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Key Points

- Severe and critical COVID-19 are associated with persistent endothelial dysfunction up to three months after recovery, characterized by a predominance of vasoconstrictive responses during flow-mediated dilation testing.
- Endothelial dysfunction is accompanied by elevated circulating levels of endothelin-1, arginase-1, and thromboxane A₂, together with reduced prostacyclin concentrations, indicating a shift toward a pro-constrictive and pro-thrombotic vascular phenotype.
- The degree of vascular impairment demonstrates a clear severity-dependent pattern, with the most pronounced abnormalities observed in patients who experienced severe or critical COVID-19.
- Significant correlations between FMD-derived parameters and vasoactive biomarkers support a mechanistic link between functional endothelial impairment and persistent biochemical dysregulation in the post-COVID period.

Future directions: Prospective longitudinal studies are warranted to determine the duration and reversibility of post-COVID endothelial dysfunction and to evaluate whether integrated vascular function and biomarker assessment can improve cardiovascular risk stratification and guide targeted preventive interventions.

Abstract

COVID-19 is frequently associated with endothelial dysfunction (ED), characterized by an imbalance between vasodilatory mediators (such as nitric oxide and prostacyclin) and vasoconstrictors (including endothelin-1 and thromboxane A₂), leading to increased vascular resistance and a prothrombotic state. This study aimed to evaluate ED in the post-COVID-19 period across different disease severity groups using flow-mediated dilation (FMD) and echocardiographic assessment. Serum levels of thromboxane A₂ (TxA₂), prostacyclin (PGI₂), endothelin-1 (ET-1), and arginase-1 (Arg1) were quantified by ELISA. Endothelial dysfunction was defined as a brachial artery (BA) dilation of <10% from baseline or the presence of vasoconstriction during FMD.

Patients were stratified into four groups according to COVID-19 severity: mild (home-quarantined, HQM, n=262), moderate (hospitalized, HMO, n=183), severe (hospitalized with oxygen therapy, HSV, n=41), and critical (ICU, HCR, n=16). Vasoconstrictive responses during FMD were significantly more frequent in patients with severe (90.24%) and critical (100.00%) disease compared to healthy control group (18.52%). These responses were associated with elevated levels of ET-1, Arg1, and TxA₂, alongside reduced PGI₂ concentrations. Significant correlations were observed between biochemical markers and FMD parameters.

Importantly, three months after a negative SARS-CoV-2 PCR test, patients continued to exhibit vasoconstrictive responses and persistent alterations in endothelial biomarkers, indicating sustained endothelial dysfunction. The severity of endothelial impairment was directly associated with the initial clinical severity of COVID-19. A strong relationship between vasoconstriction and biochemical markers further supports the mechanistic link underlying post-COVID endothelial injury.

Keywords: post-COVID-19, flow-mediated dilation, endothelin-1, arginase-1, thromboxane A₂, prostacyclin

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Institutional Review Board Statement

Protocol № 59 from June 05, 2020, of the Bioethics Commission of the I. Horbachevsky Ternopil National Medical University.

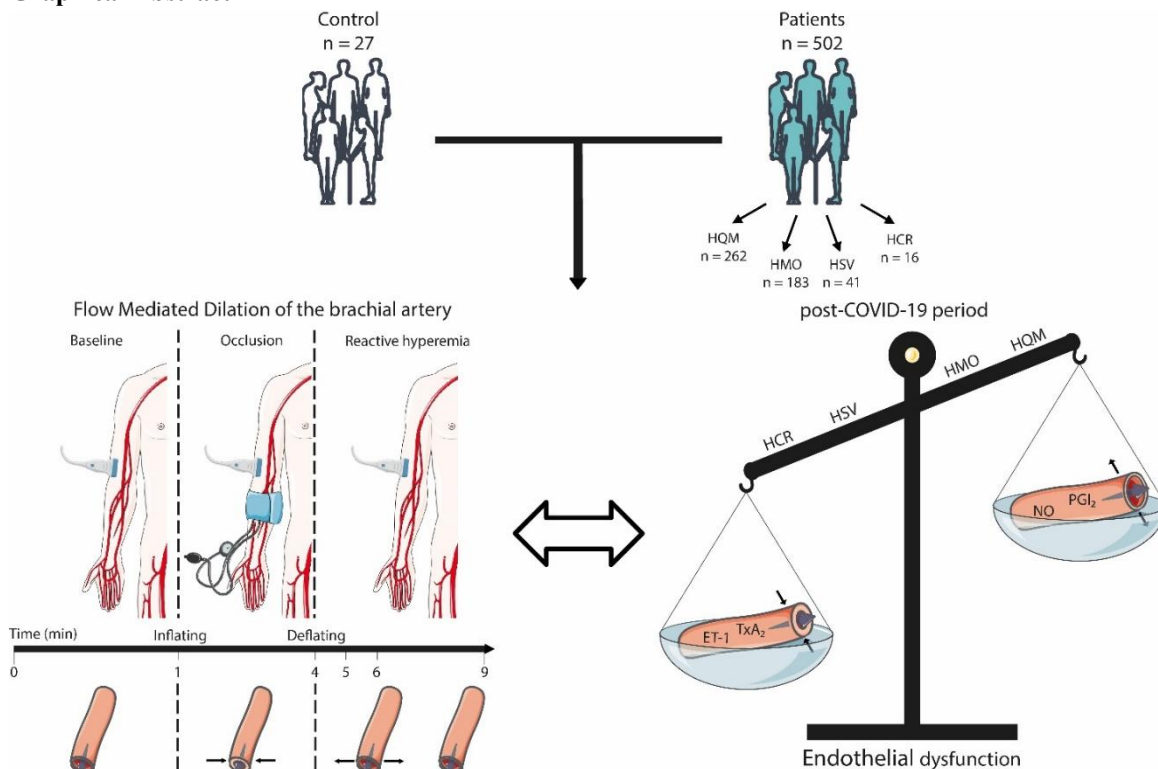
Informed Consent Statement

Signed informed consent was obtained from all involved persons.

Conflicts of Interest

The authors declare that there is no conflict of interest.

