

## REVIEW

# COVID-19 and Cardiovascular Disease

## From Bench to Bedside

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**ABSTRACT:** A pandemic of historic impact, coronavirus disease 2019 (COVID-19) has potential consequences on the cardiovascular health of millions of people who survive infection worldwide. Severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2), the etiologic agent of COVID-19, can infect the heart, vascular tissues, and circulating cells through ACE2 (angiotensin-converting enzyme 2), the host cell receptor for the viral spike protein. Acute cardiac injury is a common extrapulmonary manifestation of COVID-19 with potential chronic consequences. This update provides a review of the clinical manifestations of cardiovascular involvement, potential direct SARS-CoV-2 and indirect immune response mechanisms impacting the cardiovascular system, and implications for the management of patients after recovery from acute COVID-19 infection.

**Key Words:** angiotensin-converting enzyme 2 ■ COVID-19 ■ inflammation ■ magnetic resonance imaging ■ thrombosis

With the coronavirus disease 2019 (COVID-19) pandemic entering its second year, the extrapulmonary impact of the disease has become increasingly evident. For the cardiovascular system, infection with the etiologic virus, severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2), can manifest acutely and persist into convalescence and possibly beyond.<sup>1</sup> Clinical outcomes are worse in patients with COVID-19 with cardiovascular disease and risk factors (eg, hypertension, diabetes, and obesity). Acute cardiac injury, inferred from elevations in cTn (cardiac troponin) levels, is reported in 8% to 62% of patients hospitalized with COVID-19 and is associated with greater disease severity, including need for mechanical ventilation, and death.<sup>2–6</sup>

SARS-CoV-2 contrasts from earlier coronavirus disease outbreaks in both its global epidemiology and cardiovascular impact.<sup>7,8</sup> In 2002, the SARS-CoV pandemic arose in Guangdong province, China; of the 8098 people infected worldwide, 774 died, yielding a case fatality rate of 9.6%.<sup>9,10</sup> In 2012, the Middle East Respiratory Syndrome outbreak arose from the Arabian peninsula and

infected 2562 people, with a case fatality rate of ≈34.4% and cases occasionally still being reported.<sup>11</sup> The current SARS-CoV-2 pandemic became evident in December 2019 from Wuhan, Hubei province, China. As of February 15, 2021, SARS-CoV-2 has infected over 109 million people and caused over 2.4 million deaths.<sup>12</sup> Overall, the worldwide apparent case fatality rate is estimated to be ≈4%.<sup>13</sup> However, case fatality rates vary by country from <0.1% to >20% and are influenced by testing strategies that define the infected population, economics, health care resources, comorbidity rates, demographics, and politics. In the United States alone, there have been over 550 000 deaths out of 30.6 million cases.<sup>12</sup> Furthermore, detection of SARS-CoV-2 infection is based on convenience sampling, not randomized testing. There is also increasing awareness of asymptomatic, presymptomatic, and pauci-symptomatic COVID-19 illness, which can nevertheless transmit infection. Thus, reported cases of SARS-CoV-2 infection likely underestimate its true prevalence. Regardless, even as cardiovascular complications were reported in case series of SARS-CoV and Middle East Respiratory Syndrome,<sup>14</sup> the cardiovascular

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## Nonstandard Abbreviations and Acronyms

<b>ACE</b>	angiotensin-converting enzyme
<b>ANG II</b>	angiotensin II
<b>CMR</b>	cardiac magnetic resonance imaging
<b>COVID-19</b>	coronavirus disease 2019
<b>cTn</b>	cardiac troponin
<b>HDL</b>	high-density lipoprotein
<b>hs-TnT</b>	high-sensitivity troponin-T
<b>IFN</b>	interferon
<b>LGE</b>	late gadolinium enhancement
<b>LV</b>	left ventricle
<b>NLR</b>	NOD-like receptor
<b>NRP1 BD</b>	neuropilin-1 binding domain
<b>NRP-1</b>	neuropilin-1
<b>nsp</b>	nonstructural protein
<b>PAR</b>	proteinase-activated receptor
<b>PF4</b>	platelet factor 4
<b>RBD</b>	receptor binding domain
<b>RV</b>	right ventricle
<b>SARS-CoV-2</b>	severe acute respiratory syndrome-coronavirus 2
<b>TLR</b>	toll-like receptor
<b>TMPRSS2</b>	transmembrane protease serine 2
<b>TNF</b>	tumor necrosis factor
<b>WPB</b>	Weibel Palade Body

impact of SARS-CoV-2 seems more prominent and correlates with COVID-19 disease severity and mortality.

This update focuses on the impact of SARS-CoV-2 on the heart and vasculature. We review SARS-CoV-2 clinical data implicating cardiovascular involvement, mechanisms underlying cardiovascular involvement, and implications for clinical management and future research directions.

## SARS-COV-2 VIRAL ENTRY AND LIFE CYCLE

SARS-CoV-2 is a betacoronavirus closely related to SARS-CoV and a number of naturally occurring bat coronaviruses.<sup>15,16</sup> Like other coronaviruses, SARS-CoV-2 virions are decorated with trimers of the spike protein that resemble a halo or corona upon electron microscopy, for which the family was named. These spike trimers, along with other viral structural proteins, are expressed late in the viral life cycle and incorporated into budding virus in the endoplasmic reticulum-Golgi intermediate compartment. The spike protein contains a surface subunit (S1) that includes the signal sequence, an N-terminal domain, a receptor-binding domain (RBD), and the NRP1 (neuropilin-1)-binding domain (BD) (Figure 1A).

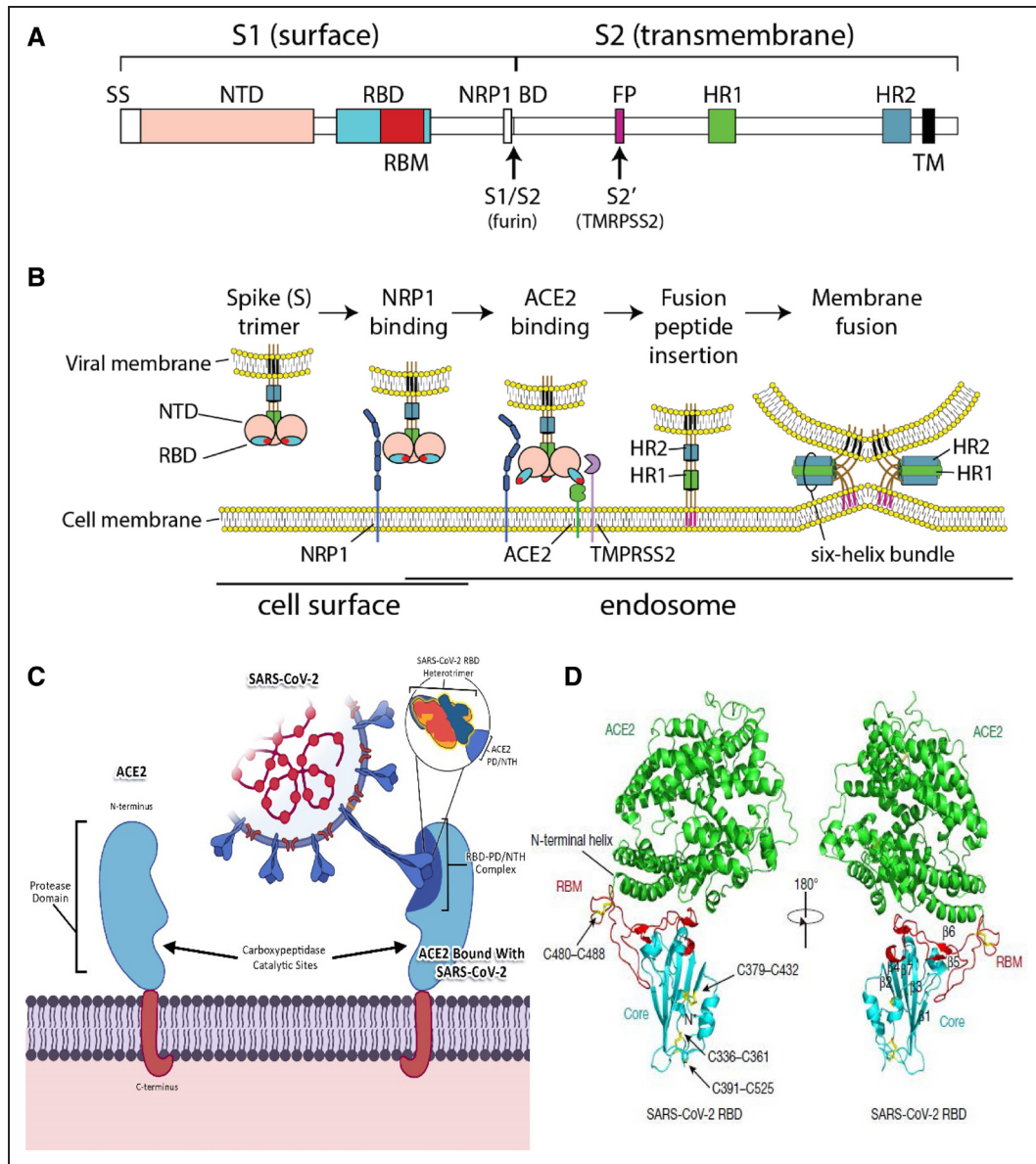
The transmembrane subunit (S2) contains the fusion peptide, 2 heptad repeat domains (HR1 and HR2), a transmembrane domain, and a short cytoplasmic tail. Within the producer cell, the SARS-CoV-2 spike protein is processed by the cellular protease furin at the S1/S2 site, separating the S1 and S2 domains<sup>18</sup> and exposing a conserved C-terminal motif, RXXR<sub>OH</sub>.

## Entry of SARS-CoV-2 Into Host Cells

### *The Host Cell Receptor for SARS CoV-2 Is the Biologically Critical Enzyme ACE2*

In 2003, 1 month after reports that it generated angiotensin 1-7 from ANG II (angiotensin II) in the intact human LV<sup>19</sup> and was upregulated at enzyme activity and protein levels in explanted human hearts,<sup>20</sup> ACE2 was surprisingly identified as the receptor for SARS-CoV.<sup>21</sup> SARS-CoV-2 also binds to ACE2. Binding of the spike protein RBD to ACE2 brings the virion into proximity with the host cell surface membrane and induces conformational changes in the RBD that initiate the process of membrane fusion.<sup>18,22</sup>

Infection of target cells (Figure 1B) is facilitated by the binding of NRP1 expressed on target cells, to the RXXR<sub>OH</sub> NRP1 BD exposed by furin cleavage. Although not required for infection, NRP1 is an attachment factor that significantly enhances viral infectivity.<sup>23</sup> Similarly, integrin  $\alpha 5$  or  $\alpha 5\beta 1$  dimer, known to mediate cell binding and entry of certain viruses,<sup>24</sup> binds to and is co-regulated with ACE2 in ventricular remodeling.<sup>25</sup> In the Vero E6 cell model, an  $\alpha 5\beta 1$  dimer inhibitor prevents SARS-CoV-2 infection and reduces binding of SARS-CoV-2 to ACE2.<sup>26</sup> Thus,  $\alpha 5\beta 1$  integrin, likely through binding to  $\alpha 5$ , may also be an attachment factor or even a co-receptor for SARS-CoV-2 entry.<sup>25-27</sup> Following attachment, SARS-CoV-2 RBD binds to the cell surface receptor ACE2. SARS-CoV-2 is subsequently taken up into target cells via endocytosis, and in the presence of low pH, the RBD undergoes dramatic structural refolding.<sup>28,29</sup> Concomitant with binding to ACE2, in many cell types, the cellular transmembrane serine protease TMPRSS2 (transmembrane protease serine 2) cleaves the S2 transmembrane subunit at the S2' position,<sup>28</sup> triggering further conformational changes in the spike protein that enable the fusion peptide to insert into the cellular membrane. Cellular cathepsins can also process the S2' position. In some cell types, such as human ventricular myocardium<sup>30</sup> and inducible pluripotent stem cell-derived cardiomyocytes,<sup>31,32</sup> where TMPRSS2 is expressed at very low levels, cathepsin inhibitors, rather than TMPRSS2 inhibitors, prevent SARS-CoV-2 cell entry. Thus, mechanisms involved in membrane fusion and cell entry may be cell-specific. The HR1 and HR2 domains of S2 subsequently interact within the trimers to form an energetically stable 6-helix bundle that brings the viral and cellular membranes into proximity



