The Pathogenisis of COVID-19 Myocardial Injury: an Immunohistochemical Study of Postmortem Biopsies

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Abstract

Rationale: Myocardial injury is significantly and independently associated with mortality in COVID-19 patients. However, the pathogenesis of myocardial injury in COVID-19 is still not clear, and cardiac involvement by SARS-CoV-2 remains a major challenge worldwide.

Objective: This histopathological and immunohistochemical study seeks to clarify the pathogenesis and propose a mechanism with pathways involved in COVID-19 myocardial injury.

Methods and Results: Postmortem minimally invasive autopsies were performed in six patients who died from COVID-19, and the myocardium samples were compared to a control patient. Histopathological analysis was performed using hematoxylin-eosin and toluidine blue staining. Immunohistochemical (IHC) staining was performed using monoclonal antibodies against the following targets: caspase-1, ICAM-1, TNF-α, IL-4, IL-6, CD163, TGF-β, MMP-9, type 1 and type 3 collagen. The samples were also subjected to a TUNEL assay to detect potential apoptosis. The histopathological analysis showed severe pericellular interstitial edema surrounding each of the cardiomyocytes and higher mast cells count by high-power field in all COVID-19 myocardium samples. The IHC analysis showed increased expression of caspase-1, ICAM-1, IL-4, IL-6, CD163, MMP-9 and type 3 collagen in the COVID-19 patients compared to the control. No difference from the control was observed in expression of TNF-α, TGF-β and type 1 collagen. The TUNEL assay was positive in all the COVID-19 samples confirming the presence of endothelial apoptosis.

Conclusions: The pathogenesis of COVID-19 myocardial injury seems to be related with pyroptosis leading to endothelial cell injury and disfunction. The subsequent inflammation with associated interstitial edema could explain the myocardial disfunction and arrythmias in these patients. Our findings also show that COVID-19 myocardial injury may cause myocardial fibrosis in the long term. These patients should be monitored for myocardial dysfunction and arrythmias after the acute phase of COVID-19.

Introduction

Since the first cases in December 2019 [1], the coronavirus disease 2019 (COVID-19) pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) continues to grow despite unprecedented worldwide efforts in the search for treatments and vaccines. COVID-19 is mainly a respiratory disease, causing viral pneumonia and acute respiratory distress syndrome. However, in most critical cases, cardiovascular manifestations have been related to poor outcomes [2]. Myocardial injury (defined by troponin blood levels above the 99th-percentile upper reference) was observed in 7 to 17% [3] of patients and was significantly and independently associated with mortality [4]. Common cardiac complications among hospitalized patients with COVID-19 include myocarditis, arrhythmias and acute heart failure. The heart failure may contribute up to 40% of deaths, and circulatory failure may be the death cause even without respiratory failure [3]. Prothrombotic coagulopathy has been also described in 25% of patients resulting in venous and arterial thromboembolic events [5].

Recently, the evidence of SARS-CoV-2 genome detection in endomyocardial biopsies [6] and autopsy cases [7] proved that SARS-CoV-2 infection directly impairs the heart [6]. However, the mechanism of cardiac damage by SARS-CoV-2 is not clear and remains a major challenge worldwide. Some autopsies of patients with COVID-19 revealed infiltration of the myocardium by interstitial mononuclear inflammatory cells [8] while others showed no increase in inflammatory cells despite the presence of the viral genome[7]. SARS-CoV-2 particles have already been observed in myocardial interstitial cells [9] and endothelial cells [10] by electron microscopy, and it has been proposed that pyroptosis may have an important role in endothelial cell injury in patients with COVID-19 [10]. Pyroptosis is a specific type of programmed pro-inflammatory cell death that culminates in caspase-1 activation, interleukin-6 (IL-6) secretion and endothelial disfunction[11]. This endothelial activation followed by pyroptosis could be the initial pathway for myocardial injury, and could also explain the involvement of various organs and tissues that has been described in COVID-19.

