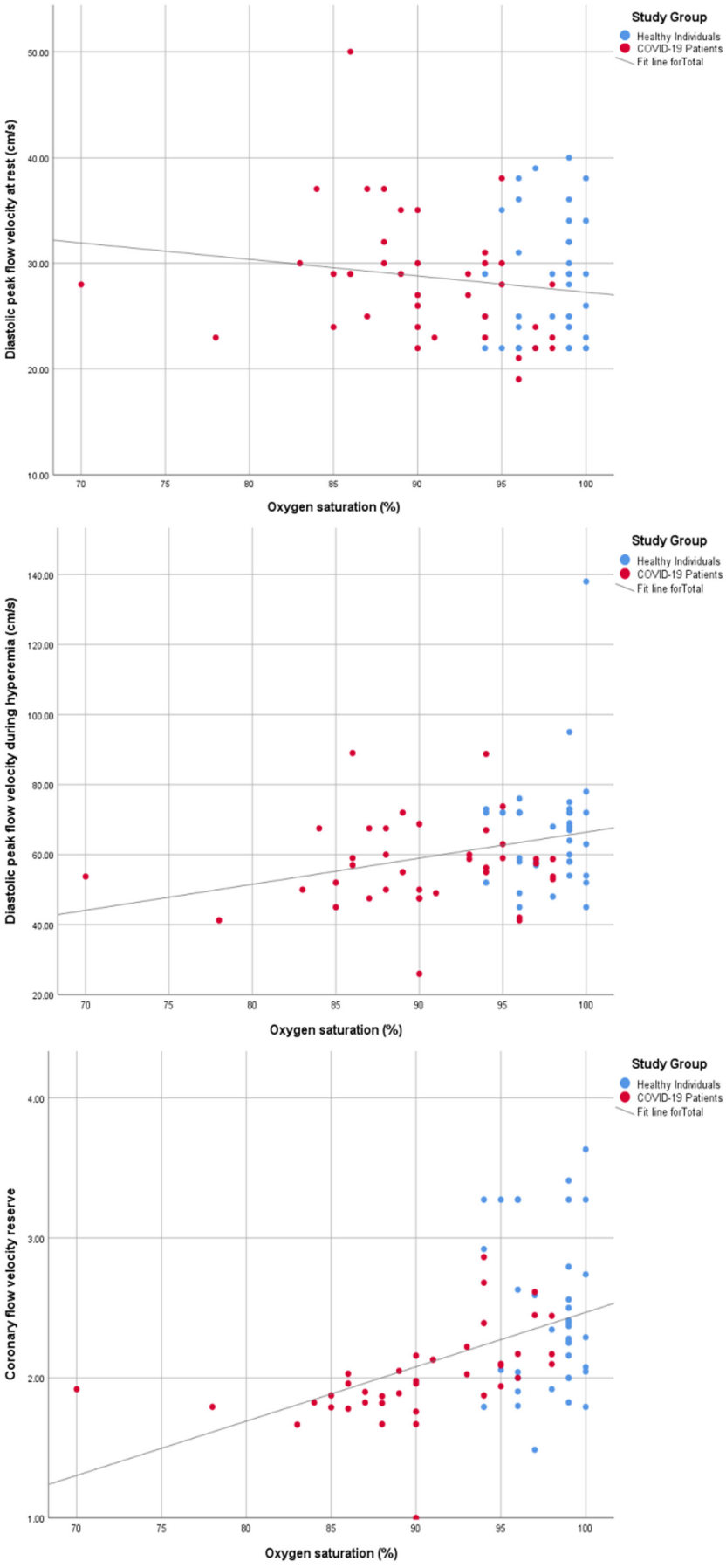


FIGURE 3 Scatter plots showing correlations between oxygen saturation and diastolic peak flow velocity at rest (top panel), diastolic peak flow velocity during hyperemic phase (middle panel), and coronary flow velocity reserve (bottom panel) in patients with and without COVID-19. Color codes show subjects within the control and study groups