**Figure 1. Structural components of the severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2) spike protein, interactions with ACE2 (angiotensin-converting enzyme 2) and host cell entry.**

**A**, Spike protein structural and functional elements. **B**, Interaction of the spike protein and processing resulting in viral entry to host cells. **C** and **D**, Structural and membrane topology of ACE2 and binding to SARS-CoV-2 S-protein receptor binding domain (RBD). **C**, ACE2 as a transmembrane protein with an N-terminal extracellular protease ectodomain and a short cytoplasmic C-terminus. Protease enzymatic activity is in the ectodomain (protease domain [PD]), containing a carboxypeptidase catalytic site. The receptor binding site for SARS-CoV-2 is more toward the N-Terminus, involving the N-terminal helix (NTH). **D**, X-ray crystallography of SARS-Cov-2 RBD bound to ACE2. ACE2 is in green, RBD in cyan and the CoV-2 receptor binding moiety (RBM) in red. Reproduced from Lan et al<sup>17</sup> with permission. Copyright©2020, Springer Nature. ACE2 indicates angiotensin-converting enzyme 2; FP, fusion peptide; HR, heptad repeat domain; NRP1 BD, neuropilin-1 binding domain; NTD, N-terminal domain; SS, signal sequence; TM, transmembrane domain; and TMPRSS2, transmembrane protease serine 2.

and initiates the formation of a fusion pore, releasing viral contents into the cytosol.<sup>33,34</sup>

Several important differences exist between entry of SARS-CoV-2 and SARS-CoV. SARS-CoV-2 contains a furin cleavage site at S1/S2 that is not present in SARS-CoV; instead, cellular cathepsins mediate cleavage of S1 and S2.<sup>35,36</sup> The furin cleavage of SARS-CoV-2 spike protein has several important consequences, including enhancing cell-cell fusion and enabling the virus to infect

human lung cells.<sup>37</sup> The SARS-CoV-2 spike protein RBD has a number of mutations that confer higher affinity for ACE2 than SARS-CoV<sup>38,39</sup> and prevent effective cross-neutralization by antibodies targeting the RBD<sup>40-42</sup> with some exceptions.<sup>43-47</sup> Conversely, genetic polymorphisms in ACE2 vary across populations,<sup>48</sup> several missense variants are predicted to disrupt ACE2 function and structure that may affect SARS-CoV-2 interactions,<sup>49</sup> and a recently reported short isoform that lacks

spike high-affinity binding sites and that is upregulated in response to IFN (interferon) stimulation may affect host resistance.<sup>50</sup> A new, more highly transmissible strain of SARS-CoV-2 emerging from the United Kingdom (20I/501Y.V1) at the end of 2020 appears to have an even higher affinity for ACE2, via a N501Y mutation at one of the 6 key RBD contact sites.<sup>51</sup> In South African (20H/501Y.V2) and Brazilian (20J/501Y.V3) variants, an E484K mutation has emerged that seems to confer heightened resistance to neutralization while only modestly increasing affinity for ACE2.<sup>52</sup> Together, these factors underlying differences in cell-cell spread, viral tropism, and affinity for ACE2 may underlie the enhanced transmission of SARS-CoV-2.

### Late Life Cycle of SARS-CoV-2

Following cell entry, the  $\approx 30$  kb SARS-CoV-2 genomic RNA is translated into large concatenated polypeptides corresponding to the *ORF1a* and *ORF1b* reading frames. These polypeptides contain the viral nsps (nonstructural proteins), including the nsp3 and nsp5 viral proteases that process the polypeptide chains into 15 to 16 nsp subunits. Most nsps form the viral replication and transcription complex responsible for replicating the full-length viral genome. The structural proteins, including spike, nucleocapsid, membrane, and envelope proteins, are expressed from a subgenomic promoter in a series of nested transcripts and assemble with the full-length viral RNA in the endoplasmic reticulum-Golgi intermediate compartment. Additional details on the coronavirus life cycle can be found in an excellent review.<sup>53</sup>

### ACE2 and Preferential Access of SARS-CoV-2 to Human Cardiac Myocytes and Other Cardiovascular System Host Cells

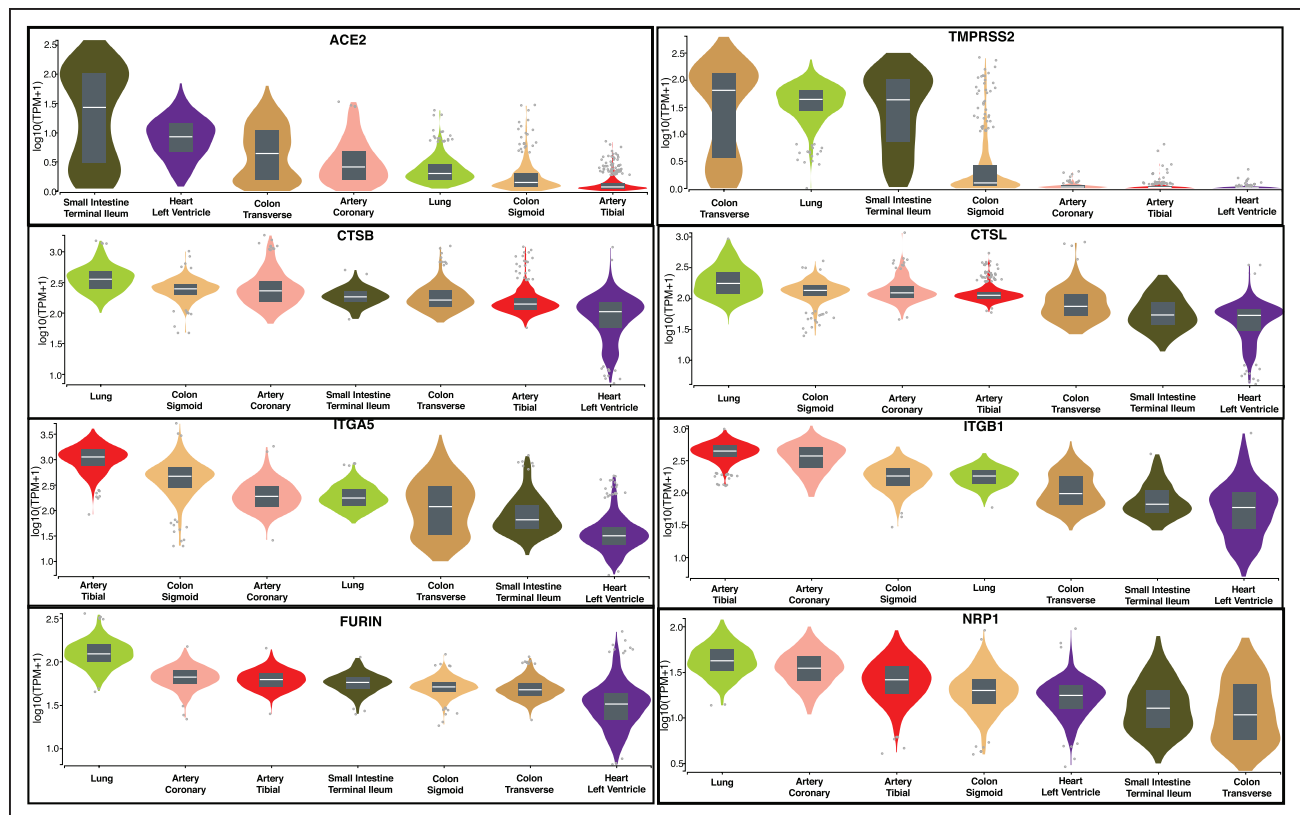
Viruses use a wide range of proteins, carbohydrates, or lipids to bind to host cells and consequently enter them for viral propagation. However, ACE2 stands as the only example of a critical myocardial enzyme used by a highly pathogenic virus for cell binding and entry. SARS-CoV, SARS-CoV-2, and 2 other coronaviruses<sup>54,55</sup> are known to use ACE2 for cell binding and entry, but no other virus families are known to utilize ACE2 as a host cell receptor. In contrast to many other highly infectious pathogenic viruses such as influenza,<sup>56</sup> the initial binding of SARS-CoV-2 spike protein to its host cell receptor ACE2 is of very high affinity<sup>18</sup>; the SARS-CoV-2 spike protein binds to human ACE2 via its RBD with reported equilibrium constants (dissociation constants, or  $K_D$ s) of 1.2<sup>18</sup> and 4.7 nmol/L.<sup>17</sup> The ACE2-binding affinity of SARS-CoV spike protein is somewhat less,  $\approx 30$  nmol/L.<sup>17</sup> Cell-specific expression and high-affinity binding of ACE2 to the SARS-CoV-2 spike protein RBD likely account for the relatively high incidence of cardiovascular involvement in COVID-19.

### Distribution of ACE2 and Other Viral Entry Proteins in Cardiovascular Tissue and Cells

Host cell receptor binding of a virus is essential to cell invasion, and cell and tissue distribution of host receptors is a major determinant of viral tropism and its clinical manifestations. The tissue distribution of ACE2 includes the heart, lung, intestines, kidney, testis, nose, and mouth.<sup>57</sup> While nasal and pulmonary epithelial cells are recognized as the first infected cells, after initial viral replication and circulation, many cardiac resident cells express the necessary components for uptake and replication. Based on gene expression studies, human ventricular myocardium contains all the requisite mediators of SARS-CoV-2 binding and entry. Although TMPRSS2 is minimally expressed in the heart, there is high expression of *ACE2*, and other proteases (eg, *FURIN*, *NRP1*, *CTSB/L*) known to participate in priming and membrane fusion, and integrin co-receptors appear to be ubiquitously expressed (Figure 2).<sup>25–27</sup> More recent studies, employing single nuclei RNA sequencing,<sup>58–60</sup> open source proteomics,<sup>61</sup> and immunostaining,<sup>61</sup> have confirmed *ACE2* expression in cardiac myocytes and pericytes with lack of evidence for *ACE2* expression in endothelial cells.<sup>30,61</sup> Lung expression is mostly confined to type 2 alveolar cells.<sup>61</sup> In ventricular myocardium cardiac myocytes and fibroblasts exhibit *ACE2* expression, but pericytes, which support the microvasculature throughout the myocardium, appear particularly susceptible with robust expression of *ACE2* (Figure 3A).<sup>60</sup>

### Functional and Structural Characterization of Human Myocardial ACE2

The renin-angiotensin system, including the balance of ACE (angiotensin-converting enzyme) and ACE2, has important physiological effects on the cardiovascular system (Figure 4). In the human heart, generation of the octapeptide ANG II from angiotensin I is mostly (85%–89%) mediated by ACE.<sup>63</sup> In human ventricular myocardium, breakdown/hydrolysis of ANG II is primarily mediated by ACE2, leading to formation of angiotensin 1-7,<sup>19</sup> a counter-regulatory peptide that is antihypertrophic and has other favorable biologic activities (Figure 4). Thus, ACE2 nullifies the pathological hypertrophy and other adverse effects of ANG II by both catalyzing its breakdown and generating a counter-regulatory peptide. The importance of ACE2's enzymatic action on ANG II is highlighted by the effects of *ACE2* gene inactivation, which leads to reduction of contractile function<sup>64</sup> or pathological ventricular hypertrophy when subjected to chronic afterload stress.<sup>65</sup> *ACE2* structure has transmembrane, cytosolic, and extracellular domains, the latter containing carboxypeptidase activity (Figure 1C).<sup>66</sup> The *ACE2* amino acid composition of the catalytic domain is 42% homologous with the ACE catalytic domain, but ACE inhibitors have no effect on *ACE2* activity.<sup>57</sup>



**Figure 2. Gene expression by tissue of genes encoding viral entry proteins that interact with severe acute respiratory syndrome-coronavirus 2 from GTEx.**

The lung and intestines express high levels of ACE2 (angiotensin-converting enzyme 2) and transmembrane protease serine 2 (TMPRSS2), whereas the heart left ventricle expresses ACE2 at high levels, but TMPRSS2 at low levels. However, CTSB and CTSL (encoding cathepsins B and L, respectively), FURIN, NRP1, and ITGA5 and ITGB1 (integrins) show expression in all tissues displayed. Obtained from the Genotype-Tissue Expression (GTEx) Portal, accessed on January 14, 2021.