Graphical Abstract



1. Introduction

Coronavirus disease-2019 (COVID-19) is a disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1]. Almost everyone is susceptible to SARS-CoV-2 infection, but the clinical spectrum ranges from an asymptomatic or mild course to a severe or even lethal critical course [2, 3]. However, as we know today, it doesn't end with an acute phase of disease. Some people who have fully recovered from coronavirus disease begin to experience its long-term consequences. According to the WHO, this accounts for approximately 10-20% of all convalescents, although the actual percentage is likely much higher. The so-called long COVID-19 (post-acute sequelae of COVID-19, PASC) is characterized by a range of symptoms. And it has drawn global attention to post-acute infection syndromes (PAIS) [4]. Millions of people are already suffering from PASC. Since the registration of new cases of COVID-19 has not stopped, its number continues to grow. PASC/Long COVID is a significant public health concern, with research actively focused on understanding its complex nature to develop effective management, treatments and rehabilitation.

Several potential long-term complications have been identified, including cardiac thrombosis and inflammation, venous thromboembolism, pulmonary fibrosis, stroke, arterial thrombosis, dermatological manifestations, and cognitive impairment ("brain fog") [5]. This list is not exhaustive, as SARS-CoV-2 has been shown to adversely affect nearly all organ systems in the body [6]. Cardiac symptoms such as shortness of breath, chest pain, and tachycardia are common complaints among those who have recovered [8]. The direct or indirect impact of SARS-CoV-2 infection, along with the subsequent development of endotheliitis or endotheliopathy, leads to multiple manifestations of endothelial dysfunction (ED), including impaired vascular tone, coagulopathy, inflammatory responses with leukocyte adhesion and cytokine storm [8]. ED occurs due to activated oxidative stress, pathophysiological shear stress, and inflammation, which leads to a decrease in the anti-inflammatory and antithrombotic functions of the endothelium [9]. Patients with COVID-19 experience more frequent and more severe clotting events than those with other common respiratory viral infections. It can occur as microthrombi or macrothrombi, leading to damage in several organs, including the lungs, heart, kidneys, and brain [10-12].

A decrease in the bioavailability of nitric oxide (NO) and an increased level of vasoconstrictor substances (such as endothelin-1 (ET-1), angiotensin II (Ang II), etc.) are observed [8]. One of the specific features of COVID-19 caused by the SARS-CoV-2 virus is the pronounced non-specificity of pathological processes that develop in almost all organs and systems of the human body, which could be the reason for a severe course [13]. For now, it has been proven that the endothelium is one of the tissues involved in the pathological process caused by the SARS-CoV-2 virus, and some papers use the term "endotheliitis" to describe pathological processes in the endothelium [14]. SARS-CoV-2 entry into host cells triggers endothelial cell (EC) injury, leading to endothelial dysfunction (ED) and perivascular inflammation, thereby amplifying vascular damage [15]. Biochemical methods for the assessment of ED provide not only quantitative measurements of specific biomarkers but also insight into the levels of factors contributing to ECs dysfunction [16, 17]. Under physiological conditions, endothelial cells express anticoagulant molecules, including nitric oxide (NO), prostacyclin (PGI₂), and thrombomodulin. However, under conditions of inflammation or stress, ECs shift toward a procoagulant phenotype, characterized by the expression of molecules such as plasminogen activator inhibitor-1 (PAI-1), tissue factor, and thromboxane A₂ (TxA₂), among others [18].

Prostanoids are cyclooxygenase (COX)-derived metabolites of arachidonic acid, including thromboxane A₂ (TxA₂) [19]. TxA₂ exerts its biological effects by binding to the prostanoid Tx receptor, activating G-protein-coupled signaling pathways that increase intracellular calcium levels, leading to vasoconstriction and platelet aggregation [2]. ET-1 is the most potent endogenous vasoconstrictor and plays a key role in maintaining basal vascular tone under physiological conditions [20]. In contrast, nitric oxide (NO), a critical endothelium-derived signaling molecule, promotes vasodilation and inhibits the release of ET-1 [21]. Arginase is a key regulator of NO bioavailability, as it competes with endothelial nitric oxide synthase (eNOS) for their common substrate, L-arginine. This enzyme hydrolyzes L-arginine into L-ornithine and urea and exists in two isoforms, arginase 1 (Arg1) and arginase 2 (Arg2). Both isoforms are expressed in endothelial cells and are upregulated under conditions of inflammation and oxidative stress. In pathophysiological states such as atherosclerosis, increased arginase activity reduces NO production by diverting L-arginine away from the eNOS pathway [22]. Prostacyclin (PGI₂), synthesized primarily by endothelial and vascular smooth muscle cells via COX-2, exerts effects opposite to those of TxA₂. While TxA₂ promotes vasoconstriction and platelet aggregation, PGI₂ inhibits platelet aggregation and induces vascular smooth muscle relaxation, thereby contributing to endothelium-dependent vasodilation [2].

However, it should be noted that laboratory-based assessment of endothelial dysfunction (ED) is often invasive, and the cost associated with measuring multiple specific biochemical markers limits its widespread application in large patient populations. In contrast, functional diagnostic methods are based on the evaluation of endothelial vasomotor function through changes in the bioavailability of vasoactive substances.

Among these, flow-mediated dilation (FMD) of the brachial artery, assessed by ultrasound, is currently considered a reliable and widely used non-invasive method for evaluating endothelial function [23]. The growing clinical interest in non-invasive approaches to ED assessment is driven by their potential to enable early detection of macro- and microvascular dysfunction, thereby facilitating timely prevention of adverse cardiovascular events in high-risk populations [24].

In this non-invasive approach, endothelial dysfunction (ED) is assessed using a pneumatic cuff to induce transient ischemia, followed by reactive hyperemia in the distal circulation. Flow-mediated dilation (FMD) of the brachial artery (BA) was first described by Anderson and Mark (1989) [25] and subsequently introduced into clinical practice by Celermajer et al. (1992) [26]. The post-ischemic increase in blood flow through the BA leads to endothelium-dependent vasodilation, reflecting endothelial function.

The present study aimed to investigate endothelial dysfunction in the post-COVID period, with a particular focus on the relationship between biomarkers of vasodilation and vasoconstriction and FMD-derived parameters.

2. Materials and methods.

A retrospective observational study was conducted among 502 patients in the post-COVID-19 period (0–90 days following a negative SARS-CoV-2 PCR test). Patient recruitment took place between July 2020 and December 2021, corresponding to the Delta wave of the pandemic in Ukraine.

All participants were stratified into four groups according to COVID-19 severity, based on national Ukrainian clinical guidelines and World Health Organization (WHO) recommendations [27, 28].