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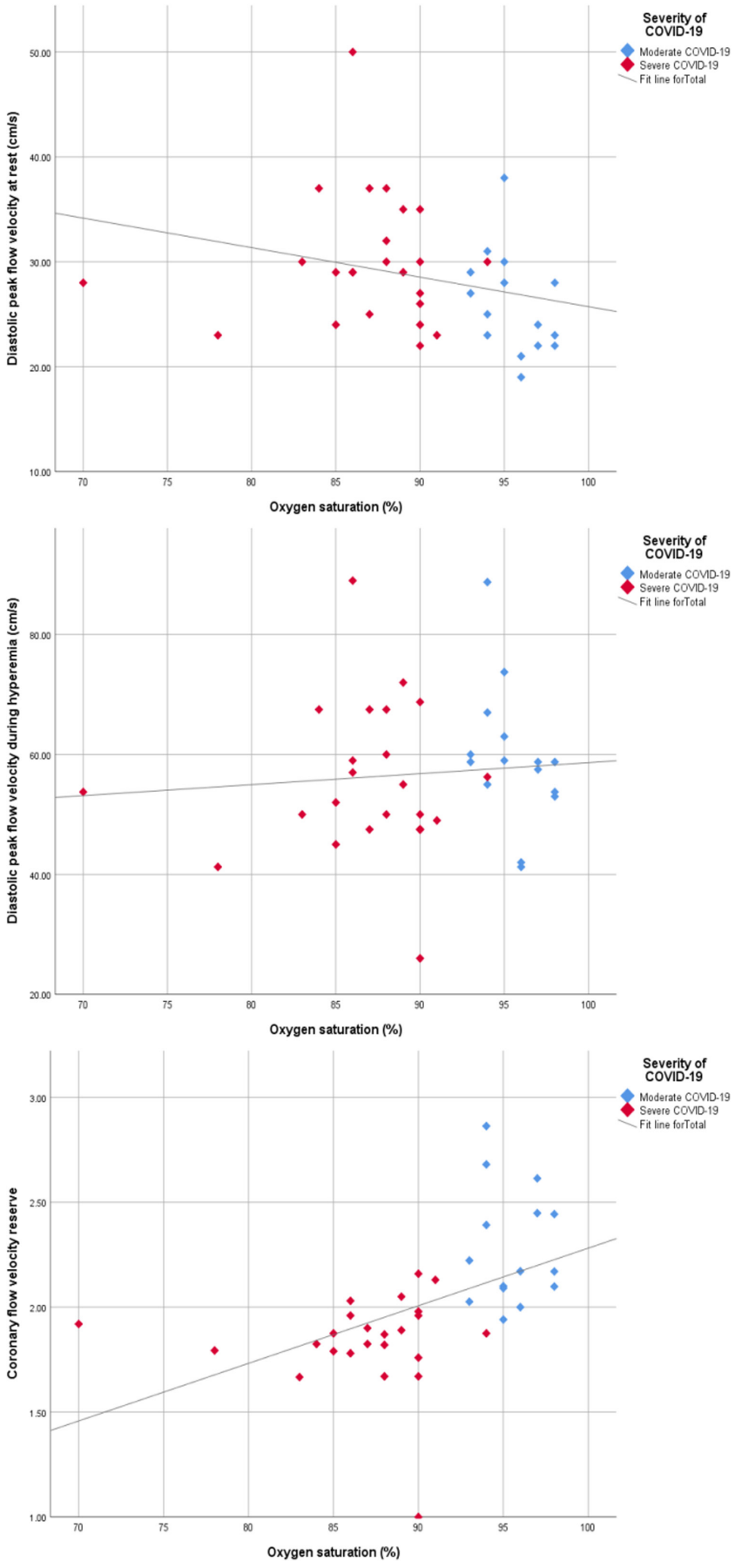


FIGURE 4 Scatter plots showing correlations between oxygen saturation and diastolic peak flow velocity at rest (top panel), diastolic peak flow velocity during hyperemic phase (middle panel), and coronary flow velocity reserve (bottom panel) in patients within the COVID-19 group. Color codes show subjects with moderate and severe COVID-19 disease

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unethical. As such, present results were obtained from the best time frame that CMD can be evaluated without harming a patient.

Our study had several limitations. This is a single-center study with a small sample size and a cross-sectional design. While controls did not have active infection at the time of echocardiographic evaluation, past asymptomatic infections (which might or might not affect microvascular function) cannot be excluded. As correlation does not imply causality, present findings do not show that inflammation or prothrombotic milieu causes CFVR or CFVR leads to myocardial injury but rather suggest an association between them. Also, elevation of an inflammatory/thrombotic biomarker does not show an organ-specific condition but rather reflects an overall inflammatory or prothrombotic state. Thus, present findings should be interpreted in this context.

5 | CONCLUSIONS

Patients with COVID-19, particularly those with severe infection, have a reduced hyperemic coronary flow and CFVR indicating the presence of CMD. The degree of CMD correlates with biomarkers of inflammation, fibrin turnover, myocardial injury, and myocyte stretch, though it remains to be determined whether these associations represent causal relationships between inflammation, thrombosis, microvascular dysfunction, and finally myocardial injury. Further work is needed to understand the clinical importance of these findings, as well as therapeutic approaches to prevent or treat CMD in COVID-19 patients.

ACKNOWLEDGEMENTS

Not applicable.

AUTHOR CONTRIBUTION

M.Ç and M.A.A. involved in study conception and design, and critical review. Ö.F.B., F.B.Ç., A.A., Y.Ç., O.K., T.İ., and Ü.Z.B. involved in data acquisition and preparation of draft. T.S.G. involved in data interpretation, preparation of draft and final manuscript. All authors have approved the final version of the manuscript.

DATA AVAILABILITY STATEMENT

The data are available from the corresponding author upon reasonable request.

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