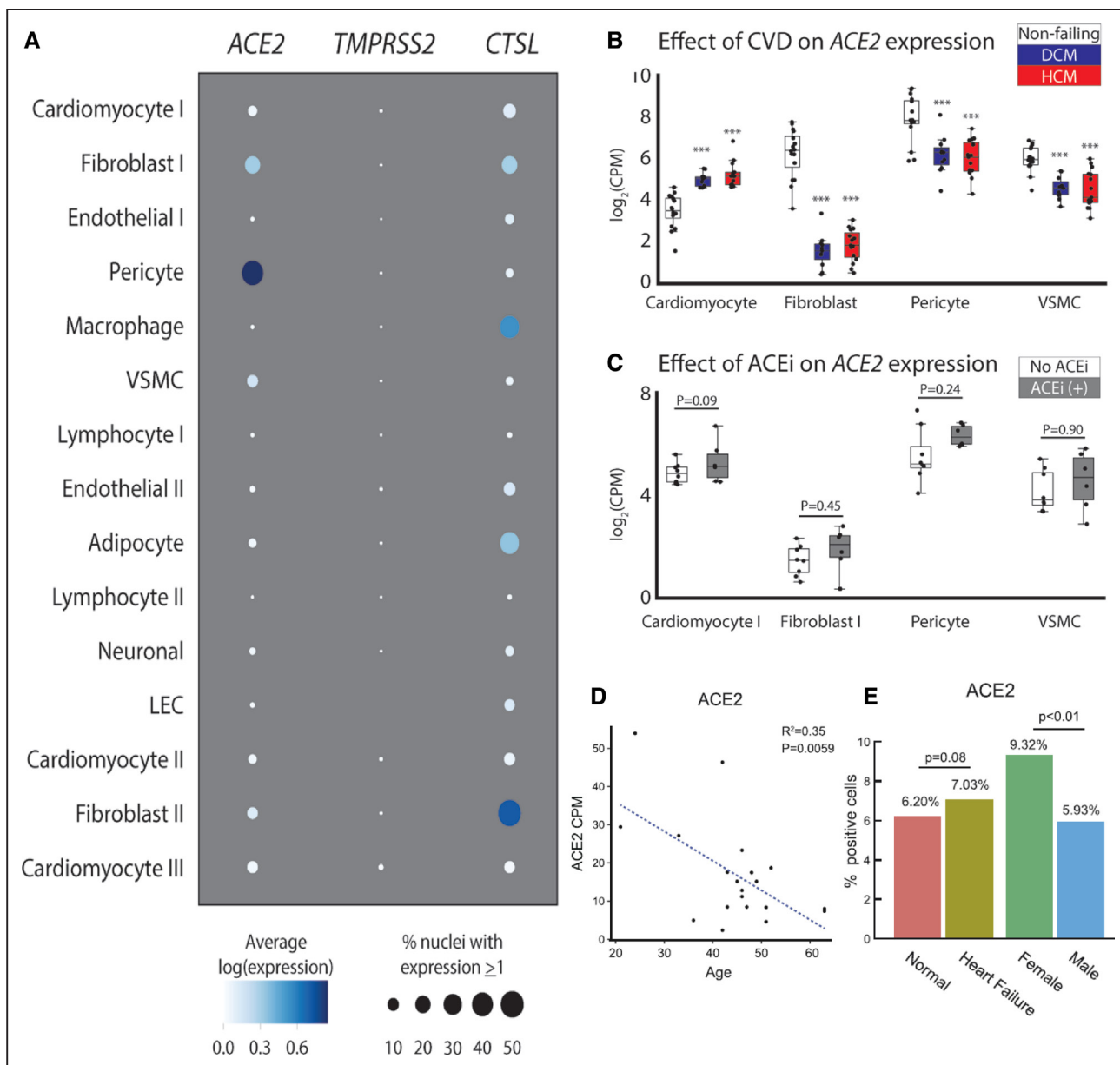
The ACE2 receptor region is in the N-terminal helix region of the extracellular domain (Figure 1C). The RBD-binding moiety in ACE2 is in a region distinct from the carboxypeptidase catalytic domain (Figure 1C and 1D),<sup>66</sup> creating the possibility of targeting this region of ACE2 to interfere with SARS-CoV-2 binding to ACE2 without reducing enzymatic activity. Given the cardiovascular protective function of ACE2 carboxypeptidase activity under normal physiological conditions, a lack of enzyme inhibition is a requisite for any ACE2-targeted therapeutic agent designed to prevent SARS-CoV-2 binding to ACE2.<sup>25</sup>

### Regulation of ACE2 in LV Myocardial Remodeling

The mechanisms involved in SARS-CoV-2 cell binding and entry seem to be modified by heart muscle disorders. Given the susceptibility of those with preexisting cardiovascular conditions to severe COVID presentations, several studies examined the expression of ACE2 in (non-COVID) failing hearts.<sup>58,60,67,68</sup> With pathological eccentric remodeling of the left ventricle (LV), ACE2 mRNA expression increases  $\approx 2$ -fold and then decreases toward normal levels on reverse remodeling.<sup>25</sup> Proteases are not regulated with remodeling, but multiple integrins are, and ITGA5 mRNA expression tracks with ACE2. More recently, single nuclei

RNA sequencing studies showed that upregulation in failing hearts was specific to cardiomyocytes (Figure 3B),<sup>60</sup> suggesting a potential mechanism for the enhanced susceptibility in patients with heart failure. The regulation of ACE2 is independent of treatment with ACE inhibitors or ARBs (Figure 3C).<sup>25,60</sup> These and other studies,<sup>20,58,69</sup> indicating that ACE2 upregulation is associated with pathological ventricular remodeling, may have implications for susceptibility or response to SARS-CoV-2 infection in those with cardiovascular comorbidities.<sup>25,70</sup> Conversely, ACE2 expression in cardiac cells seems to be lower with older age and in males, compared with females (ACE2 is also X linked), (Figure 3D and 3E) both factors associated with worse disease severity.<sup>62</sup> Whether and how these sex and age differences in ACE2 expression contribute to the sex and age differences observed in COVID-19 severity remain unclear.

The next sections explore cardiovascular manifestations of the potential direct and indirect myocardial effects of SARS-CoV-2 infection. Myocardial and/or vascular injury have been postulated to occur via direct viral infection as well as indirectly, from immune responses to viral infection.



**Figure 3. ACE2 (angiotensin-converting enzyme 2) expression in human myocardium.** **A**, Relative expression of *ACE2*, *TMPRSS2*, and *CTSL* in left ventricle by snRNASeq. **B**, snRNASeq showing expression of *ACE2* in cell subtypes, demonstrating increases in *ACE2* expression in cardiomyocytes but reduced expression in fibroblasts, pericytes, and vascular smooth muscle cells (VSMCs) in dilated cardiomyopathy (DCM) and hypertrophic cardiomyopathy (HCM) ventricles. **C**, Effects of ACE inhibitors on *ACE2* expression across cell types in HCM, showing no significant change. **D** and **E**, *ACE2* expression in adult human heart from public scRNASeq databases. **D**, Age and (**E**) normal vs heart failure and male vs female stratification. **A**, **B**, and **C** reproduced from Tucker et al<sup>60</sup> with permission. Copyright©2020, Wolters Kluwer Health, Inc. **D** and **E** reproduced from Liu et al<sup>62</sup> with permission. Copyright©2020, Oxford University Press.

## DIRECT MYOCARDIAL EFFECTS

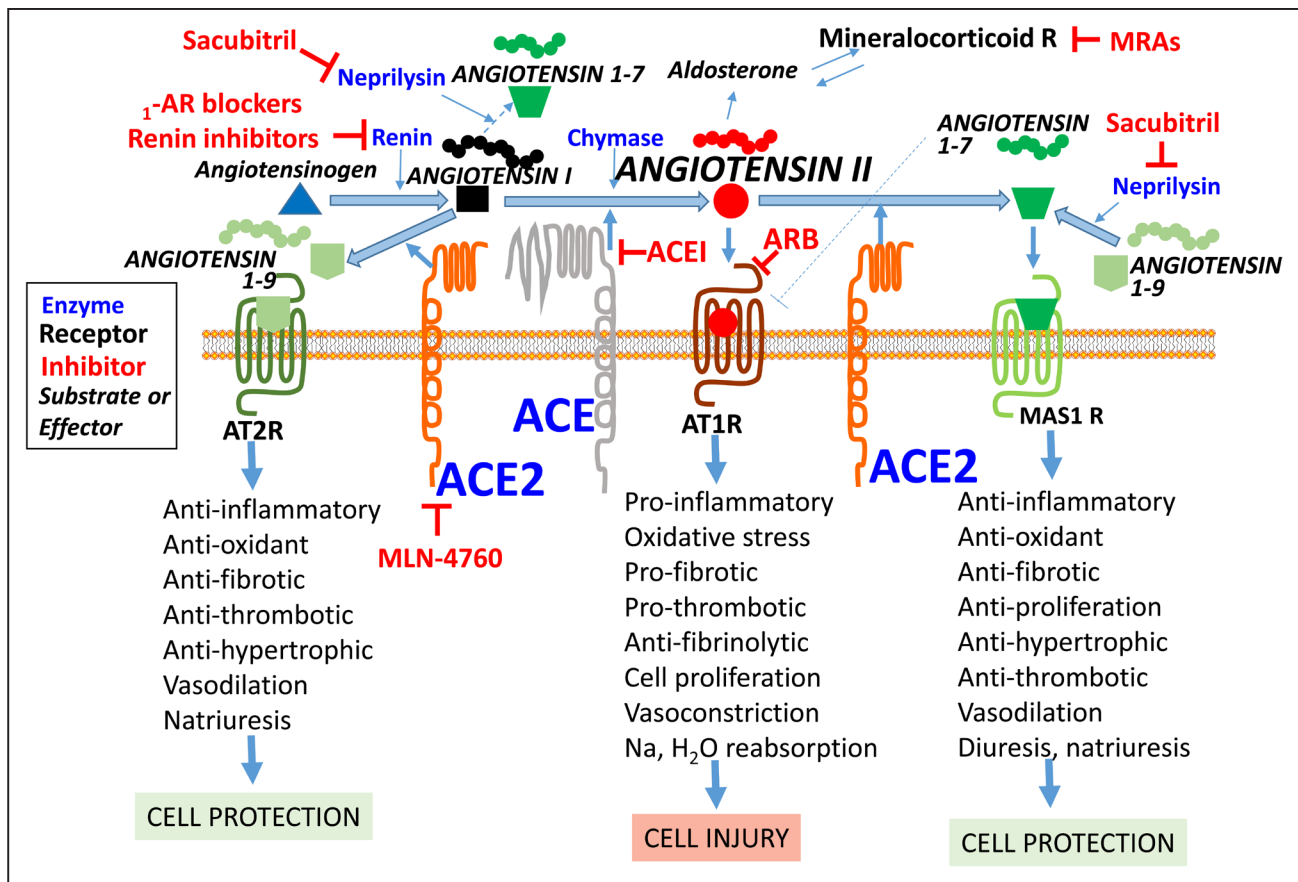
### Clinical Evidence for Acute Myocardial Injury