Interstitial myocardial fibrosis has been described as a possible consequence of myocardial injury [6,12] in a process initiated by intercellular edema, which is accompanied by endothelial dysfunction and consequent innate immune system recruitment through endothelial ICAM-1 expression [13]. Once the immune cells, notably Th2 type cells and mast cells, have migrated to the site of the injury, they start secreting interleukins and pro-fibrotic chemokines as well as inducing matrix metalloproteases and subsequent remodeling by type 1 and type 3 collagen production [14]. Myocardial fibrosis changes the cytoarchitecture and extracellular environment of the myocardium and may lead to both systolic and diastolic dysfunction, and also arrhythmias [15].

Given that the cardiac manifestations play a major role in adverse outcomes and that there is a lack of pathological studies showing myocardial injury in COVID-19, we investigated myocardium samples in a histopathological and immunohistochemical study to help clarify the pathogenesis in lethal cases. Moreover, we propose a possible mechanism involved in COVID-19 myocardial injury.

Methods

Postmortem minimally invasive autopsies were performed in six patients who died from COVID-19 in Marcelino Champagnat Hospital, Brazil. All patients tested positive for SARS-CoV-2 on nasopharyngeal swabs (RT-PCR) and had the chest computed tomography at admission suggestive of viral pulmonary infection for COVID-19. This study was approved by the National Research Ethics Committee (CONEP), protocol number 3.944.734/2020. Patients' families authorized the autopsies and signed the informed consent form before the procedures. All methods were carried out following relevant guidelines and regulations. Clinical data were obtained from medical records during hospitalization in the Intensive Care Unit (ICU).

Myocardial tissue was collected by left anterior mini thoracotomy for direct access to the left ventricle. The pericardium was sectioned and a fragment of myocardial tissue with dimensions approximately 1,5 x 1,5 cm was obtained. The tissues from the myocardial biopsies were fixed in neutral buffered formalin for over 24 hours, and then processed under conventional histological technique.

The formalin fixed paraffin embedded (FFPE) sections were subjected to hematoxylin-eosin (H&E) and toluidine blue (TB) staining. Histopathological features (H&E) were observed and described by using Olympus BX40. Mast cells (only nucleated cells with granules) were scored (TB) by counting cells per high-power field (HPF – 40x objective – 0.26mm^2) by screening 20 randomized HPFs (total area of 5.2mm^2 per case).

Immunohistochemical (IHC) staining was performed in the myocardium samples using monoclonal and polyclonal antibodies against the following targets: caspase-1, intercellular adhesion molecule-1 (ICAM-1), tumor necrosis factor alpha (TNF-α), interleukin-4 (IL-4), interleukin-6 (IL-6), CD163 (macrophage-specific protein), transforming growth factor (TGF-β), matrix metalloproteinase-9 (MMP-9), type 1, type 3 and type 4 collagen. The table in the **supplementary material** summarizes the specifications of the antibodies used to investigate the FFPE myocardial tissues. In order to detect apoptosis, the samples were also subjected to a TUNEL assay (Terminal deoxynucleotidyl transferase dUTP nick end labeling), using the 'In Situ Cell Death Detection Kit, POD' by Roche.

Scores of biomarker expression according to the IHC staining were given by an experienced pathologist and confirmed by two trained technicians. Biomarkers were analyzed using the following scoring system: 0 (-) absent; 1 (+) mild positivity; 2 (++) moderate positivity; 3 (+++) marked positivity. The postmortem myocardium biopsies of the six patients with COVID-19 were then independently compared to a myocardium sample from a control patient.

Results

Patient clinical data

Clinical data from the baseline of COVID-19 patients is presented in **Table 1**. Our cases were mainly male (5/6) with a median age of 74 years. The most prevalent underlying conditions were type 2 diabetes mellitus (5/6), systemic arterial hypertension (4/6) and coronary artery disease (4/6). Half of the patients presented some degree of obesity (3/6). The control patient had similar age (80 years), similar underlying conditions (type 2 diabetes mellitus, systemic arterial hypertension and coronary artery disease) and died of acute pulmonary thromboembolism after hip arthroplasty surgery.

