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Endothelial function, arterial stiffness and heart rate variability of patients with cardiovascular diseases hospitalized due to COVID-19

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ABSTRACT

Background: The novel coronavirus disease (COVID-19) may cause vascular (e.g., endothelial dysfunction, and arterial stiffness), cardiac, autonomic (e.g., heart rate variability [HRV]), and systemic inflammatory response via direct viral attack, hypoxia-induced injury, or immunological dysregulation, especially in those patients with pre-existing cardiovascular diseases (CVD). However, to date, no study has shown prevalence of endothelial dysfunction, arterial stiffness and heart rate variability assessed by bedside peripheral arterial tonometry in patients with previous CVD hospitalized in the acute phase of COVID-19.
Objective: This study aimed to assess the prevalence of endothelial dysfunction, arterial stiffness, and altered HRV in patients with CVD hospitalized due to COVID-19.
Methods: This cross-sectional study was conducted from July 2020 to February 2021. Included male and female adult patients aged 40 to 60 years with previous CVD and diagnosed with COVID-19. Anthropometric data, comorbidities, and blood tests were analyzed. Endothelial function, arterial stiffness, and HRV were assessed using peripheral arterial tonometry (PAT), and the statistical significance was set at 5%.
Results: Fourteen (51.8%) patients presented endothelial dysfunction (reactive hyperemia index = 1.2 ± 0.3) and enhancement in the high-frequency component of HRV ($p < 0.05$). There was a high prevalence of endothelial dysfunction, especially in patients with chronic heart failure (10 (71.4%)). Patients with preserved endothelial function showed a high augmentation index normalized to a heart rate of 75 bpm ($p < 0.01$), suggesting arterial stiffness.
Conclusion: Patients with CVD hospitalized due to COVID-19 presented endothelial dysfunction assessed using PAT, which could be used as a biomarker for arterial stiffness and altered HRV. The possibility of detecting vascular and autonomic changes during phase II of COVID-19 may help to prevent possible long-term complications.

Introduction

SARS-CoV-2 is the etiologic agent of the coronavirus disease 2019 (COVID-19), affecting mainly the respiratory system. However, clinical and epidemiological evidence suggests it also impairs the cardiovascular system (e.g., myocardial injuries, myocarditis, stress myocardiopathy, heart failure, shock, and arrhythmia).[1–3] Patients with increased inflammatory markers (e.g., diabetes mellitus and obesity) and cardiovascular diseases (CVD) with high serum levels or non-regulated expression of angiotensin-converting enzyme II present greater severity of COVID-19.[4]

COVID-19 may impair the cardiovascular system directly via viral toxicity or indirectly (e.g., hypoxemia, imbalance between high metabolic demand and low cardiac store, stimulation of sympathetic nervous system, thrombogenesis, systemic inflammation, and dysregulated immune and renin-angiotensin system).[1,2] SARS-CoV-2 acts in angiotensin-converting enzyme II receptors (the main receptor for infection), inhibiting the conversion of angiotensin II to angiotensin 1-7 and triggering harmful events to the vascular wall (e.g.,

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Abbreviations list

AIx	Augmentation index
AIx@75%	Augmentation index normalized to a heart rate of 75 beats per minute
CHF	chronic heart failure
COVID-19	novel coronavirus disease
CVD	cardiovascular diseases
HF	high frequency
HRV	heart rate variability
LF/HF	low and high ratio
LF	low frequency
LnRHI	natural logarithm of reactive hyperemia index
MeanNN	mean of NN-intervals
NN	normal sinus rhythm pulse intervals
PAT	Peripheral arterial tonometry
PAT	peripheral artery tonometry
PNN50	percentage of successive NN intervals that differ by more than 50 ms
RHI	Reactive hyperemia index
RMSSD	root-mean-square of successive differences
SDNN	standard deviation of NN-intervals
SpO2	Peripheral oxygen saturation

endothelial dysfunction, increased platelets aggregation, and endothelial thrombogenic activity).[3,5]

Viral attacks decrease nitric oxide bioavailability (i.e., by reducing production or increasing degradation by reactive oxygen species or both) and trigger immunological reactions, unbalancing the vascular homeostasis, altering the vascular wall, and inhibiting anti-proliferative, antithrombotic, and antiatherogenic phenotypes.[6] Endothelial dysfunction is observed during and after COVID-19.[7–10] After weeks of the infection, an impaired systemic vascular function was also observed in healthy adults.[8,11] Consequently, these alterations due to COVID-19 may lead to arterial stiffness even in the acute phase.[12,13]

