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# "Role of Cardiac Inflammation in the Pathology of COVID-19; relationship to the current definition of myocarditis"

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We would like to thank the Society for Cardiovascular Pathology (SCVP) Publication Committee for our receipt of the 2022 Margaret Billingham Award for the most important original paper published in 2021 in the Society's journal. We are proud to receive the award given in memory of the cardiac transplant pioneer Dr. Billingham, best remembered for developing the Billingham Criteria for the grading of heart transplant rejection. In this Commentary, we provide context for the generation of our report and discuss the concepts of myocarditis and inflammation as it relates to COVID-19 pathology.

Over two years into the pandemic, SARS-CoV-2 infection is responsible for more than 500 million cases of COVID-19 infection and over 6 million reported deaths. The first wave of SARS-CoV-2 rapidly spread to Northern Italy, followed by other parts of Europe, with the first reported mortality reported in the United States in February 2020. The earliest cases of death in the United States were likely travel related; the first documented community spread occurred in the San Francisco area in early February of 2020 (determined retrospectively). In New Orleans, the first COVID-19 deaths occurred on March 14, 2020. At University Medical Center in New Orleans, (the former Charity Hospital), we performed our first autopsy on March 19, 2020. It occurred in a 44-year-old male with no serious underlying medical conditions except for obesity and hypertension. We reported our initial pathological findings in May of 2020 in Lancet Respiratory Medicine [1].

Early reports from China indicated that the heart, specifically myocarditis, was an important cause of serious morbidity and mortality in acute COVID-19. However, early autopsy-based studies from Europe and the United States, including our results, demonstrated respiratory failure was the primary cause of death and that acute lymphocytic myocarditis was actually an unusual event. Interestingly, an early report from Basso described a subjective (i.e., not quantified) increase in the number of macrophages in COVID-19 hearts rather than increases in lymphocytes in the majority of cases they reported [2].

## 1. Reports of increased numbers of cardiac macrophages

In our earlier reports on the cardiac findings of COVID-19, we, as well as others, [3] noticed that some small capillaries contained platelet and/or fibrin clots associated with varying degrees of acute myocyte necrosis. We originally hypothesized that SARS-CoV-2 was directly infecting the endothelium causing damage and capillary and/or small vessel clot formation [4]. After recognizing the increased monocyte and/or macrophage population present in the interstitium and microvasculature of COVID-19 hearts, we proposed that COVID-19 might represent a different and/or newly recognized form of myocarditis associated with diffusely infiltrative cells of the monocyte/macrophage lineage. Our study, the subject of this commentary, was designed to investigate and quantify the inflammatory infiltrate in COVID-19 hearts to determine if macrophages were significantly greater in number and distribution compared to hearts having inflammatory myocarditis diagnosed at autopsy, and to control hearts where the cause of death was not related to COVID-19 or inflammatory myocarditis [5]. A key feature of our study was that the control group was matched for age, gender, and BMI and all decedents had one or more of the most common co-morbidities associated with severe COVID-19, that is, diabetes, hypertension, chronic kidney disease, or known cardiovascular disease. Since hypertension and diabetes are important risk factors for endothelial and/or vascular dysfunction, the control hearts provided an important measure of the baseline influence of vascular dysfunction on macrophage vascular localization.

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High resolution whole slide image scanning and quantification of CD3, CD4, CD8 and CD68 staining cells revealed a skewed distribution of CD68 staining cells in COVID-19 hearts with upper quantiles showing a significant increase compared to both control hearts and hearts with inflammatory myocarditis. It is important to recognize that this upper quantile likely represents a distinct subset of individuals with COVID-19 myocardial pathology, and may not be present the same extent in all individuals infected with the virus. Hearts from inflammatory myocarditis contained more CD4+ and CD8+ cells compared to both COVID-19 and control hearts. We concluded that the diffuse interstitial and microvascular CD68+ cell infiltrate is distinct from typical viral myocarditis and may represent a unique form of cardiac inflammation affecting COVID-19 patients.

Earlier this year, Goldman et. al published a series of six patients who presented with acute onset heart failure to their medical center in Rochester, New York from 2019 to 2021. The six patients included two patients with known systemic lupus erythematosus (SLE) and four patients without known disease. Endomyocardial biopsies were examined by three cardiac pathologists and all patients received a histologic workup that included routine stains as well as immunostains for T cells, monocyte and/or macrophages, complement C4d, and endothelium. The biopsies revealed no eosinophilic or giant cell myocarditis but rather a diffuse infiltrate of CD68 positive cells with a paucity of infiltrative T lymphocytes. They also found monocytes (by immunohistochemistry) in the microvasculature in five cases. Interestingly, they found small clusters (1-2 cells) of necrotic myocytes staining positive for C4d and C9 in two of the non-SLE patients. Based on the findings that all six cases showed inflammation composed primarily of CD68 positive macrophages and/or monocytes the authors classified them as representing histiocytic inflammatory myocardial disease (HIMD) or "histiocytic myocarditis." We agree with Goldman et. al that acute macrophage-predominant inflammatory infiltrates may represent a distinct form of inflammatory heart disease that occurs in a variety of clinical contexts [6].