#### Acute Clinical Cardiovascular Manifestations

Among hospitalized patients with COVID-19, evidence of acute cardiac compromise is common and includes acute heart failure (3%–33%),<sup>71–73</sup> cardiogenic shock (9%–17%),<sup>74</sup> myocardial ischemia or infarction (0.9%–11%),<sup>71</sup> ventricular dysfunction (left ventricular [10%–41%], right ventricular [33%–47%], biventricular

[3%–15%]),<sup>75–78</sup> stress cardiomyopathy (2%–5.6%),<sup>75,77</sup> arrhythmias (9%–17%),<sup>71,72,74,79</sup> venous thromboembolism (23%–27%),<sup>73</sup> and arterial thrombosis secondary to viral-mediated coagulopathy.<sup>80</sup>

Preexisting cardiovascular disease (coronary heart disease, heart failure, cerebrovascular disease), cardiovascular risk factors (eg, male sex, older age, hypertension, diabetes), and other comorbidities (eg, chronic obstructive pulmonary disease, chronic renal failure, and cancer) predispose patients with COVID-19 to



**Figure 4. Renin-angiotensin system and ACE2 (angiotensin-converting enzyme 2).**

more severe disease and mortality. Racial and ethnic disparities in COVID-19 outcomes are also evident. In a meta-analysis, Black, Asian, and Hispanic individuals had a higher risk of COVID-19 infection compared with White individuals; more severe disease, marked by need for intensive therapy units and death, was associated with Asian cohorts.<sup>81</sup> Other studies have highlighted health disparities in the United States with higher mortality rates in Black<sup>82,83</sup> and Latinx populations,<sup>82,83</sup> and in predominantly Black compared with White populated counties.<sup>83,84</sup> In an analysis of the American Heart Association, COVID-19 Cardiovascular Disease Registry of 7868 patients hospitalized with COVID-19, 33% were Hispanic, 25.5% non-Hispanic Black, 6.3% Asian, and 35.2% non-Hispanic White; in-hospital mortality and major adverse cardiovascular events (death, myocardial infarction, stroke, heart failure) did not differ by race or ethnicity after adjustment, although Black and Hispanic patients had a larger burden of mortality due to their higher proportions of hospitalizations.<sup>85</sup> Asian patients had the highest cardiorespiratory severity score.<sup>85</sup>

**Biomarker Evidence for Cardiac Injury**

Biomarker evidence of cardiac injury is strongly associated with worse COVID-19 outcomes. Elevation of cardiac biomarkers, such as NT-proBNP, cTn, or d-dimer,<sup>4,6,86,87</sup> predict poor clinical outcomes. In hospitalized patients

with COVID-19, the prevalence of elevated hs-TnT (high-sensitivity troponin-T) is 20% to 30%.<sup>5,88</sup> Inferred from such elevated cTn levels, acute myocardial injury reportedly ranges from 8% to 62% overall,<sup>2,3</sup> with worse disease severity associated with a higher prevalence of elevated levels. Elevated cTn levels were rare in COVID-19 survivors with an uncomplicated course (1%–20%), common in severely ill patients (46%–100%), and nearly universal in the critically ill (ie, requiring intensive care or mechanical ventilation) and nonsurvivors.<sup>2,4,5</sup> Among 2736 hospitalized patients with COVID-19 in New York City, even small elevations of cTn I (>0.03–0.09 ng/mL) were associated with higher mortality.<sup>89</sup> Furthermore, the greater the cTn elevation, the higher the mortality risk.<sup>5,89</sup> Compared with those without cTnI elevation, patients with COVID-19 with cTn elevation have higher risks of acute respiratory distress syndrome (58%–59% versus 12%–15%),<sup>4,5</sup> need for mechanical ventilation (22%–60% vs 4%–10%),<sup>4,5</sup> malignant arrhythmias (17% versus 2% VT/VF),<sup>4</sup> and death (51%–95% versus 5%–27%).<sup>4,5</sup> cTn and NT-proBNP levels increase during hospitalization in nonsurvivors but not among survivors.<sup>4,6</sup>

**Imaging Evidence for Myocardial Injury**

Biomarker evidence of myocardial injury with associated echocardiographic abnormalities correlate with higher risk of in-hospital mortality. Echocardiographic

abnormalities commonly reported in hospitalized patients with COVID-19 include right ventricular (RV) dysfunction (26.3%), LV wall motion abnormalities (23.7%), global LV dysfunction (18.4%), grade II or III diastolic dysfunction (13.2%), and pericardial effusion (7.2%).<sup>3</sup>

Abnormalities suggesting injury on cardiac magnetic resonance imaging (CMR) have also been reported commonly. CMR findings include T1 mapping abnormalities (suggesting diffuse myocardial changes such as diffuse fibrosis and/or edema); T2, short tau inversion recovery, or T2 mapping abnormalities (more specific for myocardial inflammation, as occurs in acute myocarditis); late gadolinium enhancement (LGE, suggestive of acute myocardial injury and/or myocardial fibrosis); or pericardial involvement—all of which can indicate cardiac pathologies associated with COVID-19. In a systematic review comprising 199 patients from 34 acute or postrecovery CMR studies in patients with COVID-19,<sup>90</sup> CMR diagnoses included myocarditis in 40.2%, myopericarditis in 1.5%, Takotsubo in 1.5%, ischemia in 2.5%, and dual ischemic and non-ischemic changes in 2.0%. Regional wall motion abnormalities were reported in 13/32 (40.6%), edema (on T2 or short tau inversion recovery) in 46/90 (51.1%), LGE in 85/199 (42.7%), and T1 and T2 mapping abnormalities in 109/150 (73%) and 91/144 (63%), respectively. Additionally, perfusion and extracellular volume mapping abnormalities were described in 18/21 (85%) and 21/40 (52%) patients, respectively. Pericardial involvement included pericardial effusion (43/175; 24%) and pericardial LGE (22/100; 22%). In summary, the most common CMR diagnosis was myocarditis, and imaging findings included evidence of diffuse myocardial edema, and myocardial fibrosis. However, it is important to note the majority of findings reported were mild increases in T1 and T2 times, and the clinical significance of isolated T1/T2 abnormalities related to COVID-19 still remains unknown.

### Postrecovery Cardiac Involvement

The potential for long-term cardiac sequelae of COVID-19-associated myocardial injury has been highlighted by CMR studies in recovered patients (Online Table I) with evidence of myocardial fibrosis or myocarditis reported in 9% to 78% of patients recovered from acute COVID-19. Among 100 post-COVID-19 patients who underwent CMR 2 to 3 months after the diagnosis, Puntmann et al<sup>1</sup> reported cardiac involvement in 78% with evidence of ongoing inflammation in 60%. On the day of imaging, 71% had elevated hs-TnT. Cardiac symptoms were common and included atypical chest pain (17%), palpitations (20%), and dyspnea and exhaustion (36%). Recovered patients had lower left ventricular (LV) ejection fraction and higher LV volumes compared with risk factor-matched controls. In 3 patients with severe CMR findings, endomyocardial biopsy revealed active lymphocytic infiltration but without detectable viral genome.

CMR findings were also reported in 26 patients who had recovered from COVID-19 but with cardiac symptoms after discharge, including chest pain, palpitations, or chest distress.<sup>91</sup> Abnormal CMRs with increased T2 signal and/or positive LGE were found in 15 (58%), including myocardial edema in 14 (54%), and 8 (31%) with LGE. Compared with 20 healthy controls of similar age and sex, T1, T2, and extracellular volume values were significantly elevated in the recovered patients with positive CMR findings. Only one of the abnormal CMR patients had impaired LVEF (45%) with reduced contractility of the segments with edema. Abnormal right ventricular functional parameters, including ejection fraction, cardiac output, cardiac index, stroke volume, and stroke volume/BSA were also found in patients with abnormal CMRs compared with healthy controls.

Studies in competitive collegiate athletes report a 27% to 46% prevalence of LGE.<sup>92,93</sup> In 26 competitive college athletes (mean age, 19.5 years),<sup>92</sup> CMR revealed evidence of myocarditis in 4 (15%) and LGE in 12 (46%); 8 (31%) had LGE without T2 elevation, suggesting prior myocardial injury. None had required hospitalization or received COVID-19 antiviral therapy. Twelve had only mild symptoms (sore throat, shortness of breath, myalgias, fever), and the others were asymptomatic. The study lacked a control group for comparison, used CMR criteria derived from symptomatic rather than asymptomatic patients, and included 2 (8%) with LGE at the RV insertion site that has been considered a nonspecific finding in athletes. Nevertheless, abnormalities were not detected by other measures (there were no diagnostic changes on ECG, all had normal ventricular function and volumes by transthoracic echo and CMR, all had normal cTnI). Another study of 59 collegiate athletes reported CMR findings in 16 (27%), of whom 13 (22%) had nonpathological punctate inferoseptal RV insertion abnormalities; 2 (3%) met criteria for myocarditis, 1 (2%) had other myocardial abnormalities, and 1 (2%) had pericarditis.<sup>93</sup> Mild increases in T1, T2, or extracellular volume were seen in 39% of COVID-19+ athletes, compared with 13% of 60 athletic controls and 8% of 27 healthy controls. A third study of 145 competitive student athletes with CMRs a median of 15 days after diagnosis reported that only 2 (1.4%) met criteria for myocarditis, 2 (1.4%) had LGE without T2 abnormality, 1 had pericardial enhancement, and 38 (26.2%) had LGE at the RV insertion site.<sup>94</sup> Excluding nonspecific RV insertion site LGE, the prevalence of LGE abnormalities in athletes in these studies ranged from 2.8% to 38% with 1.4% to 15% meeting criteria for myocarditis (Online Table I).

Such CMR findings of myocarditis and myocardial fibrosis raise concerns regarding potential long-term cardiac sequelae, including increased risk for heart failure and arrhythmias based on prior experience with myocarditis. The presence of LGE related to myocarditis often implies myocardial necrosis in addition to myocardial edema and has previously been associated with adverse outcomes



in multiple non-COVID related myocarditis CMR studies.<sup>95,96</sup> This risk has been shown to be further modulated by LV dysfunction (LVEF<50%)<sup>97</sup> and persistent T2 elevation or myocardial edema.<sup>98</sup> The significant prevalence of abnormal LGE (12%) in the Puntmann et al,<sup>1</sup> control group highlights the need to expand our understanding of subclinical myocardial injury in the general population and athletes. At this time, the actual risk of complications in patients with abnormal CMR findings remains undefined. Moreover, whether isolated elevations in T1/T2 times are due to capillaritis, microthrombi, or endothelial dysfunction secondary to a systemic inflammatory response, or histologically defined myocarditis remains undiscerned.