In this context, endothelial function assessment is recommended during the follow-up of patients with COVID-19 to detect vascular changes and possible long-term complications.[11,14] Endothelial function can be assessed using peripheral artery tonometry (PAT), which is new, noninvasive, reproducible, and clinically accessible. PAT is a quantitative and operator-free clinical test with a standardized method, automated analysis, and reproducible data.[15,16] It can also assess arterial stiffness using the augmentation index (AIx). The Alx is widely used as an index of pulse wave reflection using the pulse waveform at rest as a reference. Lower Aix reflects better arterial elasticity.[17]

Angiotensin-II enhances the sympathetic autonomic tonus in the central nervous system by interacting with angiotensin 1 receptors (AT_1).[18] Autonomic dysfunctions were observed after COVID-19 infection, including palpitations, fatigue, orthostatic intolerance, dizziness, hyperhidrosis, postural orthostatic tachycardia, and syncope.[19,20] Thus, monitoring the cardiac autonomic function of patients with COVID-19 may help diagnose adverse cardiovascular outcomes and autonomic dysfunctions. Heart rate variability (HRV) is a simple, objective, reproducible, low-cost, and validated tool to assess autonomic dysfunction, being useful in clinical practice as a rapid marker for diagnosis and prognosis.[21]

Literature lacks studies on vascular and autonomic dysfunctions in patients with CVD hospitalized due to COVID-19. No study showed prevalence of vascular function changes (i.e., endothelial function and markers of arterial stiffness) and heart rate variability evaluated by bedside PAT in patients with previous cardiovascular diseases hospitalized due to COVID-19. Thus, this study aimed to assess endothelial function, arterial stiffness, and HRV of adult patients with CVD hospitalized due to COVID-19.

Methods

A cross-sectional study was conducted according to the Strengthening the Report of Observational Studies in Epidemiology (STROBE) and the Declaration of Helsinki and approved by the research ethics committee of the Federal University of Pernambuco (no. 4.169.772). Data were collected between July 2020 and February 2021 from a convenience sample in the Hospital Agamenon Magalhães (Pernambuco, Brazil) along the Department of Physical Therapy of the Federal University of Pernambuco.

Adult patients of both sexes aged between 40 and 60 years with pre-existing CVD (i.e., hypertension, coronary disease, and heart failure) and stage II of COVID-19 (RT-PCR) (SIDDIQI and MEHRA, 2020) were included.[6] Patients with the following conditions were excluded: chronic obstructive pulmonary disease, asthma, previous stroke, psychiatric disorder, cancer; mastectomy, chronic kidney disease, pacemakers or arteriovenous fistula, psychomotor agitation; significant respiratory problems (respiratory frequency $>$ 25 rpm, use of accessory muscles in respiration, nasal flaring, decreased peripheral oxygen saturation ($SpO_2 < 90\%$) in pulse oximetry), hemodynamic instability (systolic blood pressure $<$ 90 mmHg, drop in systolic blood pressure $>$ 40 mmHg, heart rate $>$ 90 bpm, decreased level of consciousness, or cold skin), invasive or non invasive mechanical ventilation, or other clinical condition hindering adequate positioning for HRV assessment or endothelial function.

Patients were invited to participate in the study, informed about all stages, proceedings, possibility of interruption at any moment, and signed the informed consent form. Data were collected in the same or next day. Patients with short period of COVID-19 symptoms were prioritized.

In one day, patients were interviewed to collect anamnesis, medical history, signs and symptoms of COVID-19, comorbidities, personal and anthropometric data, and vital signs. Comorbidities (e.g., diabetes mellitus, acute kidney injury, obesity, dyslipidemia, depression, rheumatic disease, family history of coronary artery disease, arrhythmias, and Chagas disease) were also checked on medical records. Next, PAT assessed endothelial function, arterial stiffness, and HRV; and blood tests and drugs were recorded. Patients were followed-up until hospital discharge, transference to other units, or death, and adverse cardiac events or other complications were verified on medical records on the last day.