Group I (n = 262) comprised home-quarantined patients with mild symptoms (HQM).

Group II (n = 183) included hospitalized patients with moderate disease (HMO).

Group III (n = 41) consisted of hospitalized patients with severe disease requiring oxygen therapy (HSV).

Group IV (n = 16) included critically ill patients admitted to the intensive care unit (ICU) (HCR).

A control group of 27 individuals with no history of COVID-19, no detectable comorbidities, and negative anti-SARS-CoV-2 serology was also included. Detailed demographic and clinical characteristics of the study population are presented in Table 1.

Table 1. Clinical characteristics of the cohort.

Parameters		HQM (I) (n=262)	HMO (II) (n=183)	HSV (III) (n=41)	HCR (IV) (n=16)	Control Group (c) (n=27)	p
Age, years		44.86 ±12.07	53.73 ±11.49	55.46 ±12.62	58.38 ±11.05	32.15 ±5.89	p _{I-c} , p _{II-c} , p _{III-c} , p _{IV-c} , p _{I-II} , p _{I-III} , p _{I-IV} <0.05*
Sex	M	61 (23.28%)	69 (37.70%)	17 (41.46%)	9 (56.25%)	16 (59.26%)	$\chi^2=26.83$; p<0.001*
	F	201 (76.72%)	114 (62.30%)	24 (58.54%)	7 (43.75%)	11 (40.74%)	
Systolic blood pressure, mmHg		124.72 ±17.42	136.09 ±20.92	145.48 ±23.39	152.14 ±15.28	121.25 ±10.25	p _{II-c} , p _{III-c} , p _{IV-c} , p _{I-II} , p _{I-III} , p _{I-IV} , p _{II-III} , p _{II-IV} <0.05*
Diastolic blood pressure, mmHg		80.12 ±11.84	84.78 ±12.40	87.58 ±11.89	89.29 ±13.28	77.50 ±6.22	p _{II-c} , p _{III-c} , p _{IV-c} , p _{I-II} , p _{I-III} , p _{I-IV} <0.05*
BMI, kg/m ²		27.18 ±5.69	29.51 ±4.90	30.72 ±4.45	32.85 ±5.06	25.11 ± 2.06	p _{I-c} , p _{II-c} , p _{III-c} , p _{IV-c} , p _{I-II} , p _{I-III} , p _{I-IV} <0.05*
Note. * – statistically significant result.							

The study protocol was approved by the Bioethics Committee of Ivan Horbachevsky Ternopil National Medical University (protocol No. 59, dated June 5, 2020). Written informed consent was obtained from all participants prior to enrolment. The study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki and its subsequent amendments.

To ensure methodological rigor and comparability of results, patient preparation, FMD assessment of the brachial artery, and data analysis were performed according to standardized protocols described by Thijssen et al. [24].

Prior to the examination, patients were instructed to rest in a seated position for 15–20 minutes at room temperature (22–25 °C). All assessments were performed after an overnight fast. Participants were advised to abstain from food, caffeine, and alcohol for at least 12 hours before the test. The use of vasoactive medications was recorded and monitored throughout the study.

A pneumatic cuff was placed distal to the measurement site on the brachial artery, at the level between the upper and middle third of the forearm. Baseline systolic blood pressure was measured prior to cuff inflation, and the cuff pressure was set at 30–50 mmHg above systolic pressure. Arterial occlusion was maintained for a minimum of 3 minutes, with a standard duration of 5 minutes [29].

The brachial artery (BA) diameter and blood flow parameters were assessed using a Sonoscape S8 Exp ultrasound system equipped with a 7 MHz linear transducer. The measurement site was marked 3–5 cm above the antecubital fossa. The examination was performed in triplex mode, including two-dimensional imaging, color Doppler flow mapping, and spectral Doppler analysis. The BA diameter was measured using electronic calipers placed at two predefined points: the intima–lumen interface of the near wall and the lumen–intima interface of the far wall.

Flow-mediated dilation (FMD) was expressed as the percentage increase in brachial artery diameter from baseline to the maximum post-occlusion value and calculated as follows:

$$FMD (\%) = \frac{D_{max} - D_{baseline}}{D_{baseline}} \times 100$$

where $D_{baseline}$ is the baseline brachial artery diameter and D_{max} is the maximum diameter recorded after cuff release. Absolute diameter change was calculated as $D_{max} - D_{baseline}$. Negative values indicated vasoconstrictive response.

Quantitative hemodynamic parameters included peak systolic velocity (Vps), end-diastolic velocity (Ved), pulsatility index (PI), and resistance index (RI). Each parameter was assessed over three consecutive cardiac cycles.

Measurements were performed during diastole at baseline (prior to occlusion), immediately before cuff release, and at 1, 2, and 5 minutes after cuff deflation. An increase in brachial artery (BA) diameter of $\geq 10\%$ at 60 seconds following cuff release was considered a normal endothelium-dependent response. A smaller increase was interpreted as indicative of endothelial dysfunction (ED), whereas a decrease in arterial diameter (vasoconstriction) was defined as a paradoxical response, also reflecting impaired endothelial function.

Qualitative assessment of flow parameters was performed immediately after cuff release, corresponding to peak shear stress and maximal endothelial stimulation.

Serum levels of endothelin-1 (ET-1), arginase-1 (Arg1), and prostacyclin (PGI₂) were measured using commercially available ELISA kits (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA), while thromboxane A₂ (TxA₂) was quantified using kits from Abcam (Cambridge, UK).

Statistical analysis was performed using STATISTICA 6.0 for Windows (StatSoft, USA) and Microsoft Excel (Microsoft Office 2013, USA). Data was presented as mean $\pm$ standard deviation (SD) for normally distributed variables and as median with interquartile range (IQR) for non-normally distributed variables. Between-group comparisons were conducted using parametric tests (Student's t-test, one-way ANOVA) or nonparametric tests (Mann–Whitney U test, Kruskal–Wallis test), as appropriate. Categorical variables were compared using the chi-square test. Correlations were assessed using Spearman's rank correlation coefficient. A p-value < 0.05 was considered statistically significant.

3. Results

The study demonstrated a significantly higher frequency of vasoconstrictive responses in HCR and HSV patients compared with the control group ($\chi^2=113.41$; $p<0.001$) (see **Fig. 1A**).

