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editorial

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Direct SARS-CoV-2 infection of the heart potentiates the cardiovascular sequelae of COVID-19

Dear Editor,

The ongoing pandemic, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has wreaked havoc worldwide, **infecting over eight million people and killing at least 437,604 as of the mid-end of June**. Whereas the primary organ system affected by the virus is the lungs, over 20% of hospitalized patients present with significant myocardial injuries, including infection-related myocarditis with reduced systolic function and arrhythmias [1]. Unfortunately, the mechanisms responsible for these sequelae are not fully understood. While this injury could be secondary to severe lung damage (e.g., cytokine storm or acute hypoxia-induced cell death), direct infection of the heart is becoming a more plausible option, particularly for those patients with pre-existing co-morbidities.

It is thought that one of the primary mechanisms through which SARS-CoV-2 gains entry into cells to facilitate viral replication is through binding to the angiotensin-converting enzyme 2 (ACE2) [2]. To determine whether there was a direct role for ACE2 in the heart, Tucker *et al.* measured ACE2 expression levels in human myocardial samples from the Penn Human Heart Tissue Biobank using both bulk and single nucleus RNA-sequencing (RNA-seq) analyses [3]. Using bulk analysis, they found that ACE2 levels were similarly expressed in both non-diseased and diseased hearts, including in pericytes, vascular smooth muscle cells, fibroblasts, and cardiomyocytes. However, when hearts were analyzed using single-cell RNA-seq, investigators revealed that diseased hearts showed significantly increased levels of ACE2 in cardiomyocytes, but significantly reduced levels in fibroblasts, pericytes, and vascular smooth muscle cells, as compared to healthy controls. Together, these data indicate that 1) patients with underlying heart conditions have increased levels of ACE2 in cardiomyocytes, and 2) are consequently more likely to develop an infection due to SARS-CoV-2. In addition to ACE2 expression, the associated proteases transmembrane protease serine 2 (TMPRSS2) and Cathepsin L (CTSL) facilitate viral entry into cells via the priming of the spike protein [4]. Single-cell analysis across organ systems demonstrated that double-positive populations ($ACE2^{+/+}CTSL^{+/+}$) were abundant subsets of heart cells, including

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in cardiomyocytes and pericytes, further lending credence to the possibility of direct infection.

Based on data such as these, we may now begin to more fully understand the repercussions of this infection on underlying cardiac conditions. SARS-CoV-2 is 82% homologous to the human betacoronavirus SARS-CoV and uses the same mechanisms to further infection [5]. In studying the effects of the 2003 SARS outbreak, which sickened over 8,000 people and resulted in more than 800 deaths, Oudit *et al.* found that infection of ACE2 positive cells caused a rapid downregulation of *ACE2* mRNA, a loss of the protein on the cell surface, and an imbalance of the renin-angiotensin-aldosterone system (RAAS) [6]. This resulting pathophysiology was thought to be a consequence of the infection's ability to significantly increase the levels of circulating Angiotensin II.

The viral infection itself was also shown to be detrimental to the viability of cardiomyocytes, resulting in a cytopathic effect in many cardiac cell types [7]. This finding is further supported by more recent data showing that infection of inducible pluripotent stem cell (iPSC)-derived cardiomyocytes with SARS-CoV-2 increases cell death [8]. Additionally, specific viral proteases, including the main protease (M^{pro}) and a papain-like protease (PL^{pro}), of which SARS-CoV shares 90% sequence homology to SARS-CoV2, are required for viral replication [9,10]. Upon infection, the virus utilizes the host cell ribosomes to translate two large polyproteins, which are subsequently processed by proteolysis to produce protein components required for the generation of new virions. In addition to processing their target sequences, these proteases also possess the capacity to cleave a number of intracellular proteins, including dystrophin in the heart, leading to decreased sarcolemma integrity and to contractile dysfunction.

Given the plethora of targets that are being identified, researchers have begun exploring potential therapeutic strategies to slow the spread of the disease and limit its consequences. For example, camostat mesylate, a serine protease inhibitor approved for the treatment of pancreatitis in South Korea and Japan, was demonstrated to prevent SARS-CoV-2 from entering cells via the inhibition of TMPRSS2 in early March [2]. This compound has been quickly approved for a clinical trial in Denmark, which was underway as of early April. Others have turned to in vitro and virtual approaches using previous experiences with SARS-CoV, Middle East Respiratory Syndrome, and the crystal structure of SARS-CoV-2 to quickly screen known compounds for potential efficacy against this novel beta coronavirus, with hits being generated for many of the targets discussed above. Currently, there over 500 ongoing clinical trials investigating a large number of these compounds, including inhibitors of M^{pro} and PL^{pro}, so it should not be long before we begin learning of the successes and failures.

Though our social distancing efforts have effectively "flattened the curve" of new infections, hospitalization, and deaths, it will still be more than a year until there is a viable option for the prevention of COVID-19 caused by the SARS-CoV-2 virus. There is thus an urgent need to understand the cardiovascular consequences of infection, in particular, the mechanisms responsible for the observed cardiac damage. Only then will we be able to investigate potential therapeutic options for those patients in need.

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