We used a digital scale and mobile stadiometer (Welmy® W300, São Paulo, Brazil) to assess anthropometric measures and a multiparameter monitor (DIXTAL 2023, Manaus, Brazil) to assess vital signs.

Blood pressure was measured from the dominant arm five minutes before PAT. PAT is a noninvasive method for assessing pulse wave amplitude in response to reactive hyperemia. It was conducted using the EndoPAT-2000® (Itamar Medical version 3.4.4, Cesareia, Israel), a pneumatic plethysmograph that applies uniform pressure over the distal finger and assesses changes in pulsatile arterial volume. Patients were placed in a quiet room, positioned in elevated dorsal decubitus (30° of bed inclination) for at least 20 minutes before assessments, and requested to remain as quiet as possible. An occlusive cuff was positioned two centimeters above the cubital fossa in the non-dominant arm (i.e., the assessed arm), while the dominant arm was the control. Biosensors were positioned at the tip of both index fingers. After assessing the pulse wave amplitude in each finger, the cuff was inflated 60 mmHg above systolic arterial pressure (200 to 300 mmHg) to interrupt the arterial flux for five minutes. The cuff was abruptly deflated to induce reactive hyperemia, and PAT of both arms was recorded for at least five minutes.[22,23] The reactive hyperemia index (RHI) was calculated as the ratio between

hyperemic and baseline pressures in the assessed and control arms. RHI values under 1.67 (or 0.51 in the natural logarithmic [LnRHI]) indicated endothelial dysfunction.[22]

The EndoPAT-2000® (version 3.1.2) automatically calculated AIx based on the rest pulse waveform. AIx is described as the difference of pressure between the reflection wave peak (P2) and the systolic wave peak (P1), expressed as the percentage of P1 by the formula $\left(\text{AIx} = \frac{[P2-P1]}{P1}\ x\ 100\right)$. AIx was normalized to a heart rate of 75 bpm (AIx@75) for final analysis. The algorithm of EndoPAT-2000® software also automatically calculated LnRHI and AIx@75.[17]

HRV was also assessed using the EndoPAT-2000® via changes in pulsative volume based on waveforms from the plethysmography to derivate HRV parameters and estimate reflex sensibility. The frequency domain considers two spectral components and physiological origins: low-frequency (LF; 0.04 to 0.15 Hz; sympathetic modulation) and high-frequency (HF; 0.15 to 0.40 Hz; parasympathetic modulation). The LF/HF ratio reflects the sympathetic balance. The time domain considers the mean of normal sinus rhythm pulse-intervals (MeanNN), the standard deviation of NN-intervals (SDNN), percentage of successive NN-intervals above 50 ms (PNN50), root-mean-square of successive differences (RMSSD) of NN-intervals, and triangular index. RMSSD represents the predominant parasympathetic modulation in cardiac function.[24]

Blood tests (i.e., platelets, C-reactive protein, D-dimer, and troponin) were collected during hospitalization on the same day of PAT. We recorded cardiac events (arrhythmia, acute heart infarction, and cardiorespiratory arrest) and other complications (coronary angioplasty, bioprosthetic valve dysfunction, valve replacement, deep venous thrombosis, pulmonary thromboembolism, stroke, convulsion, pneumothorax, and pleural effusion). The need for oxygen therapy and invasive mechanical ventilation, transference to the intensive care unit, and hospitalization duration were also recorded.

Patients were divided according to endothelial function into preserved and dysfunctional groups. Quantitative variables were presented as mean and 95% confidence interval or median and interquartile range, and qualitative variables were presented as relative or absolute frequency. Shapiro-Wilk and Levene tests verified data normality. Unpaired t-test and Mann-Whitney test compared, respectively, parametric and non-parametric data. The Chi-square test compared qualitative variables, and Fisher's exact test compared variables without normal distribution. Pearson's or Kendall ($n < 30$) rank correlation coefficient analyzed correlations between vascular function and HRV (LnRHI *vs.* HRV; AIx@75 *vs.* HRV) and anthropometric data (weiht, sex, age, BMI). Correlation coefficients were interpreted according to a previous study (r=0 (null), $0<r\leq0,3$ (weak), $0,3<r\leq0,6$ (moderate), $0,6<r\leq0,9$ (strong), $0,9<r<1$ (very strong) e r=1 (perfect).[25] Missing data were treated as complete case analysis or listwise deletion, and statistical significance was set at 5%.