### Post-Acute COVID-19 Syndrome

Post-acute sequelae of SARS-CoV-2 infection, often termed postacute COVID syndrome, or long-COVID, can occur in recovering patients. Among 143 patients seen as outpatients after COVID-19 infection, only 12.6% were asymptomatic.<sup>22</sup> Symptoms included fatigue (53.1%), dyspnea (43.4%), joint pain (27.3%), and chest pain (21.7%); 44.1% reported worsened quality of life. Among 1733 discharged patients with COVID-19 followed up a median of 6 months after symptom onset, the most common symptoms were fatigue or muscle weakness (63%), sleep difficulties (26%), and anxiety or depression (23%).<sup>99</sup> Greater illness severity during hospitalization was associated with more impaired pulmonary diffusion capacities and abnormal chest imaging.<sup>99</sup> What remains unclear are the contributions of post-COVID cardiac involvement and acute COVID myocardial injury to the symptoms of postacute COVID-19 syndrome.

### Mechanisms of Direct Cardiac Injury

Analyses of prior coronavirus outbreaks and COVID-19 data suggest several potential mechanisms of COVID-19 myocardial injury (Figure 5). Acutely, SARS-CoV-2 may directly infect and damage cardiac cells, triggering severe cellular and organ-wide pathology and dysfunction, although fulminant myocarditis is relatively uncommon in COVID-19. A rabbit coronavirus model of myocarditis and heart failure was established over 25 years ago and demonstrated direct viral infection of the heart,<sup>100</sup> presaging the effects of SARS-CoV-2 on the human myocardium.

### Evidence of Direct Cardiac Viral Infection From Cardiac Autopsies and Endomyocardial Biopsies

Cardiac autopsies have shown cardiomegaly, RV dilation, evidence of RV strain (19%), lymphocytic myocarditis (14%–40%), focal pericarditis (19%), endocardial thrombosis (14%) or endothelitis, and small vessel thrombosis (19%).<sup>101–104</sup> Cardiac tropism of SARS-CoV-2 was initially established by quantitative RT-PCR detection of viral RNA in postmortem hearts of patients with COVID-19 and subsequently in endomyocardial biopsies

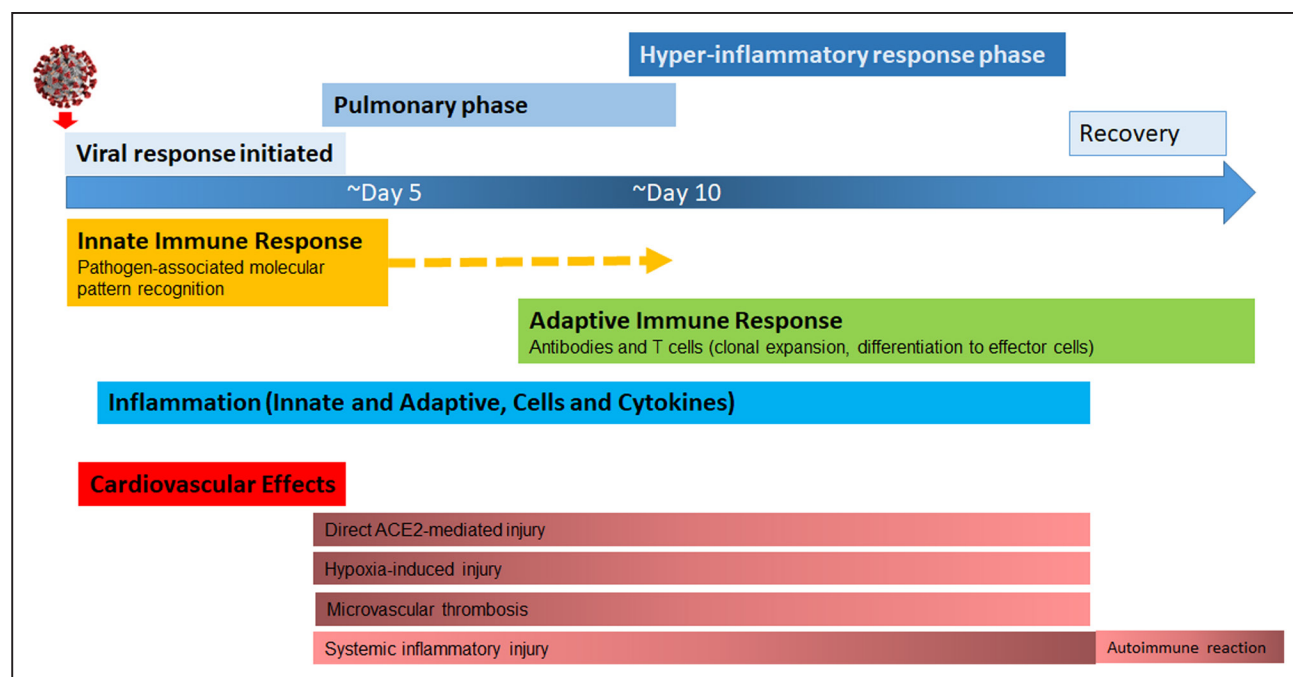
of patients with suspected myocarditis.<sup>105–107</sup> Whether the detected viral RNA reflected infected cardiac cells or circulating viral particles remained in question until more recently. Cardiac cellular tropism of SARS-CoV-2 has now been proven by in situ labeling of SARS-CoV-2 RNA and by electron microscopy detection of virus-like particles within cardiomyocytes,<sup>108</sup> interstitial cells,<sup>105,107</sup> and endothelial cells<sup>102,108,109</sup> of postmortem hearts. Putative SARS-CoV-2 viral particles have also been detected by electron microscopy on endomyocardial biopsy.<sup>110</sup> Autopsies in patients with acute myocarditis have recently demonstrated evidence of viral infection, processing, and replication within cardiomyocytes.<sup>31</sup> The preponderance of evidence suggests that SARS-CoV-2 can readily infect human cardiac myocytes<sup>31,110</sup> and can be detected in myocytes on autopsy or by endomyocardial biopsy in patients with<sup>31,110</sup> and without<sup>111</sup> clinical evidence of cardiac involvement.

Despite reports of SARS-CoV-2 in some hearts with histologically proven myocarditis,<sup>31</sup> viral RNA has been undetectable in other COVID-19 myocarditis cases or did not correlate with sites of myocarditis.<sup>102,105,112,113</sup> Furthermore, acute myocarditis as defined by Dallas criteria has rarely been detected in COVID-19 nonsurvivors. Of 277 hearts across 22 COVID-19 autopsy studies, only 20 cases of myocarditis (7.2%) were reported.<sup>114</sup> The actual prevalence of COVID-19 myocarditis was likely lower, as several cases were reportedly functionally insignificant. In contrast to the low prevalence of myocarditis, interstitial macrophage infiltration without cardiomyocyte degeneration was common in a multicenter COVID-19 autopsy series (18 of 21 cases, 86%).<sup>101</sup>

Other more common histological findings reported by COVID-19 autopsy series include perivascular and myocardial inflammatory infiltrates, endocardial and small vessel thrombosis, endotheliitis, and myocyte degeneration.<sup>101–104</sup> Whether direct SARS-CoV-2 cytotoxicity underlies these more common cardiac histopathologies remains unclear. Few autopsy series assessed SARS-CoV-2 viral load in the heart. One study of 39 postmortem hearts detected SARS-CoV-2 by qRT-PCR in 24 (61.5%) cases, with 16 hearts exhibiting a high viral load (>1000 genomic copies per  $\mu\text{g}$  of total RNA).<sup>105</sup> Although the high viral load hearts had a proinflammatory transcriptomic profile, no immune cell infiltrates were found. Whether the heterogeneity of COVID-19 cardiac histopathology signifies distinct endophenotypes of COVID-19 myocardial injury or a continuum of one pathological process remains to be determined. Also unknown is how either SARS-CoV-2 viral load or patient immunopathology relates to these diverse cardiac pathological findings in COVID-19.

### Defining Cardiac Cell Targets of SARS-CoV-2

As above, single-cell and single nuclei RNA sequencing studies have demonstrated expression of *ACE2* in pericytes, cardiomyocytes, and fibroblasts, with



**Figure 5. Approximate time course of immune response and cardiovascular effects.**

ACE2 indicates angiotensin-converting enzyme 2.

cardiomyocyte-specific upregulation of *ACE2* in failing hearts (Figure 3B).<sup>60,115</sup> The presence of severe microvascular injury in postmortem analyses supports a role for pericytes, which show high *ACE2* expression in single nuclei RNA sequencing studies, but whether the microvascular injury involves direct infection or indirect inflammatory mechanisms is unclear. Several groups have utilized human inducible pluripotent stem cell-derived cardiomyocytes, living tissue slices, and engineered heart tissue to model SARS-CoV2 infection,<sup>31,116–118</sup> demonstrating *ACE2* expression and direct cardiomyocyte susceptibility to SARS-CoV-2 infection. Infection produced cytotoxic effects<sup>110,118</sup> and activated innate immune responses, including IFN signaling, apoptosis, reactive oxygen stress, and antiviral clearance pathways, as well as inhibition of metabolic pathways and suppression of *ACE2* expression.<sup>110,118</sup> Such models are also useful in studying mechanisms of viral infection and drug effects. For example, *ACE2* antibody was demonstrated to blunt infection in cardiomyocytes,<sup>118</sup> and infection of inducible pluripotent stem cell-derived cardiomyocytes was dependent on *ACE2* and cathepsins and blocked by remdesivir.<sup>110</sup>

## INDIRECT MYOCARDIAL EFFECTS

### Mechanisms of Indirect Cardiovascular Injury

While the direct cardiovascular manifestations of COVID19 detailed above stem from unique aspects of SARS-CoV2 virology and may be specific to

coronaviruses, indirect pathological mechanisms have been reported, including hypoxia-induced myocardial injury due to hypoxic respiratory failure and hypoxemia, small vessel ischemia due to microvascular injury and thrombosis, or acute RV failure due to pulmonary embolism or in situ pulmonary artery thrombosis. Cardiac injury may also stem from a dysfunctional immune response. Canonical features of the normal immune response after viral exposure are reviewed in Figure 5 and below. Hypo- and hyper-immune responses may contribute to severity of COVID-19 disease. Systemic inflammatory responses or cytokine storm may lead to cell death and multiorgan dysfunction, and late autoimmune phenomena have been postulated to contribute to autonomic dysfunction.

The remarkable variety of symptoms, clinical severity, and manifestations after SARS-CoV2 exposure underscores our limited understanding of the heterogeneity of immune dysfunction in COVID-19. A deeper understanding of the pathways that account for immune success or failure in COVID19 would inform our understanding of the mechanisms that account for cardiovascular disease in more generalizable settings.

### Normal Immune Mechanisms of Host Protection After Viral Exposure

The innate immune system provides the first-line host defense that senses viral infection, kills virus-infected cells to minimize viral replication, induces inflammation, and enhances adaptive immunity (Figure 5). The adaptive immune response, comprised of T cell and B

cell (antibody) responses, ultimately neutralizes viral particles, clears the virus, and establishes long term immunity.