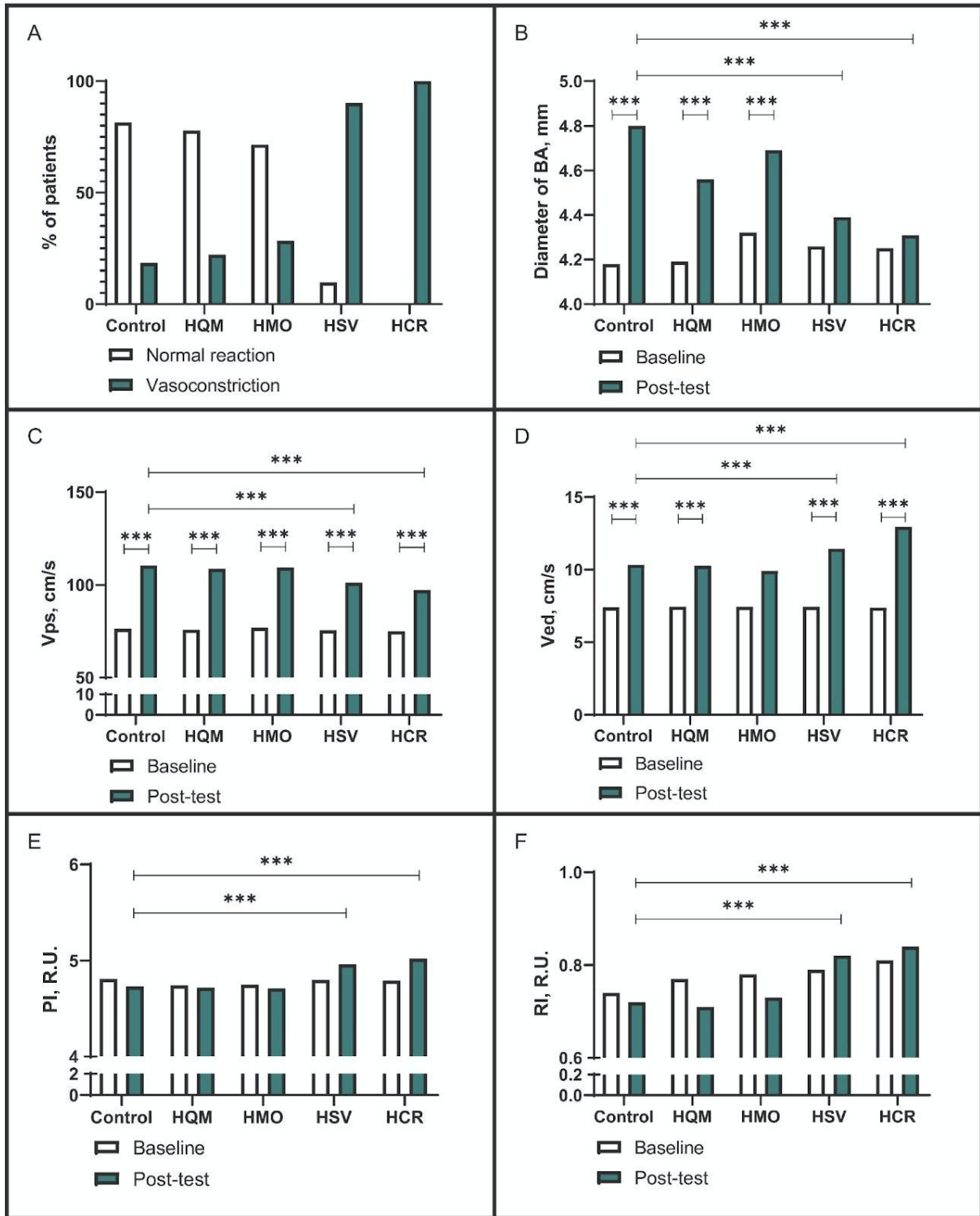


Figure 1. FMD test results in post-COVID-19 patients depend on COVID-19 severity (***) - $p < 0.001$)

The baseline diameter of the BA prior to the FMD test did not differ significantly among the study groups. However, the results of the FMD test revealed a significantly lower post-test BA diameter in patients with severe (4.39 ± 0.61 mm) and critical (4.31 ± 0.67 mm) COVID-19 compared to the control group of non-infected individuals (4.80 ± 0.65 mm; $p < 0.05$). Furthermore, in the HSV and HCR groups, the post-test BA diameter did not differ significantly from baseline. In contrast, a significant increase in BA diameter following the FMD test was observed in the control group and in patients with mild and moderate COVID-19 ($p < 0.05$) (see **Fig. 1B**).

In patients of the HCR group, Vps exceeded the baseline by 21.69% ($p < 0.05$), but at the same time, the level of increase in peak blood flow velocity was the lowest among patients included in the study (see **Fig. 1C**).

The Ved at the level of the BA was characterised by the highest level and exceeded the baseline for the group by 75.37% ($p < 0.05$) and was characterised by the highest level among all groups of patients (see **Fig. 1D**). The PI and the

RI, which characterise the elasticity of the vascular wall and the peripheral resistance of the vascular segment in comparison with the control group, had significantly higher values ($p<0.05$) (see **Fig. 1E-F**).

A similar tendency toward changes in FMD results, although to a lesser extent, was observed in patients in the HSV group. The BA diameter response to the test was less than 10%, i.e., 4.7–3.8%. Vps in patients of HSV compared to the baseline increased by 13.45% ($p<0.05$), but like in patients of HCR, were significantly lower than in patients of the control group (see **Fig. 1C**).

While evaluating the results in this group of patients, it was determined that Ved exceeded the baseline by 53.91% ($p<0.05$). Analysis of changes in PI and RI also indicated a violation of the vascular wall's elastic properties toward vasoconstriction and increased peripheral vascular resistance (see **Fig. 1E-F**). In HQM and HMO patients, all studied FMD test vasodilation parameters remained within the normal range and indicated the absence of manifestations or only a slight degree of ED (see **Fig. 1**). Detailed characteristics of the parameters are presented in Tables 2.