Results

The Hospital admitted 1,126 patients with CVD and COVID-19 during data collection, and 307 were eligible for the study. Thirteen patients refused to participate, and 39 were excluded due to symptoms hindering the assessment (e.g., dyspnea or hemodynamic instability). The main cause of sample loss (n = 203) was the delay of results from RT-PCR tests due to the high demand in the hospital, delaying the assessment up to seven days after the first COVID-19 symptom. Although 52 patients were initially included in the study, 11 were excluded due to incomplete blood tests on admission, and 14 were not followed up due to transfer to another unit. Thus, 27 patients were analyzed, and 14 (51.8%) presented endothelial dysfunction.

Anthropometric data, blood tests at admission, and medications in use were similar between both groups (Table A.1). Patients with preserved endothelial function presented high systolic blood pressure and mean blood pressure at the initial assessment. Hypertension was the most prevalent CVD in all patients with preserved endothelial function and 78.6% of patients with endothelial dysfunction.

Ten (71.4%) patients with endothelial dysfunction presented chronic heart failure (CHF) at hospital admission. The endothelial dysfunction group showed a higher prevalence of CHF with reduced ejection fraction and mildly reduced ejection fraction than the preserved endothelial function group. Diabetes mellitus was the most prevalent comorbidity in both groups (46.2% of patients with preserved endothelial function and 42.9% with endothelial dysfunction). Anthropometric data, LnRHI, and AIx@75 were not correlated in any group.

Patients in the endothelial dysfunction group had a higher percentage (57.1% versus 42.9% preserved endothelial function group. p=0.07) of adverse cardiac events, mainly acute myocardial infarction (p=0.38) and high ventricular response atrial fibrillation (p=0.48).

Fig. A.1 shows data on endothelial function. The median of LnRHI was lower in the endothelial dysfunction group than in preserved endothelial function group (0.29 [0.06 to 0.42] *vs.* 0.58 [0.54 to 0.88], respectively; $p < 0.01$). Also, AIx@75 was higher in patients with preserved endothelial function than in patients with endothelial dysfunction (-3.4 ± 16.4 *vs.* -18 ± 13.5, respectively; p=0.03), suggesting a high arterial stiffness index in the endothelial dysfunction group.

The HF component of HRV showed a negative and moderate correlation with LnRHI (r = -0.35; p = 0.01) and AIx@75 (r = -0.41; p = 0.03) in patients with endothelial dysfunction. HRV data are shown in Table A.2. Patients with endothelial dysfunction presented enhancement in the HF component. Other components of frequency and time domains showed no differences.

Discussion

The main results of this study were: the high percentage of patients with CVD hospitalized due to COVID-19 presented endothelial dysfunction, especially patients with CHF (the most prevalent CVD); many patients with preserved endothelial function had arterial stiffness, and the HF component of HRV was correlated with endothelial dysfunction.

The prevalence (51.8%) of endothelial dysfunction in patients with previous CVD in the acute phase of COVID-19 is relevant finding in this study. The endothelial dysfunction may be an important cardiovascular marker in these patients. Studies have shown endothelial dysfunction in patients with COVID-19 after weeks or even months of infection.[8,11,26,27] In Riou et al. (2021), patients presented altered endothelial function after three months of COVID-19, and half of the survivors showed decreased flow-mediated dilation. These data corroborated the evidence that endothelial dysfunction is a risk factor for COVID-19 pathogenesis since the virus targets endothelial cells, leading to systemic endothelial inflammation in the long term.[26]

In a cross-sectional observational study, patients with COVID-19 assessed using flow-mediated nitroglycerine-induced dilation presented inflammatory vasculopathy with similar values and inflammatory alterations to patients with clinically relevant CVD.[28] However, similar to our study, they did not demonstrate a causal relationship between vascular impairment and infection since the endothelial function was not studied before COVID-19. Moreover, endothelial dysfunction in this population may be related to other atherosclerotic causes influencing vascular function and reactivity (e.g., the main CVD, hyperlipidemia, diabetes mellitus, obesity, and sedentary lifestyle), which were not controlled in the present study.[29]