### **Innate Immune Activation**

Innate immune signaling is rapidly induced by pathogen-associated molecular pattern and damage-associated molecular pattern molecules, which signal through pattern recognition receptors. Examples of pattern recognition receptors include membrane-bound receptors (eg, TLRs [toll-like receptors]) and cytoplasmic receptors (eg, NLRs [NOD-like receptors]). Activation of these pathways induces type I interferon (IFN $\beta$  and multiple IFN $\alpha$  species), proinflammatory cytokines (eg, TNF [tumor necrosis factor]), and chemokines (which enhance recruitment of leukocytes). Type I IFN signals through IFN $\alpha\beta$  receptors, increasing class I major histocompatibility complex expression and antigen presentation to CD8+ T cells, which kill virus-infected cells. Type I IFN also enhances killing of infected cells by natural killer cells and inhibits mRNA translation, a critical control point in the lifecycle of RNA viruses. A parallel mechanism for local control of viral infection and cytokine activation is the assembly of inflammasomes, a set of multimeric cytoplasmic signaling complexes. For example, NLRP3 inflammasome mediates activation of caspase-1, which cleaves pro-IL-1 $\beta$  and pro-IL-18 into active proinflammatory cytokines that can synergistically activate NF- $\kappa$ B to augment IL-6 and TNF expression. Inflammasomes can also cause apoptosis or pyroptosis, a proinflammatory/lytic cell death.

### **Adaptive Immune Responses**

Immature dendritic cells resident at sites of infection (eg, the lung) sense danger signals, which induce a maturation process that results in enhanced major histocompatibility complex molecule expression and antigen presentation, accompanied by migration to lymph nodes. In the lymph node, antigens presented by class I and class II major histocompatibility complex molecules activate naive CD8+ and CD4+ T cells, respectively. CD8+ T cells contribute as cytolytic effector cells, killing virus-infected cells, while CD4+ T cells are helpers to promote B cell/antibody responses or effector cells that migrate to sites of infection to provide host defense, including production of cytokines. Thus, inflammatory mechanisms result from both innate and adaptive immune responses.

## **Dysregulation of Inflammatory or Immune Responses**

### **Hyperinflammation**

Since levels of certain proinflammatory cytokines correlate with markers of cardiac injury, adverse cardiovascular events and mortality, proinflammatory cytokines may mediate local and systemic pathology in COVID-19. Seen in chimeric antigen receptor T cell therapy, cytokine

release syndrome can cause cardiopulmonary collapse, including vasoplegic shock, transient LV dysfunction, and fulminant lymphocytic myocarditis. Cytokine release syndrome in this setting is often responsive to IL-6 interruption.<sup>32</sup> Inflammasome activation may also contribute to a hyperinflammatory milieu, and the attendant lytic cell death may contribute to tissue injury and thrombosis. Although the early observational and anecdotal experience with anticytokine therapy was promising,<sup>119</sup> randomized trials suggest the benefit of tocilizumab for the prevention of mechanical ventilation or death may be modest.<sup>120–122</sup>

### **Failure of Adequate Type I Interferon Responses**

Patients with severe COVID had lower type I IFN levels despite higher viral loads<sup>123</sup> and were also more likely to have a hyperinflammatory profile characterized by higher IL-6 and TNF plasma levels and elevated expression of NF- $\kappa$ B-related genes.<sup>123</sup> Consistent with this, plasmacytoid dendritic cells are reduced during acute illness (compared with convalescence and/or controls), especially in those with a severe course.<sup>124</sup> Impaired dendritic cell function also corresponds with delayed T cell responses in SARS-CoV-2.<sup>124</sup> Deficient-type I IFN activation may also be due to autoantibodies that neutralize the ligand and/or receptor,<sup>125</sup> but the mechanisms accounting for a potential failure of type I IFN production are unknown.<sup>126</sup> The extent to which the activity of specific cytokines (eg, IL-6, IL-1 $\beta$ , TNF) is a marker or mediator of disease severity and whether these pathways account for cardiovascular impairment is a crucial knowledge gap and may have relevance to cardiovascular resilience in the face of non-COVID critical illnesses.

### **Adaptive Immune Dysfunction and Myocarditis**

Lymphopenia at presentation is strongly prognostic of mortality in COVID-19<sup>6</sup>; patients with a fatal course had protracted lymphopenia and tended to have steep increases in D-dimer and cTn levels in association with elevated inflammatory cytokines. Lymphopenia in COVID-19 predominantly reflects T cell depletion, whereas B cell numbers are preserved.<sup>127</sup> Naive T cells tend to be diminished in the elderly, a finding associated with a poor prognosis.<sup>128</sup> Greater disease severity is also associated with lower levels of circulating spike-specific Tfh cells and lower numbers of CD8+ IFN $\gamma$ + T cells, whereas T17 cells were not generally detected. More recent reports suggest T cell function may indeed be critical to host protection and long term immunity. In those recovered from COVID-19, T cell responses appear to correlate with neutralizing antibody titers, suggesting that productive adaptive responses to SARS-CoV-2 leading to both T and B cell immunity are possible.<sup>129</sup> T cells responsive to SARS-CoV-2 have been identified from peripheral blood and persist at least 6 months after infection.<sup>130</sup> SARS-CoV also induced durable T cell memory, identifiable in many at least 4 years after infection.<sup>131</sup> Interestingly,

recent studies suggest that preexisting protection against SARS-CoV-2 may be derived from memory T cell immunity to common coronaviruses.<sup>128,132</sup>

Whether aberrant T cell responses contribute to cardiovascular manifestations has not been determined. Although effector/memory T cell activation can induce prothrombotic activation of monocytes and contribute to thrombotic risk,<sup>133</sup> whether T cells are direct contributors to other manifestations of cardiovascular injury in the setting of COVID-19 is less clear. For example, COVID-19 autopsy series have only rarely identified lymphocytic myocarditis. T regulatory cells have been implicated in myocardial fibrosis, but whether expansion of these cells during or after infection contribute to myocardial to myocardial fibrosis has not been established.

### **Potential Role of Antibody-Dependent Enhancement/Injury**

In addition to protective neutralizing activity, antibodies can recruit complement, provide an ACE2-independent route to infection via Fc receptor interactions,<sup>134</sup> or have cross-specificity to self antigens, functions with pathological consequences (ie, antibody-dependent enhancement). These humoral effects have been previously shown to play a role in autoimmune cardiomyopathies or non-COVID viral myocarditis.<sup>135,136</sup> To date, there has not been convincing evidence of antibody-dependent enhancement in SARS-CoV-2 infection or therapies. Instead, concern derives from observations that severe COVID-19 is paradoxically associated with higher SARS-CoV-2-specific antibody titers compared with those with minimal symptoms or an asymptomatic course, although differences in viral load may be an alternate explanation.<sup>137,138</sup> Nevertheless, continued vigilance and scrutiny of clinical datasets is warranted, especially as we enter into the vaccination era of COVID-19.<sup>139</sup>

Systemic immune dysfunction causes widespread endothelial injury even in tissues that are not directly infected by the virus. The presence of a systemic abnormality in immune responses in patients with COVID-19 is well documented, but these immune-related insults may not depend on local viral infection. Strikingly, Lee et al showed significant microvascular injury and fibrinogen leakage in the brain tissue of patients with COVID-19, but no evidence of virus was detected in the tissues.<sup>71</sup> It is likely that a humoral factor may be eliciting vessel damage, and further studies will be needed to uncover the mechanisms of this phenomenon.

### **Vascular Thrombosis and Platelet Activation**

Thrombosis in venous and arterial circulatory beds has been a prominent feature of SARS-CoV-2 infection.<sup>140,141</sup> Viral inflammation and degranulation of endothelial cells was demonstrated by scanning electron microscopy. Inflammation of vascular endothelial cells (endotheliitis)

leads to degranulation and exocytosis of Weibel Palade Bodies (WPBs) containing von Willebrand Factor, which promotes recruitment of platelets, as well as platelet-to-platelet aggregates through the glycoprotein 1b receptor.<sup>142</sup> Platelet activation remains a common final step in thrombus formation.

### **Indirect Activation of Platelets and Interaction With the Innate Immune System**

Disruption of the subendothelial barrier promotes tissue factor release, activating the extrinsic coagulation cascade, culminating in prothrombin to thrombin conversion. Thrombin stimulates 2 G-protein-coupled receptors on the surface of the human platelet belonging to the proteinase-activated receptor (PAR) pathway, PAR1 and PAR4.<sup>143</sup> Platelet PAR1 and PAR4 activation leads to exocytosis and secretion of alpha granules, dense granules, and WPBs.<sup>144</sup> Platelet serotonin release likely impacts the endothelium, causing endothelium- and nonendothelium-dependent changes in vascular tone and inflammation. The cause of thrombus formation in COVID-19 likely involves coordinated activation between several pathways of thrombosis and the innate immune system (thrombo-inflammation or immunothrombosis). Early in the pandemic, leukocyte count was the only reported independent predictor of thrombosis.<sup>141</sup> Both platelet and endothelial cells activate and recruit circulating leukocytes. Several investigators have now demonstrated in patients with COVID-19 that activated neutrophils release de-condensed chromatin into the extracellular milieu in a mesh-like, prothrombotic network, called neutrophil extracellular traps.<sup>145-147</sup>

### **Direct Platelet Reprogramming**

The platelet phenotype is hyper-reactive in patients with COVID-19, at least in part from a divergent circulating platelet phenotype.<sup>148-150</sup> A coordinated SARS-CoV-2 receptor access module through surface ACE2 and TMPRSS2 is clear in multiple cells, and demonstrated in platelets from patients with COVID-19 through immunologic techniques permitting direct and indirect visualization.<sup>150-152</sup> Interestingly, one group did not convincingly demonstrate ACE2 protein on the surface of platelets following a leukocyte CD45-depletion step.<sup>148</sup> CD45 is present on the surface of platelets in health and disease<sup>153</sup> and so could have diminished the signal required to detect ACE2. Other SARS-CoV-2 receptors have been demonstrated in various cells, including the HDL (high-density lipoprotein) scavenger receptor BI and CD147. These receptors were previously reported to be expressed on the surface of platelets, with a post-receptor signal transduction pathway that increases platelet reactivity and promotes thrombosis.<sup>154-158</sup> Importantly, in situ end organ thrombosis, especially in the lung and heart, is a signature of SARS-CoV-2, and the oxygen-reduced microvasculature is a region where platelets are especially reactive.<sup>159,160</sup>

### Autoimmune Phenomena and Adaptive Immune Dysfunction in COVID-19-Associated Vascular Thrombosis

Immunologic dysfunction is an important contributor to the vascular complications that arise in patients with COVID-19, and thrombotic arterial and venous occlusions are a major cause of end-organ damage.<sup>161</sup> Abnormal coagulation characteristics associate with severity of COVID-19 disease<sup>162,170</sup> and high-plasma D-dimer concentration is a risk factor for death.<sup>163,164</sup> Implicating adaptive immune system dysfunction, antiplatelet, antiphospholipid, and antiendothelial cell autoantibodies have been demonstrated in patients with SARS-CoV-2.<sup>165–168</sup> Prothrombotic autoantibodies targeting phospholipids and phospholipid binding proteins (aPL antibodies) were found in 52% of 172 hospitalized patients with COVID-19<sup>169</sup> and included anticardiolipin IgG, IgM, and IgA; anti-B2 glycoprotein I IgG, IgM, and IgA; and antiphosphatidylserine/prothrombin (aPS/PT) IgG and IgM. Antiphospholipid antibodies also activated neutrophils and initiated neutrophil extracellular trap extrusion, consistent with the proposed immunothrombosis mechanism in COVID-19.<sup>170</sup> Early case reports indicated thrombocytopenia in some patients with COVID-19 may be caused by easier haptenization of platelet antigens, including the cytokine CLCL4, also known as PF4 (platelet factor 4), as evidenced by circulating anti-PF4 antibodies<sup>165,171</sup> and earlier observations in which light transmission aggregometry functional assays with the additional of heparin to donor platelets mixed with serum from patients with COVID-19 promoted platelet activations.<sup>165</sup> These observations present a true treatment dilemma, given the propensity of patients with COVID-19 to form thrombi, and the need for anticoagulation. Curiously, several patients with COVID-19, meeting clinical diagnostic criteria for heparin-induced thrombocytopenia, subsequently tested positive for anti-PF4 antibodies, but in heparin-induced platelet aggregation assays were negative using the heparin-induced thrombocytopenia confirmatory test platelet serotonin release assay.<sup>172,173</sup> Most recently, it was revealed that circulating blood IgG in patients with COVID-19 promotes a procoagulant phenotype and thrombocytopenia through platelet apoptosis by stimulating platelet Fc gamma receptor IIA.<sup>174</sup> Overall, a pattern of autoantibody production in COVID-19 that simultaneously activates neutrophils and promotes thrombosis seems clear and may account for the morbidity and mortality benefit shown in the CoDEX and Recovery trials, respectively, when patients were randomized to immune suppression with dexamethasone.<sup>175,176</sup>