Table 2 – The results of the FMD test to determine endothelium-dependent vasodilation

		HQM (I) (n=262)	HMO (II) (n=183)	HSV (III) (n=41)	HCR (IV) (n=16)	Control group (n=27)
Normal reaction		204 (77.86%)	131 (71.58%)	4 (9.76%)	0	22 (81.48%)
Vasoconstriction		58 (22.14%)	52 (28.42%)	37 (90.24%)	16 (100.00%)	5 (18.52%)
Note 1. $\chi^2=113.41$; $p<0.001$ *. Note 2. * – statistically significant result.						
Diameter of the brachial artery, mm	Baseline	4,19±0,81	4,32±0,83	4,26±0,53	4,25±0,69	4,18±0,59
	Post-test	4,56±0,83 ²	4,69±0,79 ²	4,39±0,61 ¹	4,31±0,67 ¹	4,80±0,65 ²
Vps, cm/s	Baseline	75,84±8,91	76,91±9,03	75,48±7,81	74,95±8,12	76,23±9,11
	Post-test	108,61±9,54 ²	109,38±10,26 ²	101,12±7,92 ^{1,2}	97,29±8,12 ^{1,2}	110,37±12,49 ²
Ved, cm/s	Baseline	7,46±1,29	7,43±1,31	7,44±1,30	7,39±1,38	7,41±1,33
	Post-test	10,27 ±1,68 ²	9,92 ±1,93	11,45±2,1 ^{1,2}	12,96±2,27 ^{1,2}	10,35±1,81 ²
PI	Baseline	4,74±0,27	4,75±0,25	4,80±0,24	4,79±0,29	4,81±0,26
	Post-test	4,72±0,21	4,71±0,24	4,96±0,15 ¹	5,02±0,29 ¹	4,73±0,23
RI	Baseline	0,77±0,06	0,78±0,05	0,79±0,03	0,81±0,05	0,74±0,02
	Post-test	0,71±0,04	0,73±0,03	0,82±0,04 ¹	0,84±0,03 ¹	0,72±0,03
Note. ¹ – $p<0,05$ compared with the control group; ² – $p<0,05$ compared to the initial data for the group. Vps – peak systolic velocity, Ved – end-diastolic velocity, PI – pulsatility index, RI – resistance index.						

The study revealed significantly higher levels of ET-1, Arg1, and TxA2 in patients with vasoconstriction during the FMD test ($p<0.05$). At the same time, vasoconstriction was associated with significantly lower PGI₂ levels ($p<0.05$) (see **Fig. 2**).

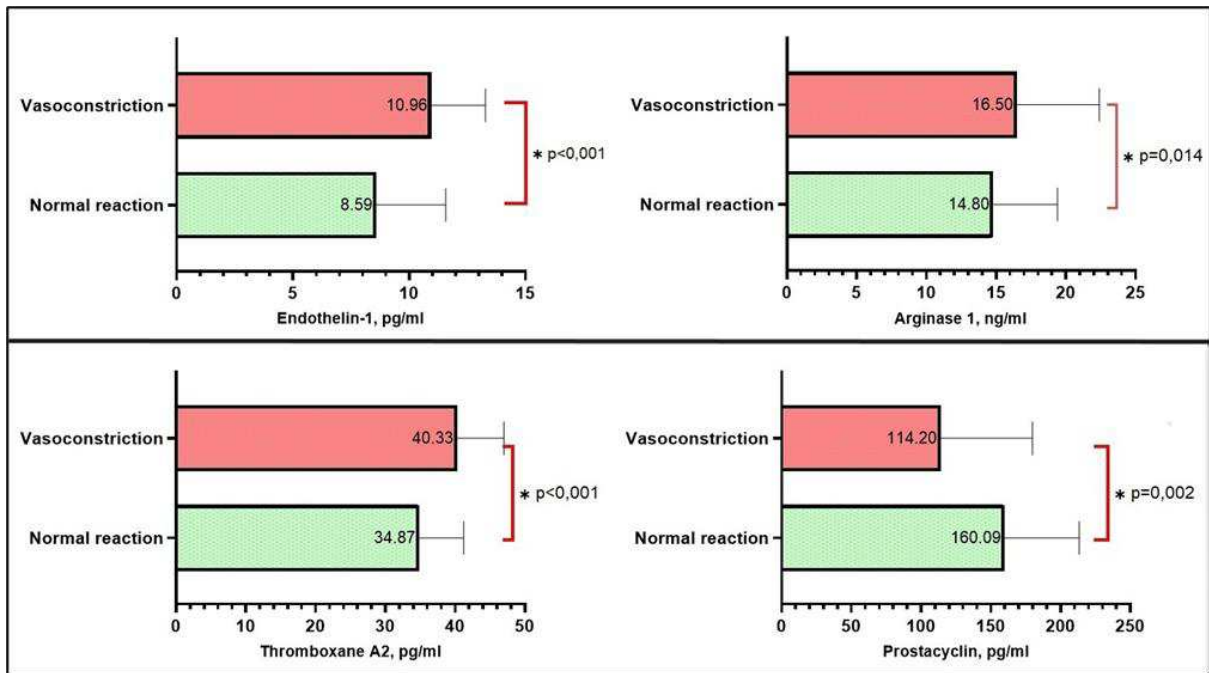


Figure 2. ET-1, Arg-1, and TxA₂ and PGI₂ levels in post-COVID-19 patients depending on the FMD test.

Given differences in biochemical markers of vasodilation and vasoconstriction, we conducted a correlation analysis with FMD test parameters (see Table 3). The study found a positive correlation between ET-1 levels and both Ved and RI ($p < 0.05$). Additionally, elevated Arg1 levels were associated with a smaller brachial artery (BA) diameter after FMD and higher RI ($p < 0.05$). Furthermore, a high RI was positively correlated with TxA₂. Notably, an increase in Ved was associated with lower PGI₂ levels among the studied groups ($p < 0.05$).

Table 3 – Correlation matrices between ET-1, Arg1, and TxA₂ and PGI₂ levels and parameters of FMD test

Parameter		Endothelin-1	Arginase 1	Thromboxane A2	Prostacyclin
Diameter of the brachial artery, mm	Baseline	$r = 0.01$	$r = -0.04$	$r = 0.08$	$r = -0.07$
	Post-test	$r = -0.09$	$r = -0.12^*$	$r = -0.08$	$r = 0.11$
Vps, cm/s	Baseline	$r = 0.01$	$r = -0.04$	$r = -0.02$	$r = -0.02$
	Post-test	$r = -0.01$	$r = -0.01$	$r = 0.02$	$r = 0.04$
Ved, cm/s	Baseline	$r = 0.18^*$	$r = 0.06$	$r = 0.17^*$	$r = -0.18$
	Post-test	$r = 0.18^*$	$r = 0.05$	$r = 0.18^*$	$r = -0.32$
PI	Baseline	$r = -0.04$	$r = 0.06$	$r = -0.07$	$r = 0.08$
	Post-test	$r = 0.07$	$r = 0.07$	$r = 0.08$	$r = -0.07$
RI	Baseline	$r = -0.03$	$r = -0.01$	$r = 0.15$	$r = -0.04$
	Post-test	$r = 0.15^*$	$r = 0.13^*$	$r = 0.17^*$	$r = -0.05$

Note. * – statistically significant result.