Several methods to assess endothelial function cause reactive hyperemia via the functional biosensor of the endothelium, nitric oxide (directly or by the subsequent cytokine storm), activation of immune cells, oxidative stress, inflammation, or alternative pathways (e.g., K^+ channels and Na/K-ATPase).[8,30] In the present study, endothelial dysfunction was detected using bedside PAT, hich may be

useful for diagnosing alterations in the vascular wall. PAT is easy-to-use, operator-free, and has acceptable reproducibility in adults compared with other noninvasive assessment methods.[16] Also, it may early diagnose endothelial alterations and was considered an independent predictor for adverse cardiovascular events.[31–33]

COVID-19 may cause endothelial injury and alterations in the vascular wall (i.e., damage to tunica media), resulting in arterial stiffness.[12] In this study, patients with preserved endothelial function presented AIx between -10% and 10% and increased AIx@75, characterizing arterial stiffness.[15] This finding may be related to the high prevalence of hypertension in the group, which is one of the main determinants of arterial stiffness.

Hypertension is characterized by early vascular function and structure alterations more severe than expected in the normal aging process.[34] The severity of inflammation from CVD is directly related to arterial stiffness, leading to a cycle.[35] In this cycle, the increase of intravascular pulsatile pressure damages the vascular wall and causes arterial stiffness, favoring atherosclerosis and increasing the inflammatory component and stiffening effect.[35] Thus, pre-existing atherosclerosis is an independent risk factor for severe COVID-19. However, whether patients with COVID-19 are predisposed to early atherosclerosis is not fully understood.[35]

Patients in the endothelial dysfunction group had a higher percentage of adverse cardiac events (eg, acute myocardial infarction) but we believe that we cannot establish a cause-effect relationship arising from the change in endothelial function caused by COVID-19 due to the underlying disease of these patients.

No differences were found between groups regarding the clinical severity of COVID-19. Riou et al. (2021) found impaired endothelial function in patients needing conventional and intensive care unit hospitalization after COVID-19. In their study, impaired endothelial function was not associated with COVID-19, intensive care unit hospitalization, prolonged hospitalization, or pulmonary damage, despite the inexistence of previous CVD in the studied population.[26] Endothelial dysfunction may be related to the severity of symptoms and clinical evolution in patients with CHF.[36] Matsue et al. (2013) demonstrated that LnRHI and PAT might predict adverse cardiovascular events in patients with CHF, highlighting their significant role in diagnosing high-risk populations.

Patients with endothelial dysfunction presented enhancement of HF component of HRV; however, other components did not differ between groups. Also, HF showed a negative and moderate correlation with LnRHI and AIx@75. HF, SDNN, RMSSD, and PNN50 represent the parasympathetic activity, while LF represents the sympathetic and parasympathetic activities.[24,37] Thus, patients with COVID-19 and endothelial dysfunction may have imbalanced autonomic responses with parasympathetic predominance.

Kalivaperumal et al. (2020) also indicated increased parasympathetic activity in patients with COVID-19; however, they only assessed SDNN and RMSSD components. Aragón-Benedí et al. (2021) suggested that the suppressed sympathetic and predominant parasympathetic activity induced compensatory anti-inflammatory response in patients with COVID-19.[38] Cardiovascular dysautonomia in post-COVID-19 is characterized by imbalanced sympathetic or parasympathetic activity and may be attributed to virus-related damage, cytokine storm, or immune-mediated dysregulation in the autonomic nervous system. Also, autonomic symptoms of COVID-19 (e.g., deconditioning, hypovolemia, hyperadrenergic state, or immune-mediated viral damage) are often multifactorial.[39]

In view of the above, we highlight the importance of evaluating vascular bed alterations in the acute phase of COVID-19 in order to prevent endothelial dysfunction and/or its evolution (e.g. arterial stiffness), especially in those patients who already have some degree of dysfunction, such as those with previous cardiovascular diseases. In addition, autonomic alterations such as cardiovascular dysautonomia may already be present at the time of hospitalization and be better controlled when diagnosed as early as possible. Thus, the detection of changes in the acute phase can contribute to better drug management of the disease, resulting in a decrease in its severity. For this, the PAT proved to be an instrument that can be used for the diagnosis of autonomic and vascular alterations still in the acute phase of COVID-19.