### Renin-Angiotensin System Dysfunction

ACE2 has a robust physiological role in regulating angiotensin II, bradykinin activity, and protection against pulmonary capillary leakage and heart failure. However,

ACE2 is critical for SARS-CoV-2 cell entry, and since treatment with ACEI/ARBs might increase ACE2 expression, early concerns centered on whether these medications may increase the risk of SARS-CoV-2 infection. However, large observational studies reported that these medications, when prescribed chronically for CVD antecedent to COVID-19 testing, are not associated with greater infectivity.<sup>177</sup> Some reports suggest abrogation of the RAS may be associated with protection from severe COVID-19.<sup>178</sup> ACEI/ARB-associated protection against COVID-19 is plausible since SARS-CoV-2 engagement and internalization via ACE2 may cause ACE2 shedding or depletion<sup>179</sup> that may promote unregulated angiotensin II and/or bradykinin activity. Indeed, ANG II levels appear to be elevated in patients with severe COVID-19,<sup>180,181</sup> and infusion of human recombinant soluble ACE2 may be sufficient to suppress these levels which was attended by marked reductions in inflammatory indices.<sup>182</sup> Thus, ACE2 is a critical gateway for SARS-COV2 binding and entry, but its functional disruption may lead to further disruption of cardiopulmonary homeostasis during COVID-19.

The impact of SARS-CoV-2 infection on myocardial *ACE2* expression is unclear. Lung expression of *ACE2* is reduced in a murine system with SARS-Co-V spike protein administration,<sup>183</sup> either because of cell internalization of spike-ACE2 and/or membrane shedding after cleavage by proteases. *ACE2* downregulation was associated with an increase in lung tissue ANG II levels.<sup>183</sup> Thus, in COVID-19, ANG II levels may also be increased,<sup>181</sup> due to ACE2 downregulation and reduced enzyme activity. Theoretically, these predicted increases in ANG II levels could be countered by delivering maximal doses of ACE inhibitors and AT<sub>1</sub> receptor blockers. However, in the absence of supporting evidence, such an approach is unwarranted and needs to be studied. Myocardial *ACE2* expression during and after SARS-CoV-2 infection is currently under investigation.

### TRANSLATING TO THERAPIES FOR SARS-COV-2

Based on growing knowledge of the life cycle of SARS-CoV-2 and its interactions with host cells, it is useful to define preventive or therapeutic strategies on the basis of the time sequence of pathogenic events: prevention upon exposure, inhibition of viral proliferation, and attenuation of exuberant host inflammatory response. Within the latter 2 categories are included 3 specific subclasses of therapies: biologics, new small-molecule therapeutics, and repurposed or repositioned approved therapeutics. Last, organ (system)-specific therapies should also be considered for individuals with complicated infections that lead to specific pathologies, such as a prothrombotic state, stroke syndromes, or acute kidney injury, the

treatment of which at the current time is not specific for SARS-CoV-2.

## Preventive Therapies

Vaccination is the cornerstone of prevention against SARS-CoV-2 infection. The World Health Organization reported that as of September, 2020, there were 36 vaccine candidates in clinical trials and 146 other candidates currently in preclinical evaluation.<sup>184</sup> By any measure, the rapidity of vaccine candidate development and initial clinical trial implementation is an amazing accomplishment, given that this virus has only been recognized for  $\approx 1$  year. The vaccines currently in clinical trials comprise 5 different subclasses: inactivated virion-based vaccines, RNA vaccines, DNA vaccines, nonreplicating and replicating viral vector-based vaccines, and recombinant protein subunit-based vaccines. Among these candidates, almost all require a second dose of vaccine after the initial dose, usually at 2, 3, or 4 weeks, for optimal protection.

The benefits and risks of these different types of vaccine have been extensively reviewed<sup>184</sup> and will only be briefly summarized here. Inactivated and live attenuated vaccines have been the mainstays of vaccinology since its inception. While these can be readily produced and stably express antigenic epitopes that are in the appropriate conformation, the expression of a highly antigenic but pathogenically less important antigen may skew the immune response. In addition, these vaccines are more difficult to produce owing to the requirement for Biosafety Level 3 facilities. Both DNA and (m)RNA vaccines, by contrast, can be rapidly produced, requiring only conventional nucleic acid synthesis, and their ability to generate specific viral proteins that can be processed by antigen-presenting cells (in the skin—dendritic cells) into a variety of potentially immunogenic conformations is a clear advantage over conventional vaccines. Chemical instability of mRNA is a limiting feature that requires special storage or modification with stabilizing vehicles in their preparation. Although mRNA vaccines are novel, recent phase 1 and 2/3 randomized trials have reported  $\approx 95\%$  efficacy with very low incidence of serious adverse events and efficacy demonstrated across race, ethnic, and age groups.<sup>185,186</sup> With limited experience thus far using these nucleic acid-based vaccines, however, their precise adverse event rates, vaccine efficacy among different populations with differing risks of infectious complications, long-term safety, and durability of protection remain to be determined. Viral vector-based vaccines involve the use of a carrier virus (adenovirus, poxvirus) engineered to carry an immunogenically relevant protein from the virus of interest. The advantage to these vaccines is that they can infect antigen-presenting cells directly, and they tend to be chemically and biologically stable. These vaccines can induce an anamnestic

response in an immune system previously exposed to these common carrier viruses. Last, vaccines that incorporate specific recombinant proteins of interest (such as the spike protein) can stimulate an effective immune response. As the protein is not exposed to the immune system in its complex biological context, however, its protection may be limited, or the immune response relatively unbalanced.

## Therapeutic Approaches Directed at ACE2 and Other Entry Proteins

An obvious therapeutic approach to treating any viral infection including SARS-CoV-2 is to prevent virus-host cell receptor binding.<sup>187</sup> This could be achieved by targeting the spike protein (as antibodies do in the course of the immune response) or by targeting of the ACE2 domains that interact with the spike protein. Use of the latter approach must avoid loss of ACE2 physiological function. Use of decoy soluble ACE2 receptors might be a way to circumvent this problem, and such an agent is currently in clinical trials.<sup>188</sup> Other approaches include administering an antibody that blocks RBD-ACE2 binding without affecting enzyme activity, or administering an ACE2 enzyme activity agonist, such as diminazene,<sup>189</sup> as blockade is attempted.

Inhibition of SARS-CoV-2 viral entry into host cells in the (upper) airway has been recently achieved using a lipid-conjugated peptide derived from the spike protein's C-terminal heptad repeat domain. In preclinical studies, this lipopeptide has been shown to inhibit cell-cell fusion mediated by the spike protein, block infection in cultured cells, and inhibit the spread of virus in human airway epithelial cells.<sup>190</sup> Similarly, other entry inhibitors have been identified from generic screens of approved drugs (*vide infra*), including clemastine, amiodarone, trimeprazine, busitinib, toremifene, flupenthixol, and azelastine, likely via histamine receptor antagonism.<sup>191</sup> Last, owing to the now established role of the integrin  $\alpha 5 \beta 1$  as a ligand for the spike protein and for ACE2, efforts to interrupt those interactions represent another strategy for inhibiting viral entry. To this end, the integrin-binding peptide, ATN-161, has recently been shown to inhibit these critical interactions in early infection.<sup>26</sup>

## Drug Development Strategies

In general, 2 broad strategies can identify potential pharmacotherapies for SARS-CoV-2: target-based drug development and unbiased drug screening. In the former, a detailed understanding of the SARS-CoV-2 life cycle and its molecular determinants is essential, with considerable knowledge gleaned from experiments and by analogy with previous SARS-CoV studies. Each segment of SARS-CoV-2 life cycle of SARS-CoV-2 is a potential therapeutic target: host cell attachment, membrane fusion, uncoating, RNA translation, replication, structural

protein assembly, and virion constitution, and exocytosis. Specific targets have been identified for some of these steps, including the following: viral entry—spike protein (neutralizing antibodies, SARS-CoV-2-HR2-derived decoy peptides, peptide fusion inhibitor EK1), ACE2 (human recombinant soluble ACE2—APN01), TMPRSS2 (camostat, mafamostat, bromohexine, rubitecan, loprozalam), CD147 (meplazumab, metuximab, metuzumab), integrin  $\alpha 5\beta 1$  (ATN-161), adaptor-related protein complex 2 or AAK1 (baricitinib), membrane lipids (umifenovir), and specific neutralizing antibodies; and viral replication—3CL protease (lopinavir/ritonavir, darunavir), and RNA-dependent RNA-polymerase (remdesivir, favipiravir, ribavirin).<sup>192</sup> Some are novel therapies specific to SARS-CoV-2 (eg, APN01) while others are repositioned drugs previously approved for other purposes (eg, ribavirin). Importantly, some treatments target viral proteins (eg, camostat) while others target host proteins (ATN-161). An additional therapeutic strategy has been to focus on the impaired innate immune response early in the infection or on the exuberant immune response observed in some patients later in the course of infection, leading to serious, systemic complications such as a prothrombotic state. Targets considered in these domains and potential treatments include: interferons  $\beta 1a$  and  $\beta 1b$ ; IL-6 receptor (tocilizumab, sarilumab, situximab); IL-1 receptor/IL-1 $\beta$  (anakinra, canakinumab); less specific immunosuppressive therapies (dexamethasone); and ongoing clinical trials of immunomodulatory antibodies.<sup>193</sup>

While this conventional strategy is a time-honored approach, it suffers from excessive reliance on a single target, and inadequate assessment of off-target effects. The most specific drugs are promiscuous, a fact that has been increasingly documented in comprehensive databases such as DrugBank. This promiscuity and the complex interactions among (protein) targets provide a rationale for a molecular network-based strategy for drug repurposing, including for SARS-CoV-2.