ET-1 levels increased with disease severity and were higher in patients with moderate, severe, and critical cases than in the control group (see Table 4). Similar patterns were observed for Arg1 and TxA₂ levels. Importantly, TxA₂ levels were elevated in COVID-19 patients, regardless of disease severity; even those with mild cases showed elevated levels. Additionally, the PGI₂ levels were significantly lower in COVID-19 patients compared to non-infected individuals ($p < 0.05$).

Table 4 – ET-1, Arg1, and TxA₂ and PGI₂ levels depending on COVID-19 severity

Group	Endothelin-1, pg/mL	Arginase 1, ng/mL	Thromboxane A2, pg/mL	Prostacyclin, pg/mL
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HQM (I)	6.41 (2.70; 10.54)	14.80 (10.80; 19.20)	33.46 (28.98; 37.65)	157.27 (102.30; 201.20)
HMO (II)	10.93 (8.53; 13.60)	16.00 (11.50; 22.40)	38.56 (33.83; 44.34)	125.20 (99.70; 213.10)
HSV (III)	11.90 (9.25; 16.39)	18.50 (14.10; 25.60)	43.11 (38.77; 47.65)	104.10 (89.20; 162.22)
HCR (IV)	13.51 (12.31; 16.31)	19.70 (14.00; 27.90)	65.09 (42.77; 77.99)	98.50 (80.25; 111.35)
Control group	5.44 (2.02; 7.28)	13.00 (10.70; 15.70)	25.22 (21.56; 28.97)	231.80 (176.40; 301.40)
H, p	H=97.00; p<0.001*	H=19.77; p<0.001*	H=88.94; p<0.001*	H=38.74; p<0.001*
Multiple groups' comparisons	p _{I-II, I-III, I-IV} <0.001* p _{II-c, III-c, IV-c} <0.001*	p _{I-III} <0.05* p _{II-c, III-c, IV-c} <0.05*	p _{I-II, I-III, I-IV, I-c} <0.05* p _{II-IV, II-c} <0.05* p _{III-c, IV-c} <0.001*	p _{I-III, I-IV, I-c} <0.05* p _{II-IV, II-c} <0.05* p _{III-c, IV-c} <0.05*
Note 1. H – Kruskal-Wallis test and p-level of its significance. Note 2. * – statistically significant result.				

Analysis of biomarkers of vasoconstriction (ET-1, Arg1, and TxA₂) depending on the results of FMD in different COVID-19 groups revealed the same patterns: disease severity was associated with higher levels of ET-1, Arg-1, and TxA₂. Notably, ET-1 levels were significantly higher in the vasoconstriction-only groups HQM and HMO (see Table 5). In contrast, for Arg1 and TxA₂, such vasoconstriction-related elevations were typical in patients with severe COVID-19 (Table 5). Concurrently, severe COVID-19 leads to decreased PGI₂ synthesis and vasoconstriction.

Table 5 – ET-1, Arg-1, TxA₂ and PGI₂ levels depending on COVID-19 severity and results of FMD test

Parameter		HQM (I)	HMO (II)	HSV (III)	HCR (IV)	Control group	H, p	p
Endothelin-1, pg/mL	Vaso-constriction (VC)	8.72# (3.72; 10.94)	11.67# (9.11; 14.08)	11.83 (9.25; 16.67)	13.51 (12.31; 16.31)	5.44 (0.44; 8.62)	H=33.95; p<0.001*	p _{I-II, I-III, I-IV} <0.05* p _{II-c, III-c, IV-c} <0.05*
	Normal	5.97 (2.55; 10.38)	10.65 (8.42; 13.53)	11.93 (10.27; 13.34)	–	5.89 (2.02; 7.28)	H=52.11; p<0.001*	p _{I-II} <0.05* p _{II-c, III-c} <0.05*
Arginase 1, ng/mL	VC	16.25# (11.45; 21.10)	15.60 (12.40; 21.90)	20.30# (13.80; 25.60)	19.70 (14.00; 27.90)	13.60 (9.60; 15.70)	H=9.14; p=0.058	–
	Normal	14.75 (10.60; 19.05)	16.20 (11.20; 23.00)	16.60 (14.10; 16.70)	–	12.90 (10.80; 15.50)	H=6.62; p=0.085	–
Thromboxane A ₂ , pg/mL	VC	30.81 (25.67; 35.67)	38.00 (33.23; 45.46)	53.34# (43.16; 59.53)	65.09 (42.77; 77.99)	24.48 (23.37; 31.44)	H=41.08; p<0.001*	p _{I-III, I-IV} <0.05* p _{II-IV, II-c, III-IV, III-c, IV-c} <0.05*
	Normal	33.92 (29.34; 39.08)	38.61 (34.56; 43.67)	42.31 (38.77; 46.44)	–	25.38 (19.87; 28.97)	H=44.39; p<0.001*	p _{I-II, I-III, I-c} <0.05* p _{II-c, III-c, IV-c} <0.05*
Prostacyclin, pg/mL	VC	179.80 (128.70; 263.85)	120.70 (98.38; 198.45)	100.60# (89.20; 138.10)	98.50 (80.25; 111.35)	199.53 (171.88; 251.35)	H=19.42; p<0.001*	p _{I-III, I-IV} <0.05* p _{IV-c} <0.05*

	Normal	156.70 (102.30; 189.30)	131.85 (99.75; 213.10)	156.45 (107.00; 231.30)	–	232.10 (176.40; 301.40)	H=12.29; p=0.007*	p I-c, II-c, III-c, <0.05*
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Note 1. VC – vasoconstriction in Flow Mediated Dilation (FMD) test.
Note 2. H – Kruskal-Wallis test and p-level of its significance.
Note 3. * – significant results were observed.
Note 4. # – statistically significant difference between vasoconstriction and normal reaction in FMD.