Limitations

We could not assess endothelial function and arterial stiffness before COVID-19 and after hospitalization (e.g., after discharge). Thus, the causes and actual consequences of endothelial dysfunction could not be determined in these patients with CVD (i.e., due to the underlying disease or vascular alterations from COVID-19). A high sample loss occurred due to a delay in RT-PCR results, mainly because of the high hospital demand at the beginning of the pandemic. Thus, the small sample size hindered data extrapolation. Also, CHF and hypertension may be confounding variables. Although cardiovascular risk factors were not significantly different in patients, we could not exclude the potential bias from these factors affecting endothelial dysfunction results.

Conclusion

The present study suggested that bedside PAT was useful for assessing endothelial dysfunction, which may be an early marker of arterial stiffness and altered HRV in patients with CVD hospitalized due to stage II of COVID-19. Further longitudinal studies with longer follow-ups may help elucidate the potential role of endothelial function in the prognosis of patients with COVID-19 and whether its dysfunctions are persistent among susceptible populations. Moreover, results may help develop therapies to prevent endothelial deterioration and improve patient outcomes.

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Conflict of Interest

None

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Appendices

Fig. A1
Tables A1 and A2.

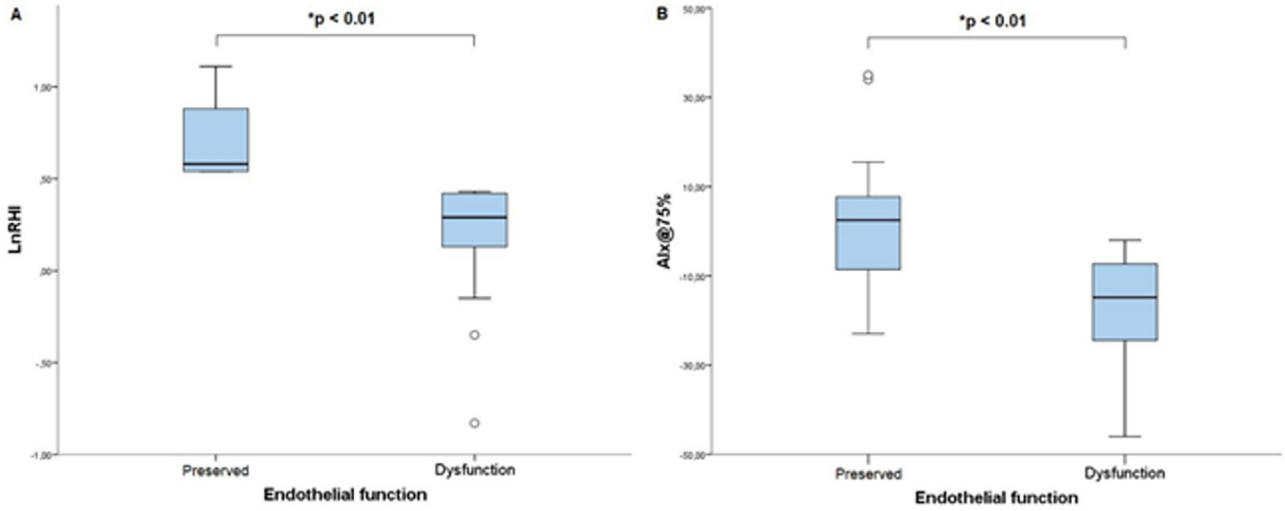

Fig. A1. Endothelial function of patients with cardiovascular diseases hospitalized due to COVID-19. A) Endothelial function assessed using the natural logarithm of reactive hyperemia (LnRHI) in preserved endothelial function and endothelial dysfunction groups. B) Comparison of endothelial function between groups using the augmentation index normalized to a heart rate of 75 beats per minute (AIx@75). Data are expressed as median and interquartile ranges.

Table A1
Sample characteristics (anthropometric data, vital signs, medications, blood tests, cardiovascular diseases, comorbidities, adverse cardiac events, and complications).

