COVID-19 Myocarditis: Quantitative Analysis of the Inflammatory Infiltrate and a Proposed Mechanism

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Introduction

- The SARS-CoV-2 coronavirus has resulted in a global pandemic and the loss of over 3.5 million lives. Cardiovascular involvement has been described as a significant cause of morbidity and mortality in COVID-19 patients, with early studies focused on the role myocarditis in the pathogenesis.
- Early descriptions of viral myocarditis in hospitalized and even patients that had recovered from COVID-19 were derived from clinical, radiological, and laboratory measurements, rather than tissue diagnosis.
- There have been several autopsy case series that have documented varying histopathologic changes, including what is considered viral myocarditis. While there are differences in what is considered myocarditis in the published reports, the largest autopsy series published to date indicates that the overall rate of lymphocytic myocarditis is low (<2%).
- Growing concern regarding the reported occurrence of cardiac symptoms in this patient population emphasizes the importance in determining whether subtle changes in COVID-19 hearts may yield important clues to susceptibility to long term cardiac consequences.

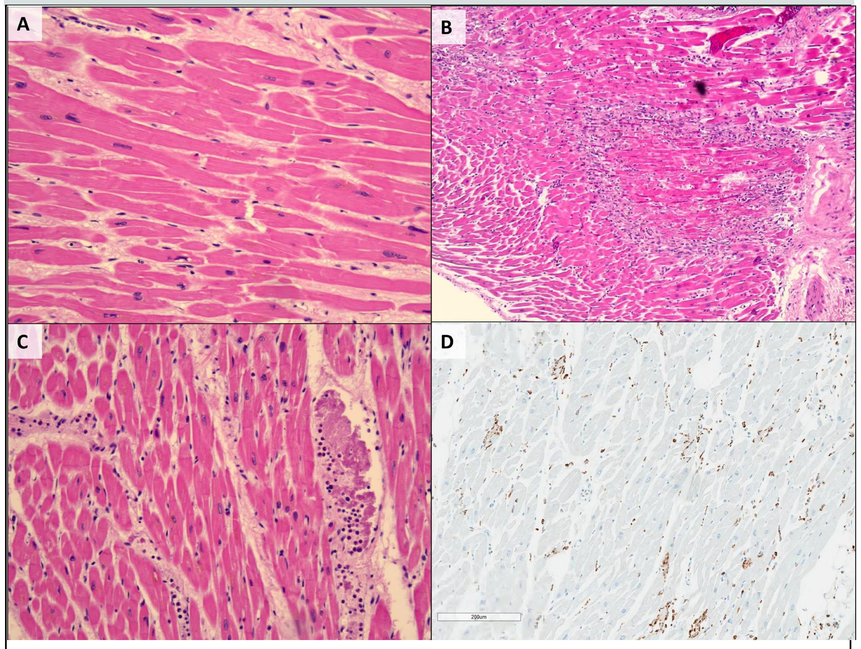
Methods

- We identified 10 non-consecutive decedents whose death was due to COVID-19 infection.
- A control group of 10 decedents (5 male and 5 female) was selected, all of whom had pre-mortem diagnosis of HTN, DM2, and CKD and had died and had an autopsy performed during the same period.
- The myocarditis control group consisted of 5 patients with a confirmed diagnosis of myocarditis who had an autopsy during the years 2015-2020
- The COVID-19 and control groups were compared for age, BMI, percentage of coronary artery stenosis as well as serum troponin, d-dimer, and BNP levels. Some demographic and laboratory data was not available for each included decedent.