Analysis of echocardiographic data showed no significant deviations from control parameters in patients in the HQM, HMO, and HSV groups. Only in patients of the HCR significant deviations of all echocardiography parameters were found, except for the ejection fraction (see **Fig. 3**).

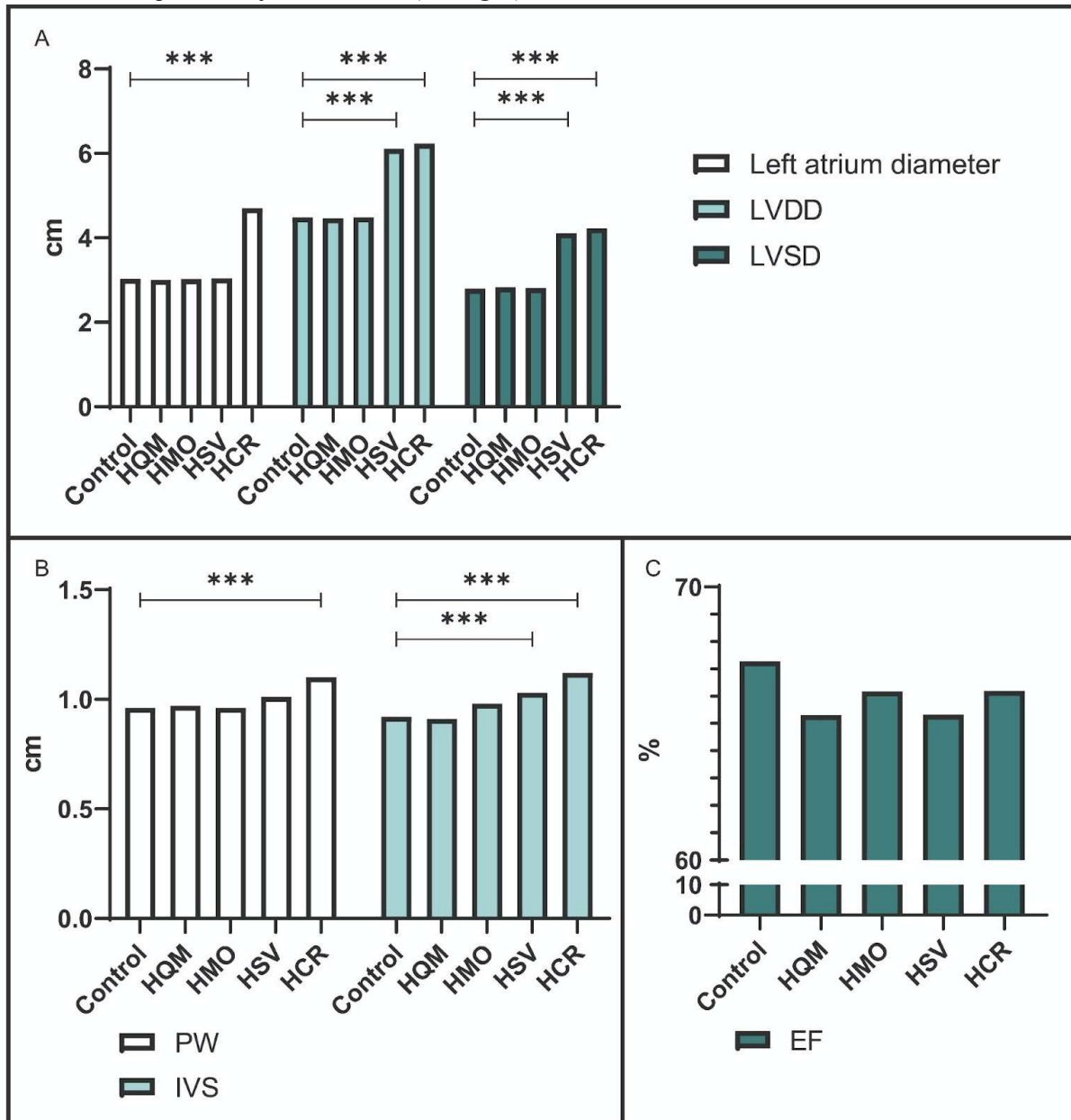


Figure 3. Echocardiography Parameters: A- Left atrium diameter, LVDD – left ventricular diastolic diameter and LVSD – Left ventricular systolic diameter; B - PW – posterior wall, IVS – interventricular septum; C - EF – Ejection fraction.

4. Discussion.

Patients with COVID-19 are at an increased risk of thrombotic complications, which may arise through multiple pathophysiological pathways, including ED [30, 31, 32]. This is largely attributable to the fact that endothelial cells represent one of the primary targets of SARS-CoV-2. Endothelial injury promotes activation of the coagulation cascade, thereby facilitating thrombus formation [33].

Vasoconstriction represents a key step in primary hemostasis and is regulated by a complex interplay of mediators, many of which are derived from the endothelium [34]. Under physiological conditions, vascular tone is maintained by a balance between vasoconstrictive and vasodilatory factors. Endothelium-derived mediators such as ET-1, TxA₂, and NO play a critical role in this regulation and have been implicated in various pathological states, including obesity, hypertension, and diabetes mellitus [34, 35, 36].

In the present study, we demonstrated that, in the post-COVID-19 period, levels of endothelium-derived biomarkers are closely associated with disease severity, supporting the concept of persistent endothelial dysfunction following SARS-CoV-2 infection.

Non-invasive assessment of ED using flow-mediated dilation (FMD) of the brachial artery (BA) in response to ischemia-reperfusion is widely applied in clinical practice [37]. This method provides a reliable functional assessment of the arterial system and is considered a valid surrogate marker of endothelium-dependent vasodilation [38, 39]. Previous studies have demonstrated a strong correlation between brachial artery FMD and coronary endothelial function, suggesting its utility in assessing coronary artery dysfunction [40]. Moreover, FMD has been proposed as a diagnostic tool comparable to exercise ECG for identifying patients at risk of coronary artery disease [41]. Impaired FMD responses have been associated with established cardiovascular risk factors, including smoking, diabetes, aging, and hypercholesterolemia, as well as with overt cardiovascular disease [37].

However, several limitations of the FMD test should be considered when interpreting the results. The method may be influenced by cardiac arrhythmias, reduced left ventricular ejection fraction, bronchial obstructive diseases, and peripheral arterial occlusive disease of the upper limbs, as well as by increased psycho-emotional stress [37, 42]. In addition, FMD primarily reflects NO-mediated vasodilatory capacity and does not fully capture other aspects of endothelial function.