	Preserved endothelial function (n=13)		Endothelial dysfunction (n=14)	
	Mean [CI] or median (p25-p75) or n (%)		Mean [CI] or median (p25-p75) or n (%)	P-value
Age (Years)	58 (57–59)		53 (50–59)	0.22[α]
Sexo (%)				
Women	4 (30.8%)		5 (35.7%)	1.00[†]
Men	9 (69.2%)		9 (64.3%)	
Weight (kg)	72 [65.5-79.8]		80.6 [68.9-102.1]	0.23*
Body mass index (kg/m^2)	24.5 (23.6–27.8)		27.3 (24.9–34.3)	0.22[α]
Waist circunference (cm)	96 [85.4-106.7]		93.9 [77.5-110.2]	0.80*
Vital Signs				
Systolic blood pressure (mmHg)	138.4 [127.2-149.7]		120 [108.9-131]	0,01*
Diastolic blood pressure (mmHg)	79.2 [74.6-83.8]		76.4 [71.5-81.2]	0,37*
Mean blood pressure (mmHg)	99.4 [93.8-105.1]		90.9 [84.4-97.4]	0.04*
Heart rate (bpm)	81.7 [72.7-90.7]		83.6 [72.8-94.4]	0,77*
Breath rate (ipm)	18,4 [16.9-19.9]		18,3 [16.9-19.7]	0.91*
Peripheral oxygen saturation (%)	95.2 [93.9-96.5]		95.3 [93.9-96.8]	0.89*
Laboratory Tests				
C-reactive protein (mg/L)	7.7 (5.3–9)		6.3 (4.2–9)	0.47[α]
Platelets (mil/mm^3)	229.1 [188.8-269.4]		259 [204.4-313.5]	0.35*
D-dimero (μd/mL)	0.71 (0.54–1.26)		0.44 (0.2–2.0)	0.36[α]
Troponin (ng/L)	27.7 (6.4–373)		14.4 (5.8–332.8)	0.96[α]
CVD Prevalence (%)				
Hypertension	Yes	13 (100%)	11 (78.6%)	0.22[†]
Coronary disease	Yes	3 (23.1%)	4 (28.6%)	1.00[†]
CHF[‡]	Yes	3 (23.1%)	10 (71.4%)	<0.01[†]
CHF with reduced ejection fraction		0	3 (30%)	
CHF with mildly reduced ejection fraction		2 (66.7%)	4 (40%)	
CHF with preserved ejection fraction		1 (33.3%)	3 (30%)	
LVEF – Patients with CHF (%)		42.6 ± 5.03	41.1 ± 18.6	0.84*
Comorbidities (%)				
Diabetes Mellitus	Yes	6 (46.2%)	6 (42.9%)	0.03[†]
Acute renal lesion	Yes	1 (7.7%)	3 (21.4%)	0.59[†]
Obesity	Yes	1 (7.7%)	2 (14.3%)	1.00[†]
Dyslipidemia	Yes	0 (0%)	2 (14.3%)	0.48[†]
Depression	Yes	0 (0%)	1 (7.1%)	1.00[†]
Rheumatic Disease	Yes	1 (7.7%)	0 (0%)	0.48[†]
Family history of coronary artery disease	Yes	0 (0%)	1 (7.1%)	1.00[†]
Arrhythmias	Yes	1 (7.7%)	4 (28.6%)	0.32[†]
Chagas Disease	Yes	0 (0%)	1 (7.1%)	1.00[†]
Medications				
Angiotensin Receptor blockers		4 (30.8%)	6 (42.9%)	0.69[†]
Calcium Channel Blockers		4 (30.8%)	3 (21.4%)	0.67[†]
Adrenergic agents		1 (7.7%)	1 (7.1%)	1.00[†]

(*continued*)

Table A1 (*Continued*)