## 2. Definition of myocarditis revisited

The preponderance of CD68 positive cells in COVID-19 hearts and in the Goldman study highlight the current limitations of the definition of myocarditis; especially when applied to autopsy specimens. Several reports have shown large numbers of macrophages infiltrating the myocardium but these cases are in the context of the chronic myocarditis phase following acute myocarditis, and are associated with significant fibrosis [7]. According to the World Health Organization and/or International Society and Federation of Cardiology Task Force on the Definition and Classification of Cardiomyopathies, *inflammatory cardiomyopathy* is myocarditis in association with cardiac dysfunction. *Myocarditis* is "an inflammatory disease of the myocardium" that is diagnosed by established histological, immunological, and immunohistochemical criteria [8]. In the position statement of the European Society of Cardiology, the WHO is cited as stating that an endomyocardial biopsy could be diagnosed as myocarditis after immunohistochemical detection of focal or diffuse mononuclear infiltrates with >14 leukocytes per 1 $mm^2$ ($CD3^+$ T lymphocytes and/or $CD68^+$ macrophages). The consortium further refined the definition to include ≥14 leucocytes/$mm^2$, including up to four monocytes/$mm^2$, with the presence of CD3-positive T-lymphocytes at ≥7 cells/$mm^2$ [9]. By these currently accepted criteria, COVID-19 cases would not qualify for acute myocarditis, because they lack both CD3+ T lymphocytes (independent from the presence of macrophages) and the macrophages present do not cluster at >14 leukocytes per 1 $mm^2$ . The Dallas Criteria generally considered the gold standard for endomyocardial diagnosis of myocarditis, state that the diagnosis requires the presence of an inflammatory infiltrate and definitive myocyte damage but also defines "borderline myocarditis" which is understood to be inflammatory infiltrates without evidence of myocyte necrosis [10]. Some have suggested that the increased numbers of macrophages without significant myocyte necrosis seen in COVID-19 hearts may best fit with this definition. However, the term "borderline myocarditis" predated the use of immunohistochemistry to characterize myocardial inflammatory infiltrates. A report by Kindermann et al. identified the prognostic indicators of myocarditis in 181 consecutive German patients being evaluated for clinically suspected viral myocarditis [11]. They diagnosed borderline myocarditis in 64 (35%) of the patients using Dallas criteria supplemented with immunohistology for CD3, CD68, and HLA-DR-alpha stains. By their definition, borderline myocarditis consistently contained focal areas of T lymphocyte infiltration associated with macrophages and increased expression of HLA class II molecules. In COVID-19 hearts, no significant T cell aggregates were identified compared to control hearts. Furthermore, the presence of necrotic myocytes in COVID-19 hearts, occasionally seen in direct contact with macrophages, makes the use of borderline myocarditis unsatisfactory.

With respect to tissue macrophage infiltration and myocarditis, several interesting correlations exist between COVID-19 and Ebola virus disease (EVD). Similar to COVID-19, the clinical presentation of EVD is characterized by fever, endothelial dysfunction, coagulopathy, shock, and multisystem organ dysfunction. McElroy et. al showed that the macrophage activation marker soluble CD163 (sCD163) is associated with the severity and the overall outcome of severe EVD [16]. Autopsy results in this study showed that Ebola does not cause extensive areas of tissue necrosis where viral antigen is co-localized and hypothesized that virally mediated activation of macrophages and the associated dysregulated (hyper) immune response correlates best with EVD severity and fatality. Examination of autopsy hearts from fatal EVD showed no significant necrosis but increased CD163 staining in resident interstitial macrophages indicating activation of macrophages in the absence of necrosis and again raising the question of what constitutes "myocarditis" in systemic viral illness.