All the COVID-19 patients presented symptoms of dyspnea with progressive worsening and had the chest computed tomography at admission suggestive of viral pulmonary infection for COVID-19. They were all admitted into the ICU and evolved into respiratory failure requiring mechanical ventilation. The median length of the mechanical ventilation was 12 days. During hospitalization, three patients developed acute kidney failure and patient 1 already had chronic kidney disease in need of hemodialysis; two patients presented incident acute atrial fibrillation; and one patient presented acute pulmonary embolism. In the laboratory tests, all patients had high levels of D-dimer and troponin, the latter demonstrating the presence of myocardial injury. The transthoracic echocardiograms were heterogeneous and were described in Table 1. Patients 2 and 5 presented normal echocardiographic parameters with normal ejection fraction.

Histopathological analysis

The sample tissues from the COVID-19 patients were compared to the control and histological assessment showed severe pericellular interstitial edema surrounding each of the cardiomyocytes in all the COVID-19 patients. Histological analysis also showed neutrophilic myocarditis according to the Dallas criteria[16] in the patient 1. All the other COVID-19 myocardium samples showed neither massive inflammatory cellular infiltration nor necrosis indicating the absence of typical histological myocarditis. In contrast, TB staining revealed that the perivascular and interstitial mast cell score was higher in all COVID-19 myocardium samples, most of them in degranulating process. Lipofucsin pigment and mild signs of cardiomyocyte hypertrophy were seen in COVID-19 and control patients.

Immunohistochemical analysis

The IHC analysis showed increased expression for caspase-1, ICAM-1, IL-4, IL-6, CD163, MMP-9 and type 3 collagen in the COVID-19 patients compared to the control. No substantial differences from the control were observed in expression of TNF-α, TGF-β, and type 1 and type 4 collagen. Scores of biomarker expression according to the IHC analysis are shown in Table 3. Images of the caspase-1, IL-4, MMP-9 and type 3 collagen slides, for both COVID-19 and control, are shown in **Figure 1.**

The TUNEL assay in COVID-19 myocardium samples was positive for endothelial cell apoptosis, differently from the control sample, which tested negative. Apoptosis was not observed in cardiomyocytes. Figure 1 also shows an image of the TUNEL assay slide.

A few other aspects of topography in the samples are worth noting. Firstly, caspase-1 and IL-6 were present in the cytoplasm, whereas ICAM-1 was present in the membrane of endothelial cells. Secondly, MMP-9 and type 1 collagen were observed in large quantities in the interstitial and perivascular spaces. All the results were analyzed and integrated to the previous pathological knowledge. Then, our proposed mechanism is shown in **Figure 2** with pathways involved in COVID-19 myocardial injury.

Discussion

Our main findings from the six myocardial postmortem biopsies of COVID-19 patients show myocardial interstitial edema, higher mast cell scores, and increased expression of caspase-1, ICAM-1, IL-6, CD163, MMP-9, IL-4 and type 3 collagen when compared to the control. Additionally, the TUNEL assay confirms the presence of endothelial apoptosis, and the presence of caspase-1 in the endothelial cells suggests that the mechanism is by pyroptosis. The

increased expression of ICAM-1 and IL-6 indicates endothelial activation, which alongside the higher mast cell scores can explain the increased capillary permeability, microvascular leakage and the consequent formation of myocardial interstitial edema. The increased expression of MMP-9, CD163, IL-4 and IL-6 demonstrates the presence of myocardial inflammatory response in the myocardial tissue. More specifically, CD163 signalizes macrophage recruitment and MMP-9 promotes Th2 cell recruitment and matrix remodeling. The persisting Th2 (IL-4) cytokine-driven immune mechanism is relevant to the process of myocardial fibrosis. Finally, the presence of mast cells, MMP-9, IL-4, IL-6 and type 3 collagen expression suggests that the inflammatory interstitial myocardial edema may progress to remodeling and a later myocardial fibrosis. Further implications of these events will be discussed next.