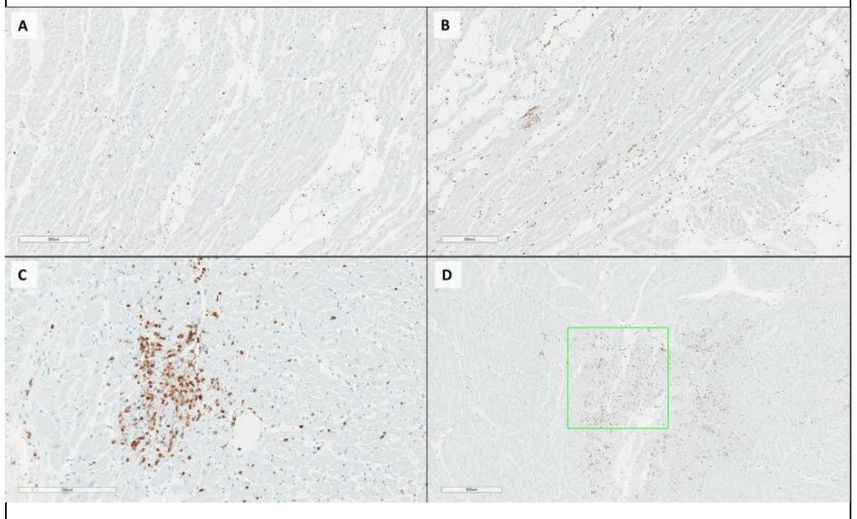
Demographic/Histopathologic Findings

	Control	Myocarditis	
COVID-19			
Study Characteristic			
Gender (M/F)	5/5	5/5	3/2 (one not known)
Age (mean; range)	64 (49-79)	56 (30-79)	49 (30-59)
BMI (mean; range)	33 (22-45)	32 (17-45)	Not recorded
Race (AA/Caucasian/Hispanic)	6/3/1	9/1/0	3/1/?
Known Heart Disease (incl. Afib)	3	0	Not recorded
Hypertension	8	10	Not recorded
Type 2 Diabetes	4	10	Not recorded
Renal disease	2	10	Not recorded
Cancer	1	0	Not recorded
Obesity (BMI>30)	4	4	Not recorded
Coronary stenosis (maximal lesion any vessel) (mean; range)	22% (0-75%)	18% (0-75%)	Not recorded
Laboratory Parameter			
Elevated Troponin I nl<0.04	6/10	2/10	3/4
Elevated BNP (pg/mL) nl<100	6/10	5/8	2/2
Elevated D-dimer (ng/mL DDU) nl<250	8/8	4/4	2/2

Histopathologic Images

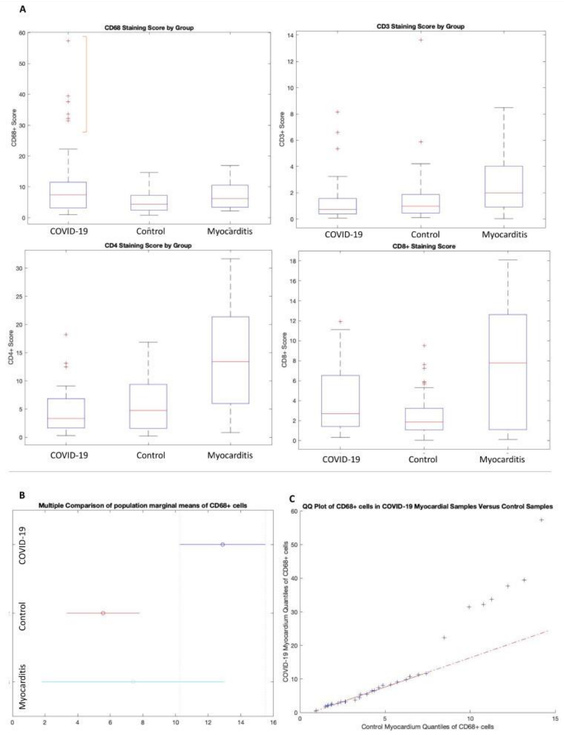


(A) Cardiac myocytes from control patient (H&E). (B) Myocarditis, characterized by patchy, dense inflammation within the myocardium (H&E). (C) Endotheliitis and diffuse, perivascular distribution of inflammation in COVID-19. (D) CD68 immunostaining highlighting the presence of CD68 + cells in a mild, diffuse intravascular and perivascular distribution in a case of COVID-19.



(**A)** CD3 immunostaining, demonstrating a paucity of CD3+ lymphocytes in COVID-19. (**B)** CD68 immunostaining in COVID-19, highlighting a mild, diffuse distribution of CD68+ cells. (**C)** CD3 immunostaining in a case of myocarditis, demonstrating intense staining for CD3+ lymphocytes in a patchy distribution. (**D)** CD68 immunostaining in myocarditis, in a similar patchy distribution to that of lymphocytes, with example of region selection box for analysis.

Analyses



(A) Boxplots showing median and range of CD68, CD3, CD4, and CD8 staining cells for each Patient Group. A red bracket highlights the numerous outliers of CD68+ cells in the upper quantile of the COVID-19 group, which may represent a subset of COVID-19 patients with greater myocardial inflammation. (B) Population marginal means after correction for multiple comparisons within the nested ANOVA performed on CD68+ cells. The population marginal mean of CD68+ cells is significantly higher for the COVID-19 group as compared to controls. C) Q-Q plot of CD68+ cell quantiles in the COVID-19 myocardium samples as compared to control quantiles. The relationship is non-linear at higher quantiles, indicating a difference in distribution of CD68 positivity among the COVID-19 group, with higher values at the upper quantiles

Conclusions

- There was a skewed distribution of the number of CD68+ cells in COVID-19 hearts, with upper quantiles showing a significant increase as compared to both matched control hearts, and those with myocarditis.
- In contrast, hearts from typical inflammatory myocarditis contained increased numbers of CD4+, and CD8+ cells compared to both COVID-19 and control cohorts.
- The presence of an increased number of CD68+ cells suggests that COVID-19 may incite a form of myocarditis different from typical viral myocarditis associated with diffusely infiltrative cells of monocytes/macrophage lineage.
- Monocyte infiltration can lead to endothelial injury in the heart, leading to clotting at the arteriole, venule and capillary level, initiating thrombosis and resulting ischemia/reperfusion injury.
- Alternatively, the presence of endothelial injury could attract non-classical monocytes (i.e., M1) to the site resulting in macrophage-induced activation of the complement pathway and generating apoptotic injury.
- Whether one of these proposed pathways is more important and/or which local conditions lead to primary activation of one or the other pathway is the subject of our ongoing investigations.