## 3. Monocyte/macrophages in COVID-19 disease

The monocyte and/or macrophage line is an important player in SARS-CoV-2 infection/COVID-19. After exposure, the virus infects type II pneumocytes (epithelial cells) through attachment to ACEII receptors. Following infection, the pneumocytes undergo cell death followed by an intense acute inflammatory response characterized by numerous macrophages filling the alveolar septae and spaces, a response similar to fatal cases of both SARS-CoV-1 and MERS-CoV [12]. The severity of the acute respiratory illness is related to the underlying comorbidities and the local concentrations of cytokines and inflammatory mediators. If severe, the infection produces severe damage to the alveoli and the endothelium causing leakage of fibrin into the alveolar spaces, and typically triggering the changes associated with diffuse alveolar damage. Although the virus is capable of infecting monocyte and/or macrophages [13] most studies show that such infection is abortive; that is, the macrophages undergo pyroptosis and the virus cannot replicate further yet can contribute to the systemic inflammatory response [14]. Interestingly, patients with severe COVID disease have increased numbers of inflammatory monocyte-derived macrophages (v.v. resident macrophages); a macrophage subset that has been shown to express gene programs associated with tissue repair and fibrosis [15]. This raises the possibility that rather than modulating the acute inflammatory response, the macrophages seen in COVID-19 hearts could potentially produce detrimental long-term effects such as chronic inflammation and/or cardiac fibrosis.

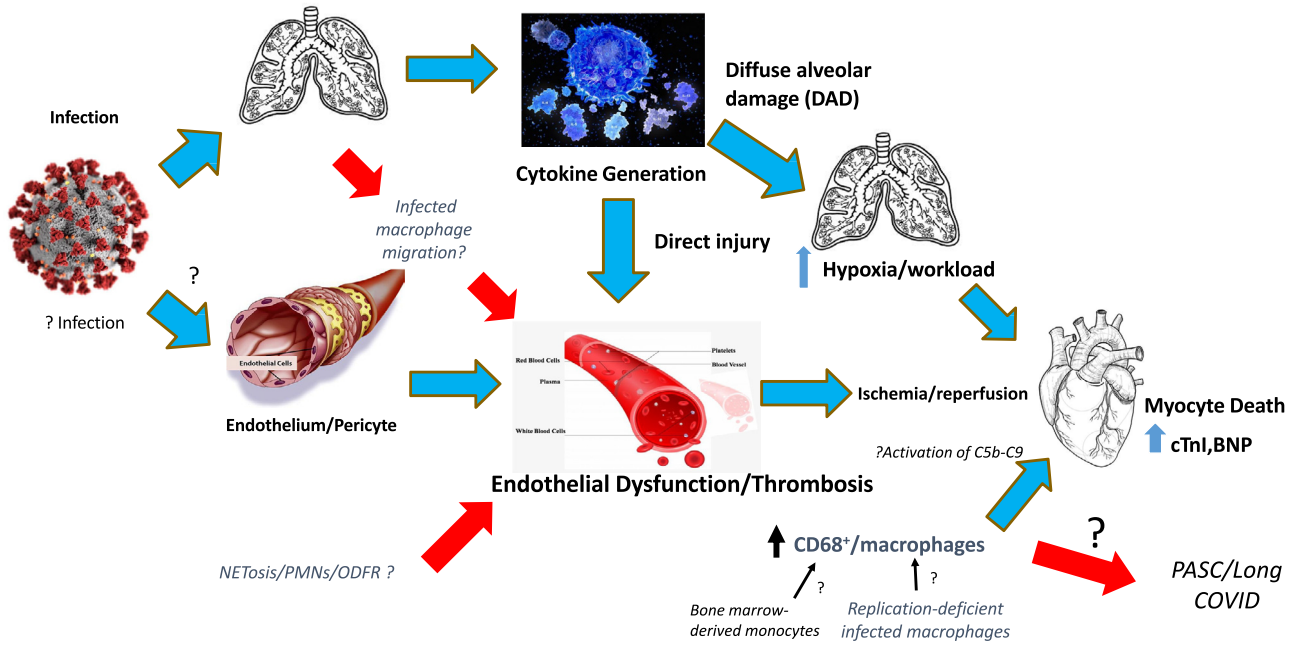


**Fig. 1.** Diagram outlining author's current hypothesis regarding COVID-19 related cardiac pathophysiology.
Blue arrows indicate pathways with experimental and/or clinical support. Red arrows indicate pathways proposed by the authors. Endothelial damage/dysfunction is key to acute cardiac injury in severe COVID infection. Macrophages/CD68+ cells are hypothesized to be important mediators of cardiac inflammation as well as contributors to potential PASC/Long COVID effects.

## 4. Summary

In summary, we propose the findings reported in our most recent study, significantly increased numbers of cardiac macrophages in a relevant subset of patients with SARS-CoV-2 infection, may represent a different and perhaps unique form of cardiac inflammation in patients with severe acute COVID-19 and/or systemic viral illness. Given the emergence of PASC and the unknown long-term consequences of mild to moderate COVID-19 infections, ongoing investigation of the role of macrophages in the heart seems warranted (Fig. 1).

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