The TUNEL assay resulting positive in all the COVID-19 samples proves that this disease promotes endothelial cell apoptosis (programmed cell death). The probable mechanism is by pyroptosis, a specific inflammatory form of apoptosis that occurs most frequently upon infection by intracellular pathogens (like SARS-CoV-2) and requires the function of the enzyme caspase-1 [17]. Caspase-1 is activated as part of a multiprotein signaling platform, the inflammasome complex, and subsequently mediates the activation and secretion of various interleukins as well as the rupture of the cell membrane [18]. We observed higher levels of caspase-1 adjacent to endothelial cells in the COVID-19 samples demonstrating endothelial infection, pyroptosis and injury in these patients. Moreover, SARS-CoV-2 particles have been described in endothelial cells by electron microscopy [10] and the caspase-1 identification is in accordance with Varga et al. [10], who suggested that pyroptosis might have an important role in endothelial cell injury in patients with COVID-19. These findings are also in line with previous biopsy studies which had already shown that the inflammatory process in cardiac tissue permeates the vascular wall [6,11]. SARS-CoV-2 potentially causes endotheliitis [10], which is determinant of microvascular dysfunction by shifting the vascular equilibrium towards more vasoconstriction with subsequent organ ischemia, inflammation with associated tissue edema, and a procoagulant state [19].

The expression of IL-6 and ICAM-1 was increased in the endothelial cells and indicates endothelial activation as well as immune cell recruitment and response. When endothelial cells are activated, they produce NF-κB and adhesion molecules such as IL-6-induced ICAM-1. These molecules have the function of attracting leukocytes to the infected region and transmitting intracellular signals, which leads to the pro-inflammatory status[20]. Activation of endothelial cells is also required for the regulation of vascular permeability and blood flow to the site[19,21]. At rest, the endothelium is highly impermeable to large molecules. However, acute changes in vascular permeability result in loss of fluid and plasma proteins from the intravascular space into the interstitium, leading to edema[19,21,22].When comparing the sample tissues from the COVID-19 patients to the control, we observed severe pericellular interstitial edema in between the cardiomyocytes, causing them to separate. The maintenance of cytoarchitecture and extracellular environment of the myocardium is fundamental for the electrical and contractile function of the heart [15]. Therefore, the myocardial interstitial edema observed in the samples, and consequent loss of structure of the syncytium [21], may account for the cardiac dysfunction and arrhythmias associated with myocardial injury in COVID-19.

According to our findings, another mechanism besides endothelial activation could explain the increased capillary permeability and the consequent tissue edema: mast cell degranulation, as we observed higher mast cells scores in the COVID-19 patients, most of them in the degranulating process. Mast cell degranulation is associated with proinflammatory effects, primarily due to release of histamine, TNF and proteases, which functionally overlap in promoting enhanced expression of adhesion molecules on endothelial cells, as well as increased vascular permeability and blood flow[22]. Mast cell degranulation is an important contributor to inflammatory processes and occurs not only in the context of allergy, but also in viral infection[23].

Interestingly, an increased number of mast cells in the myocardium has already been seen in autopsies of patients with Chagas' disease, an infectious disease caused by the protozoan parasite *Trypanosoma cruzi*.[24]. It is believed that the increase of mast cells in the Chagas' patients would play an important role in containing the infection by inducing apoptosis of the infected cells. Then, the chronic phase of Chagas' disease is characterized by apoptosis, inflammatory infiltrate and collagen neoformation being associated with an increase in the number of mast cells[24]. Mast cells release proteases that stimulate fibroblast proliferation and collagen synthesis, being increased in numbers in fibrotic areas.[23,24] Mast cells also serve as sources of TNF, which is released during degranulation and promotes cardiac fibrosis via induction of cardiomyocyte apoptosis, inflammation and MMP-9 production[23]. It is possible that the cardiac lesions of COVID-19 are more similar to Chagas' disease than other virus myocarditis.

A previous autopsy study showed that the presence of SARS-CoV-2 genome in the myocardial tissue was not associated with increased infiltration of mononuclear cells compared with the virus negative group[7]. Although most of our COVID-19 myocardium samples also showed neither inflammatory cellular infiltration nor necrosis, which would be expected in typical histological myocarditis, the high levels of MMP-9, CD163, IL-4 and IL-6 demonstrate the presence of myocardial inflammatory response in this tissue.