	Preserved endothelial function (n=13)	Endothelial dysfunction (n=14)	
Adrenergic beta-Antagonists	4 (30.8%)	7 (50%)	1.03†
Angiotensin-Converting Enzyme Inhibitors	7 (53.8%)	6 (42.9%)	0.52†
Diuretics	6 (46.2%)	11 (78.6%)	0.12†
Antiarrhythmics	0 (0%)	1 (7.1%)	1.00†
Hydroxymethylglutary-CoA reductase inhibitors	2 (15.4%)	8 (57.1%)	0.46†
Isosorbide dinitrate	1 (7.7%)	2 (14.3%)	1.00†
Dexamethasone	7 (53.8%)	7 (50%)	1.00†
Hydrocortisone	1 (7.7%)	2 (14.3%)	1.00†
Methylprednisolone	0 (0%)	1 (7.1%)	1.00†
Ceftriaxone	13 (100%)	14 (100%)	-
Azithromycin	13 (100%)	13 (9.2%)	1.00†
Piperacilin	1 (7.7%)	3 (21.4%)	0.59†
Oseltamivir	0 (0%)	1 (7.1%)	1.00†
Ivermectin	3 (23.1%)	7 (50%)	0.23†
Aspirin	3 (23.1%)	6 (42.9%)	0.42†
Clopidogrel	2 (15.4%)	2 (14.3%)	1.00†
Enoxaparin	13 (100%)	14 (100%)	-
Anticonvulsants	1 (7.7%)	1 (7.7%)	1.00†
Hypnotics and Sedatives	2 (15.4%)	3 (21.4%)	1.00†
Neuromuscular Blocking Agents	2 (15.4%)	2 (14.3%)	1.00†
Bronchdilatador agents	8 (61.5%)	6 (42.9%)	0.94†
Need for oxygen therapy	12 (92.3%)	13 (92.9%)	1.00†
Duration of oxygen therapy (days)	4.9 [3.3-6.5]	5.9 [3.7-8.0]	0.46*
Need for invasive mechanic ventilation	2 (15.4%)	5 (35.7%)	0.38†
ICU admission	5 (38.5%)	9 (64.3%)	0.18†
Days in ICU	10 (5–25,5)	8 (2,5–13,5)	0.43α
Hospitalization length (days)	11 (8–13)	17 (8–29.2)	0.16α
Adverse cardiac events	4 (30.7%)	8 (57.1%)	0.07†
Acute myocardial infarction	2 (15.4%)	5 (35.7%)	0.38†
Fibrilation with high ventricular rate	0 (0%)	2 (14.3%)	0.48†
Cardiorespiratory arrest	2 (15.4%)	1 (7.1%)	0.59†
Complications/Proceedings			
Coronary angioplasty	1 (7.7%)	2 (14.3%)	1.00†
Bioprosthesis dysfunction or valve replacement	0 (0%)	1 (7.1%)	1.00†
Pulmonary thromboembolism	0 (0%)	1 (7.1%)	1.00†
Stroke	1 (7.7%)	1 (7.1%)	1.00†
Convulsion	1 (7.7%)	0 (0%)	0.48†
Pneumothorax	1 (7.7%)	0 (0%)	0.48†
Pleural effusion	1 (7.7%)	0 (0%)	0.48†

95% CI: 95% confidence interval; CVD: cardiovascular diseases; CHF: chronic heart failure; LVEF: left ventricular ejection fraction; ICU: intensive care unit. * = unpaired t-test; † = Chi-square test

α = Mann-Whitney test

1 = classification according to Heidenreich et al., 2022 (CHF with reduced eject fraction: LVEF ≤ 40%; CHF with mildly reduced eject fraction: LVEF between 41% and 19%; CHF with preserved eject fraction: LVEF ≥ 50%).

Table A2
Comparison of the assessment of heart rate variability between groups

	Preserved endothelial function (n=13) Mean [CI] or median (p25-p75)	Endothelial dysfunction (n=14) Mean [CI] or median (p25-p75)	P-value
MeanNN (ms)	757.6 [649.2-865.9]	700.4 [606.2-794.5]	0.39*
SDNN (ms)	29.4 (14.1–41.8)	40.6 (16.1–123.3)	0.23α
RMSSD (ms)	19.3 (12.4–35.8)	30.8 (18.3–147.2)	0.18α
PNN50 (ms)	0 (0–0.6)	0.1 (0–0.4)	0.30α
Triangular index	8.5 (4.1–11.2)	9 (4.5–19.1)	0.55α
LF (ms^2)	97.1 [58.4-135.9]	151.6 [104-199.3]	0.06*
HF (ms^2)	125.9 [55.8-196]	216.1 [160.8-271.5]	0.03*
LF/HF	0.9 (0.3–1.8)	0.5 (0.5–1.1)	0.43α

95% CI: 95% confidence interval; MeanNN: mean of all NN intervals; SDNN: standard deviation of all normal NN intervals; rMSSD: root mean square of successive NN interval differences; pNN50: percentage of successive NN intervals that differ by more than 50 ms; HRV: heart rate variability; HF: high frequency; LF: low frequency; LF/HF: low frequency to high frequency ratio; ms: milliseconds; ms^2: milliseconds squared; p* = unpaired t-test; pα =Mann-Whitney

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