One approach for a repurposing strategy can begin with the comprehensive protein-protein interaction network. Previous work analyzed this interactome to assess where proteins that govern specific disease phenotypes are located and showed that these disease-specific proteins cluster in different subnetworks or modules throughout the interactome.<sup>194</sup> These disease modules can be used to guide drug repurposing by identifying the proximity of the target of a drug approved for a different disease to the disease module of interest. This approach has successfully identified repurposable drugs for coronary heart disease,<sup>195</sup> malignancies,<sup>196</sup> and cardiovascular calcification.<sup>197</sup>

In the case of SARS-CoV-2, the interactions of viral proteins with human host proteins add complexity within the interactome. To address this issue, Loscalzo and

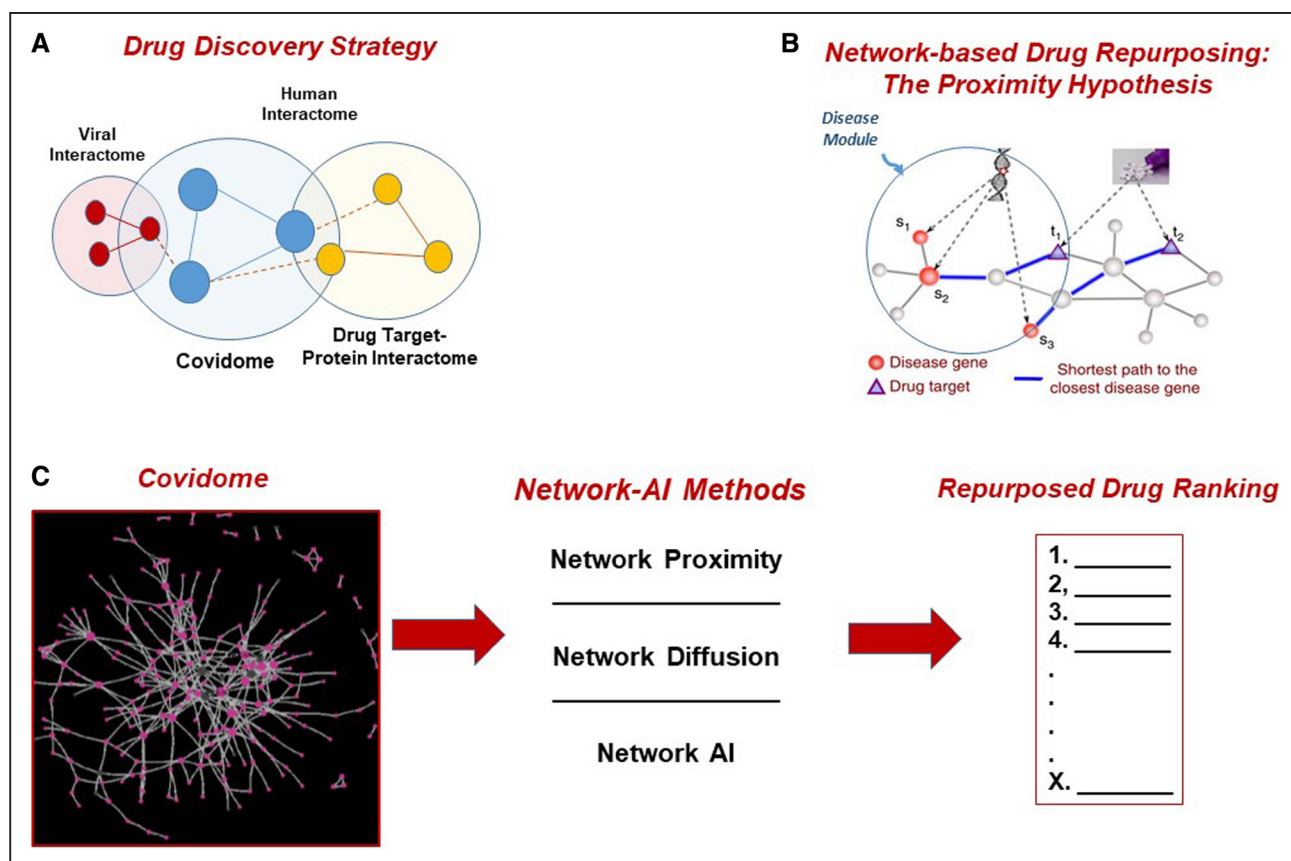
colleagues mapped the 332 human proteins that Gordon et al<sup>169</sup> showed bound to 26 (of 29) SARS-CoV-2 proteins to the protein interactome and demonstrated that they agglomerate as a cluster or disease module.<sup>198</sup> Based on the COVID-related proteins expressed in the lung (214 of 332, or 64%), 3 analytical strategies to identify potential drug targets for consideration were applied: a network proximity strategy, a network diffusion strategy, and an artificial intelligence-based neural network strategy, to rank-order a list of 6340 FDA-approved drugs. The 3 different methods provided complementary ranking information, leading to the development of a combined or aggregated ranking algorithm, which gave the best predictive accuracy. Curation of the top 10% of the rank list yielded 74 candidate drugs that were next tested in a high-throughput assay to identify those drugs that are viricidal with no or minimal (host cell) cytotoxicity where from the 74 screened compounds, 28% were shown to be effective.<sup>198</sup> Among the drugs identified were azelastine, folic acid, auranofin, fluvastatin, ivermectin, and aminolevulinic acid, to highlight but a few. This strategy is depicted in Figure 6. Other network medicine-based strategies for drug repurposing for SARS-CoV-2 that include tissue-specific transcriptomics have also been recently published, demonstrating a potential role for melatonin.<sup>199</sup>

The benefit of drug repurposing is that prior drug approval obviates, or at least limits, the need for preclinical animal studies. These compounds have already been used in humans, and their toxicity is well known. Presumably, they can be used directly and safely in patients with SARS-CoV-2 infection. This approach, however, presumes that there is no unique interaction between this novel infection and the repurposed drug that could lead to an unpredictable toxicity. For this reason, understanding the biology implicit in the network architecture of the COVID interactome can provide some guidance in considering potential toxicities that warrant study before human trials.

## TRANSLATING TO STRATEGIES FOR MANAGEMENT OF PATIENTS AFTER COVID-19 INFECTION

### Implications of CMR Findings After Recovery From COVID-19 Acute Infection

CMR findings have highlighted uncertainties in the evaluation and management of patients recovering from COVID-19. Despite normal ECG and echocardiographic findings, normal levels of myocardial biomarkers, and minimal or no symptoms, myocardial findings on CMR may still be present. Nevertheless, the clinical significance of CMR abnormalities, which in most of the reported studies were mild, remains unknown.



**Figure 6. Network-based drug repurposing paradigm for severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2).**

**A**, Network-based drug discovery strategy for SARS-CoV-2 in which the viral proteome is depicted overlapping with the human protein interactome, which, in turn, is shown overlapping with the drug-target protein interactome. **B**, Network-based proximity strategy for drug repurposing in which the proximity of a drug target for another disease to the disease module of interest serves as the basis for pursuing repositioning of that drug. **C**, The covidome, or the subnetwork or disease module in the protein interactome, is used as the basis for identifying drug targets for drug repurposing, which is evaluated through 3 complementary methods—network proximity, network diffusion, and AI prioritization, leading to a ranking algorithm that yields repurposable drugs for experimental analysis.

Moreover, the pathophysiologic mechanisms of late inflammatory changes and the long-term impact of myocarditis or myocardial fibrosis implicated by CMR on heart failure<sup>200,201</sup> or arrhythmic risk<sup>202</sup> remain uncertain. Longitudinal studies are needed to determine the natural history and clinical significance of the described CMR findings in patients with COVID-19-induced myocarditis, as LGE and myocardial edema can be dynamic.<sup>98</sup> In addition to including matched healthy controls in MRI studies, matched patients with recent non-COVID-19 viral infections would help elucidate the presence of potential differential prevalence of myocarditis related to viral etiology.

### Prolonged Exertional Intolerance and Dysautonomia

There is increasing evidence of long COVID-19 symptoms beyond the period of acute infection with prolonged exertional intolerance becoming a frequent finding in not only competitive athletes and active individuals, but many young and older survivors of COVID-19. Common

symptoms associated with myocarditis and post-COVID syndrome include chest pain, dyspnea, and palpitations. Besides concerns over CMR findings of cardiac injury, COVID-19-related small fiber neuropathy and dysautonomia are now being reported in individual cases.<sup>203–205</sup> COVID-19-related postural orthostatic tachycardia syndrome has also been identified. Relative cardiac deconditioning during a period of exercise and training restriction is a confounder when trying to delineate the cause for exertional intolerance.

### Evaluation Post-COVID-19

For investigation of COVID-19-mediated cardiac involvement or dysautonomia in patients with post-acute-COVID-19 symptoms or for cardiac risk assessment for return to exercise or sports participation, an algorithm and suggested evaluation for acute and chronic assessment of cardiac involvement are presented in Online Table II. There remains uncertainty as to the yield of noninvasive testing. Serum biomarkers suggestive of myocardial damage are typically elevated



in acute myocarditis, although 3 studies reported normal troponin levels post-COVID-19, despite abnormal CMRs.<sup>91,92,206</sup> Noninvasive testing, including the ECG and echocardiogram, may provide additional signs suggestive of COVID-19-mediated cardiac involvement. However, a post-COVID-19 CMR study of collegiate athletes noted no definitive ECG or echocardiographic abnormalities.<sup>92</sup> Nevertheless, a recent expert consensus statement on screening for potential cardiac involvement in competitive athletes recovering from COVID-19 recommends a targeted approach based on the presence and nature of symptoms with a combination of ECG, biomarkers, and echocardiography for athletes with prolonged or more than mild symptoms.<sup>207</sup> A similar targeted approach could be considered for nonathletes with such symptoms, as well.

### Implications on Return to Exercise or Sports Participation After COVID-19 Infection

The potential for heightened risk of sudden cardiac death in post-COVID myocardial fibrosis or inflammation is of concern for athletes or active individuals returning to exercise. The wide range of LGE prevalence post-COVID-19 has produced controversy over routine versus targeted use of CMR. Risk stratification with noninvasive biomarkers, ECG, or echocardiography may be insensitive for detection of CMR abnormalities. Conversely, ECG changes considered abnormal in nonathletes may represent normal variants in athletes.<sup>208</sup>

According to the American College of Cardiology Sports and Exercise Cardiology Section, athletes who have recovered from COVID-19 may return to sports participation based on biomarker and noninvasive cardiac imaging, including an ECG and echocardiogram.<sup>209</sup> Athletes are advised to restrict exercise for 10 to 14 days with gradual escalation in exercise intensity. Cardiovascular risk assessment is recommended for mild symptoms lasting longer than 10 days; for moderate or severe symptoms, including hospitalization, advanced cardiac testing is dependent upon symptoms and abnormal findings in baseline testing.<sup>210</sup> Patients with COVID-19 myocarditis are advised to follow published return-to-play guidelines for competitive athletes with myocarditis.<sup>211</sup> Whether these recommendations will be adequate remains to be determined.

### Uncertainty of Long-Term Consequences, Gaps, and Future Needs

The potential for long-term evolution into chronic myocardial disease/cardiomyopathy and other cardiovascular complications, including heart failure, chronic sinus tachycardia, autonomic dysfunction, and arrhythmias, awaits further definition and may have significant implications. Additionally, studies are needed to

determine if therapeutic interventions to mitigate the inflammatory response can also limit the extent of intermediate to long-term myocardial injury related to COVID-19. Evaluation of postacute COVID-19 syndrome (long-COVID-19) and recommendations for long-term surveillance, monitoring, and return to exercise or sports participation remain areas in need of further study.

## CONCLUDING REMARKS

The COVID-19 pandemic has produced devastating effects worldwide with loss of health, life, and livelihoods, particularly in people of color, the underserved, the vulnerable elderly, and those with prior cardiovascular disease. Further understanding of the basic viral-host interactions mediating the varied responses to infection are yet needed to improve prevention and treatment strategies, including those for the long-term cardiovascular effects of the infection. Preventive vaccines offer hope that the pandemic may wane over the next year, and their rapid, successful development within less than a year coupled with the increasing identification of effective treatments are a testament to the massive commitment and tireless efforts of the scientific community, front-line caregivers, and health care leadership. Lessons learned in our response to COVID-19 will hopefully prepare us for future pandemics.

## ARTICLE INFORMATION

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J. Loscalzo is scientific co-founder of Scipher Medicine, Inc, a company that focuses on utilizing network medicine approaches to understanding disease mechanisms and identifying therapeutic targets. The other authors report no conflicts.

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