MMP-9 is an endopeptidase which cleaves structural elements of the extracellular matrix and also plays important roles in immune cell function [25]. MMP-9 promotes Th2 cell recruitment and has been shown to be significantly increased during several cardiovascular diseases, including hypertension, atherosclerosis and myocardial infarction [25]. This, along with the high CD163 expressing macrophages on the tissue samples, are signs of cell recruitment, which is characteristic of immune inflammatory response [26]. Recruited monocytes and macrophages are capable of producing and secreting large amounts of pro-inflammatory mediators and pro-fibrotic growth factors, promoting remodeling [27]. In fact, SARS-CoV-2 particles have already been observed in a cytopathic interstitial inflammatory cell in myocardial tissue [9], and other autopsies of patients with COVID-19 revealed infiltration of the myocardium by interstitial mononuclear inflammatory cells [6,9].

Additionally, the persisting Th2 (IL-4) cytokine-driven immune mechanism is relevant to the process of myocardial fibrosis [28]. In fact, IL-6 and IL-4, which were increased in the COVID-19 samples, have already been shown to be two profibrotic cytokines, as they induce MMP-9 expression and collagen synthesis through gene transcription modulation [28–30]. MMP-9 also stimulates cardiac fibroblast migration, increases collagen synthesis, upregulates angiogenic factors, and induces the transition of cardiac fibroblasts to myofibroblasts [14,27].

As expected, we found no difference in TNF-α between cases and control, since this nonspecific proinflammatory cytokine is involved in several pathological processes such as acute and chronic inflammation, autoimmunity and malignant disease [31]. We also observed no difference in TGF-β. This is a cytokine with major roles in cardiac fibrogenesis [27,32,33] which activates SMAD2/3 pathways, stimulating alternative pathogenetic pathways and regulating cell synthesis and differentiation, promoting fibrogenesis [33]. We hypothesize that the TGF-β pathway was still not activated in these cases. If not TGF-β, an alternative pathway for myocardial fibrosis, such as the activation of macrophages via IL-4 [27,33] or mast cell degranulation [27], might be involved in the pathophysiology of COVID-19. Mast cell tryptase is a protease that can directly induce fibroblast activation, myofibroblast differentiation and collagen synthesis independently of TGF-β [34].

Myocardial fibrosis is characterized by dysregulated collagen turnover and excessive fibrillar collagen accumulation in the interstitial and perivascular spaces [27,32]. Synthesis of both type 1 and type 3 collagen is markedly increased in the remodeling fibrotic heart regardless of the etiology of fibrosis [27]. In our study, type 3 collagen was observed in large quantities in the interstitial and perivascular spaces in the COVID-19 samples when compared to the control. Type 1 and type 4 collagen, in contrast, showed no difference between cases and control. Type 1 collagen cross-links with type 3 collagen to form the final fibers in myocardial fibrosis which is primarily associated with thick fibers that confer tensile strength, and because of that, takes longer to form [27,35]. Type 3 collagen, on the other hand, typically forms thin fibers and, because of that, it takes less time to build [27,35]. Type 4 collagen is structural and was observed in the samples to validate the collagen assays, as a control.

Our observation of type 3 collagen in the COVID-19 samples, but not in the control, along with the presence of mast cells and the increased expression of MMP-9, IL-6, IL-4, is consistent with the hypothesis that COVID-19 acute myocardial injury may cause myocardial fibrosis in the long term. This is further supported by the observation of chronic myocardial interstitial edema in the COVID-19 samples but not in the control, since it results in deposition of interstitial collagen, causing interstitial fibrosis [13]. Also, these findings may indicate an early stage myocardial fibrotic response as opposed to a pre-existing fibrosis, which would be marked by a higher level of type 1 collagen expression and little IL-4 and MMP-9 expression. A genetic response study on experimental autoimmune myocarditis showed that myocardial fibrosis had formed on day 21, but not before [36]. In addition, advanced age and chronic illnesses are known to lead to myocardial fibrosis, and both our control and COVID-19 samples were mainly elderly.