Our findings indicate that patients in the post-COVID-19 period exhibit significant alterations in endothelium-dependent vasodilation, including changes in brachial artery diameter and peak systolic velocity. These alterations were strongly associated with disease severity and were most pronounced in patients with severe (Group III, HSV) and critical (Group IV, HCR) COVID-19. Baseline hemodynamic parameters in these groups further revealed increased peripheral vascular resistance and impaired arterial elasticity, consistent with a vasoconstrictive phenotype.

Biochemical markers play a crucial role in assessing vascular wall integrity and its capacity for vasodilation and vasoconstriction. ED represents an early pathogenic event in the development of vascular complications and is primarily associated with reduced bioavailability of vasodilators, particularly NO and PGI₂ [43]. NO is synthesized from L-arginine by three nitric oxide synthase (NOS) isoforms: endothelial (eNOS), neuronal (nNOS), and inducible (iNOS). Arginase 1 (Arg1), an enzyme that hydrolyzes L-arginine, competes with NOS for this common substrate, thereby limiting NO production [44]. This mechanism has important clinical implications. For example, adjunctive L-arginine therapy has been shown to significantly reduce hospital stay and the need for respiratory support in patients with severe COVID-19 [45]. Increased Arg1 expression has also been documented in conditions such as diabetes mellitus, where it contributes to reduced NO-mediated vasodilation and promotes a vasoconstrictive state [49]. Similarly, in obesity, Arg1 is implicated in the development of ED and hypertension through reduced NO bioavailability and increased oxidative stress [47, 48]. Reduced NO levels further shift the vascular balance toward vasoconstriction, partly mediated by ET-1.

ET-1 is the predominant endothelin isoform produced by the vascular endothelium and is one of the most potent endogenous vasoconstrictors [49, 50]. Elevated ET-1 levels have been associated with impaired coronary perfusion and are considered a key marker of ED in coronary microvascular dysfunction [51]. In the DAPA-HF trial, higher baseline ET-1 levels were linked to an increased risk of worsening heart failure, cardiovascular death, and progressive renal dysfunction [52]. In our study, elevated ET-1 levels were associated with greater COVID-19 severity, supporting its role as a marker of persistent endothelial dysfunction in the post-COVID-19 period.

Thromboxane A₂ (TxA₂) is another key mediator involved in both physiological and pathological processes, including hypertension, coronary and cerebral vasospasm, atherosclerosis, myocardial infarction, and arterial thrombosis [53]. TxA₂ is synthesized from arachidonic acid through sequential enzymatic action involving phospholipase A₂, cyclooxygenases (COX-1/COX-2), and thromboxane synthase (TxAS) [54, 55]. Experimental studies suggest that increased vascular responsiveness to TxA₂ contributes to enhanced vasoconstriction and elevated blood pressure [56, 57].

In contrast, prostacyclin (PGI₂) exerts vasodilatory and antiplatelet effects, counterbalancing the actions of TxA₂ and maintaining vascular homeostasis [58]. PGI₂ binds to the prostacyclin (IP) receptor, activating G protein-coupled signaling pathways that increase intracellular cAMP levels and reduce calcium concentration, leading to vascular smooth muscle relaxation [59, 60]. *In vivo*, PGI₂ administration decreases coronary vascular resistance, increases coronary blood flow, and lowers aortic pressure [61].

Our findings demonstrate that post-COVID-19 patients with vasoconstrictive responses identified by the FMD test exhibit significantly elevated levels of vasoconstrictor markers, including ET-1, Arg1, and TxA₂, alongside reduced levels of the vasodilator PGI₂. Importantly, these alterations were strongly associated with disease severity. As COVID-

19 severity increased, a progressive rise in vasoconstrictor mediators and a concomitant decline in vasodilatory capacity were observed. These results support the presence of a sustained imbalance between vasoconstrictive and vasodilatory pathways in the post-COVID-19 period, reflecting persistent endothelial dysfunction and contributing to increased cardiovascular risk.

Echocardiographic findings in patients from Groups I (HQM), II (HMO), and III (HSV) were not statistically significant, suggesting a limited impact of COVID-19 on central hemodynamic parameters in these groups. But patients in Group IV (HCR) demonstrated significant echocardiographic alterations, which may be attributed to severe disease, older age, and a higher burden of underlying cardiovascular pathology. Notably, patients in this group were older (mean age 58.38 ± 11.05 years) and exhibited higher systolic and diastolic blood pressure.

Assessment of endothelium-dependent vasodilation using FMD represents a practical and cost-effective non-invasive approach for detecting endothelial dysfunction in post-COVID-19 patients. This method enables the identification of individuals at increased risk of cardiovascular complications.

However, several limitations of FMD should be acknowledged. Test accuracy may be affected by factors such as respiratory pathology, heart rate variability, and peripheral arterial disease of the upper extremities. Despite these limitations, the integration of FMD with biochemical markers, including ET-1, Arg1, TxA₂, and PGI₂, enhances the overall reliability of endothelial function assessment and provides a more comprehensive evaluation of vascular health.

5. Conclusions

Convalescent patients in the early post-COVID-19 period exhibit pronounced vasoconstrictive responses during flow-mediated dilation (FMD), accompanied by persistent alterations in biochemical markers of endothelial dysfunction. These vascular abnormalities are strongly associated with the initial severity of COVID-19 and reflect a sustained shift toward a vasoconstrictive phenotype, characterized by elevated levels of ET-1, TxA₂, and Arg1 alongside reduced prostacyclin (PGI₂). The concordance between functional impairment and biochemical dysregulation underscores a mechanistic link driving persistent endothelial injury following SARS-CoV-2 infection.

From a clinical standpoint, FMD represents a practical, non-invasive, and cost-effective tool for the early detection of endothelial dysfunction and identification of patients at increased cardiovascular risk. Importantly, the integration of functional vascular assessment with biomarker profiling provides a more comprehensive evaluation of endothelial health, enabling improved risk stratification and personalized follow-up strategies. These findings highlight the importance of incorporating endothelial function assessment into post-COVID care pathways and support the development of targeted preventive interventions aimed at reducing long-term cardiovascular complications.

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Institutional Review Board Statement

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Informed Consent Statement

Signed informed consent was obtained from all involved persons.

Conflicts of Interest

The authors declare that there is no conflict of interest.

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