Taken together our findings indicate that the microvascular dysfunction may lead to thrombosis and justifies the rational use of anticoagulant and anti-aggregating therapy [37], and myocardial interstitial edema presented here may be one explanation for the high prevalence of cardiac arrhythmia in COVID-19 patients. Furthermore, our findings suggest that COVID-19 myocardial injury may cause myocardial fibrosis in the long term. Based on laboratory tests, individualized cardiac magnetic resonance could be useful to assess patients' cardiac involvement, and thus guide treatment. Additionally, drugs which act in cardiac remodeling, such as angiotensin-converting enzyme inhibitors or mineralocorticoid receptor antagonists, could be useful in a long-term myocardial protective effect[38,39]. However, further studies evaluating cardiac sequelae and mortality following hospital discharge are needed.

We present a panel of immunohistochemical markers with high expression in COVID-19 myocardium samples and, to the best of our knowledge, this is the first study with TUNEL assay which proves endothelial cell apoptosis in COVID-19 myocardial tissue. Moreover, our study brings light to the pathogenesis of the disease by putting all the biomarkers together and showing mast cell degranulation in myocardium samples. There are, however, a few limitations to our study. The COVID-19 patients were mainly elderly and had underlying conditions that could be confounders. Also, interpretation of our findings should take into account that autopsies do not allow the observation of the entire pathological process, and therefore cannot predict the evolution of the disease.

In conclusion, the multifactorial pathogenesis of COVID-19 myocardial injury seems to be related with pyroptosis leading to endothelial cell injury and disfunction, which contributes to the impaired microcirculatory function. Subsequently, the inflammation with associated interstitial edema would explain the myocardial disfunction and arrythmias in COVID-19 patients. These patients could be more prone to thrombotic diseases, heart failure and even death. Our hypothesis seems to be an explanation as to why patients with underlying heart conditions are predisposed to developing more severe manifestations of COVID-19. Finally, our findings show that COVID-19 myocardial injury may cause myocardial fibrosis in the long term. These patients should be monitored for systolic and diastolic dysfunction and arrythmias leading to heart failure after the acute phase of COVID-19.

Declarations

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Tables

Table 1– Clinical data from the baseline of patients

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Control
Gender, Age (years)	Male, 73	Male, 80	Male, 57	Female, 81	Male, 75	Male, 70	Female
Underlying Conditions	Type 2 Diabetes Mellitus Chronic Kidney Disease Atrial Fibrillation Coronary Artery Disease Heart Failure Peripheral Obstructive Artery Disease	Arterial Hypertension Coronary Artery Disease Heart Failure Class III obesity	Type 2 Diabetes Mellitus Arterial Hypertension Coronary Artery Disease Hepatic Steatosis	Type 2 Diabetes Mellitus Arterial Hypertension Dyslipidemia	Type 2 Diabetes Mellitus Arterial Hypertension Dyslipidemia Hyperuricemia Coronary Artery Disease Myocardial infarction (April-2020)	Type 2 Diabetes Mellitus Arterial Hypertension Atrial Fibrillation Interstitial Pulmonary Fibrosis Pulmonary Hypertension Former smoker	Type 2 Mellitu Arterial Hyperte Corona Diseas
Medications	Acetylsalicylic Acid Clopidogrel Rosuvastatin Losartan Hydrochlorothiazide Metoprolol Insulin NPH Cilostazol Erythropoietin	Acetylsalicylic Acid Metoprolol Rivaroxaban Ezetimibe Pitavastatin Trimetazidine Carbamazepine Trazodone 150 Inhaled Beclomethasone Inhaled Formoterol 12	Acetylsalicylic Acid Rosuvastatin Losartan Metformin Bupropion	Acetylsalicylic Enalapril Atorvastatin Bisoprolol Metformin Glibenclamide Empagliflozin Linagliptin Sodium Alendronate	Acetylsalicylic Acid Clopidogrel Candesartan Levanlodipine Chlortalidone Atenolol Atorvastatin Metformin Dapagliflozin Glimepiride Alogliptin Pioglitazone	Ramipril Metoprolol Rosuvastatin Warfarin 2.5mg Glicazide Dapagliflozin Linagliptin Duloxetine	Enalap Metfor
Length of stay on Mechanical Ventilation	10 days	21 days	9 days	14 days	9 days	15 days	Does n
Chest Computed tomography at admission	Diffuse and bilateral “opacities with ground-glass attenuation”, suggestive of viral pulmonary infection	Diffuse and bilateral “opacities with ground-glass attenuation”, suggestive of viral pulmonary infection	Peripheral, multifocal and bilateral “opacities with groundglass attenuation”, suggestive of viral pulmonary infection. Presence of bronchial thickening.	Diffuse and bilateral “opacities with groundglass attenuation”, thickening of the pulmonary septum, suggestive of viral pulmonary infection.	Diffuse and bilateral “opacities with groundglass attenuation”, thickening of the pulmonary septum, suggestive of viral pulmonary infection. Presence of bronchial thickening. Presence of diffuse bilateral bronchiectasis. Presence of parasseptal emphysema.	Peripheral, multifocal and bilateral “opacities with groundglass attenuation”, suggestive of viral pulmonary infection. Interstitial Pulmonary Fibrosis. Cardiomegaly. Increased Pulmonary Artery Diameter (32mm).	Pulmor Throml
Relevant initial laboratory tests	C-Reactive Protein = 83 mg/dL D-dimer = 3436 µg/mL hs-Troponin I = 12,6 pg/mL Creatinine = 7.45 mg/dL Globular volume = 25% Hemoglobin = 8.6 g/dL Leukocytes = 9,200	C-Reactive Protein = 52 mg/dL D-dimer = 816 µg/mL hs-Troponin I = 10.9 pg/dL Creatinine = 0.74 mg/dL Globular volume = 37.5% Hemoglobin = 12.8 g/dL Leukocytes = 4,700	C-Reactive Protein = 154.2 mg /dL D-dimer = 628 µg/mL hs-Troponin I = 3.9 pg/mL Creatinine = 0.82 mg/dL Globular volume = 38.3% Hemoglobin = 14g/dL Leukocytes = 14,500	C-Reactive Protein = 199.4 mg /dL D-dimer = 83,143 µg/mL hs-Troponin I = 42.1 pg/mL Creatinine = 1.33 mg/dL Globular volume = 41.3% Hemoglobin = 13.7 g/dL Leukocytes = 22,100	C-Reactive Protein = 267mg/dL D-dimer = 152,174 µg/mL hs-Troponin I = 13.3pg/mL Creatinine = 1.61 mg/dL Globular volume = 43.1% Hemoglobin = 14.9 g/dL Leukocytes = 13,100	C-Reactive Protein = 156.9 mg/dL D-dimer = 1,848 µg/mL hs-Troponin I = 1750.2 pg/mL Creatinine = 1.37 mg/dL Globular volume = 52.8% Hemoglobin = 18.2 g/dL Leukocytes = 16,000	Data n

Laboratory tests 24 hours before death	C-Reactive protein = 270 mg/dL D-dimer = 4,858 µg/mL hs-Troponin I = 87.4 pg/dL Creatinine = 5.08 mg/dL Globular volume = 23% Hemoglobin = 8.0 g/dL Leukocytes = 22,000	C-Reactive protein = 407 mg/dL D-dimer = 4,507 µg/mL hs-Troponin I = 32.7 pg/dL Creatinine = 1.81 mg/dL Globular volume = 29.4% Hemoglobin = 9.7 g/dL Leukocytes = 9,400	C-Reactive Protein = 267.2 mg/dL D-dimer = 6,571 µg/mL hs-Troponin I = 19.9 pg/mL Creatinine = 2.43 mg/dL Globular volume = 27% Hemoglobin = 9 g/dL Leukocytes = 15,300	C-Reactive Protein = 16.3 mg/dL D-dimer = 19,137 µg/mL hs-Troponin I = 324,7 pg/mL Creatinine = 1.06 mg/dL Globular volume = 29.2% Hemoglobin = 9.5 g/dL Leukocytes = 28,900	C-Reactive Protein = 226.8 mg/dL Troponina = 21,2 µg/mL Creatinine = 2.14 mg/dL Globular volume = 19.7% Hemoglobin = 11 g/dL Leukocytes = 19,100	C-Reactive Protein = 8.7 mg/dL Troponina = 245,2 pg/mL Creatinine = 1.66 mg/dL Globular volume = 32% Hemoglobin = 11.3 g/dL Leukocytes = 10,500	Data n
Echocardiogram 24 hours before death	Ejection fraction = 43% Left ventricle = mild eccentric hypertrophy; akinesia of the infero-lateral and basal lower walls. Right ventricle = increased basal dimension and normal systolic function. sPAP = 68 mmHg.	Ejection fraction = 65% Left ventricle = preserved dimensions. Right ventricle = preserved dimensions and normal systolic function. sPAP = normal.	Ejection fraction = 64% Left ventricle = preserved dimensions. Right ventricle = Increased dimensions and slightly reduced systolic function. sPAP = 51 mmHg.	Ejection fraction = 45%. Left ventricle = mild eccentric hypertrophy; hypokinesia of the lower-basal and inferoseptal walls. Right ventricle = preserved dimensions and normal systolic function. sPAP = 34 mmHg.	Ejection fraction = 66% Left ventricle = preserved dimensions. Right ventricle = preserved dimensions and normal systolic function. sPAP = 28 mmHg.	Ejection fraction = 57% Left ventricle = severe eccentric hypertrophy. Right ventricle = Increased dimensions and compromised systolic function. sPAP = 70 mmHg.	Preserv fractio Left ve preserv dimens Echoca with pharma stress: ischem
Therapeutic drugs	Hydroxychloroquine Azithromycin Oseltamivir Metronidazole Meropenem Linezolid	Hydroxychloroquine Azithromycin Oseltamivir Ceftriaxone	Azithromycin Ceftriaxone Dexamethasone Enoxaparin (prophylactic) Piperacillin + Tazobactam Alteplase for thrombolysis of PE	Azithromycin Ceftriaxone Oseltamivir Dexamethasone Enoxaparin Piperacillin + Tazobactam	Azithromycin Ceftriaxone Dexamethasone Enoxaparin (full)	Ceftriaxone Azithromycin Tocilizumabe Methylprednisolone Piperacillin + Tazobactam Enoxaparin (full)	Data n
Invasive procedure	Hemodialysis 3 times a week	Tracheostomy	Chemical Thrombolysis	Tracheostomy	Chest Tube Right (pneumothorax)	Tracheostomy	Hip Art Surger

* Reference values: hs-Troponin I < 19,8 pg/mL, D-dimer < 500 µg/mL. The choice of the antibiotics was done according to the diagnosis and protocol for the patient's profile. sPAP = systolic pressure in pulmonary artery.

Table 2 – Scores of biomarker expression in the myocardium samples according to the immunohistochemical analysis and TUNEL assay.

	Patient 1 (COVID-19)	Patient 2 (COVID-19)	Patient 3 (COVID-19)	Patient 4 (COVID-19)	Patient 5 (COVID-19)	Patient 6 (COVID-19)	Control (PE)
H&E	edema; neutrophilic myocarditis	edema	edema	edema	edema	edema	no edema
Toluidine Blue	25/20	10/20	9/20	11/20	4/20	8/20	2/20
Caspase-1	+++	++	++	++	++	+++	+/-
CD 163	++	++	+++	+++	+++	+++	+/-
Collagen 1	++	++	+++	+++	+++	+++	++
Collagen 3	+++	+++	+++	+++	+++	+++	+
Collagen 4	++	++	++	++	+	+	++
ICAM-1	+++	++	+++	++	++	+++	+
IL-4	+++	+++	+++	+++	+++	+++	+/-
IL-6	+++	+++	+++	+++	+++	+++	+/-
MMP-9	+++	+++	++	+	++	++	++
TGF-β	+	+	+	+	++	++	+/-
TNF-α	++	+	+++	+++	+++	+++	++
TUNEL assay	positive	positive	positive	positive	positive	positive	negative

* Toluidine Blue: the count of mast cells degranulating per 20 high-power field under the microscope.