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EDITORIAL COMMENT

COVID-19 and the Heart

ACE2 Level and the Company it Keeps Hold the Key*

Han-Bin Lin, PhD,[a,b] Peter P. Liu, MD[a,b]

Since the first report of COVID-19 from Wuhan, China, the pandemic's infectivity and diverse outcomes have stunned the world. A consistent feature of COVID-19 is its predilection of inflicting adverse outcomes in patients with cardiovascular disease or risk factors for cardiovascular disease (1). Although many factors contribute to this association, a major mechanistic underpinning is the fact that the novel SARS-Cov-2 virus uses the transmembrane enzyme angiotensin-converting enzyme 2 (ACE2) as its key internalizing receptor (2).

ACE2 has a key salutary function in the renin-angiotensin system (RAS) by converting the pro-inflammatory vasoconstrictive octapeptide angiotensin II (1–8), to anti-inflammatory vasodilatory angiotensin (1–7). This has raised several key questions about COVID-19, along with controversy. First, does the increase ACE2 expression in cardiovascular diseases contribute to the worse outcomes of COVID-19 in cardiovascular patients? Second, is the ACE2 expression affected by cardiovascular medications? (There is a raging controversy that involves the use of RAS inhibitors.) Third, does the SARS-CoV-2 virus preferentially target the myocytes in the heart if they express the ACE2 receptor?

The study by Bristow et al. (3) in this issue of *JACC: Basic to Translational Science* helps to shed light on some of these questions. This carefully performed study in a small cohort of patients with nonischemic dilated cardiomyopathy (DCM) helps to answer the question of ACE2 expression in disease and treatment, as well as which of the co-receptors for COVID-19 infection is present with ACE2 in the heart.

Bristow et al. (3) studied 46 patients with DCM with serial septal myocardial biopsies subjected to extensive gene expression analysis after treatment with β-blockade. During follow-up, 30 of the 46 patients were classified as responders to β-blockade, with ejection fraction improving ≥8% at 12 months. The remainder were classified as nonresponders. The analysis showed that ACE2 gene expression was up-regulated by an average of 1.97-fold in all of the patients with DCM compared with normal control subjects. Surprisingly, the patients who responded to β-blockade experienced reverse left ventricular (LV) remodeling, and the initial elevated ACE2 expression actually returned to normal at 12-month follow-up (**Figure 1**). This was not observed with RAS inhibitor usage and/or dosage.

From an infection permissiveness point of view, the 5 proteases that function as the SARS-CoV-2 co-receptor (including TMPRSS2, which allows membrane fusion with the virus to facilitate transfer of the viral RNA to the host) were all present but showed no change with DCM. However, recently, integrins were found to be an alternate co-receptor that binds the Arginylglycylaspartic acid (RGD) domain on the exposed loops of the viral capsid protein (4). Interestingly, integrin ITGA5 was tracked in line with ACE2 and was up-regulated with DCM and down-regulated with β-blockade particularly in those who had reverse

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From the [a]University of Ottawa Heart Institute, Ottawa, Ontario, Canada; and the [b]Departments of Medicine and Cellular and Molecular Medicine, University of Ottawa, Ontario, Canada. This work was supported through research grants from the Canadian Institutes for Health Research, Genome Canada, and the Heart and Stroke Foundation of Canada. Both authors have reported that they have no relationships relevant to the contents of this paper to disclose.

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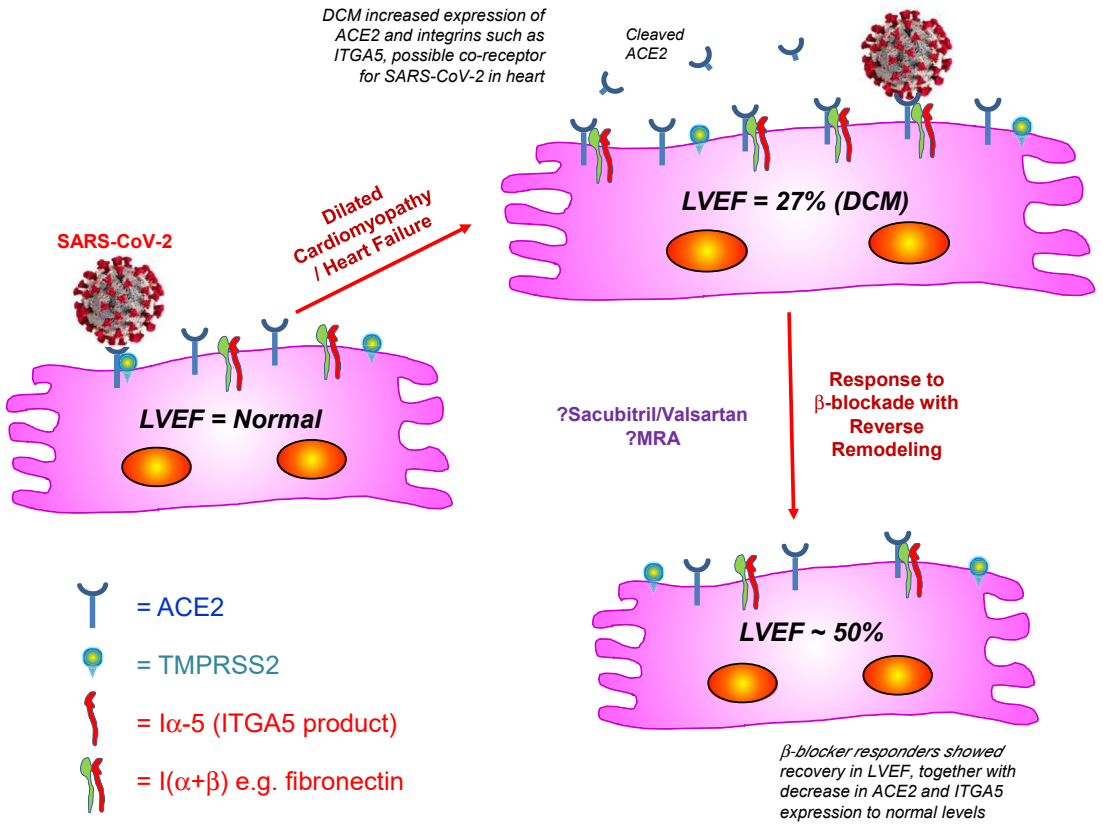
FIGURE 1 Dynamic Regulation of ACE2 and Co-Receptors for SAR-CoV-2 in Patients With DCM Treated With β-Blockade



In normal hearts, there is baseline angiotensin-converting enzyme 2 (ACE2) expression, which functions as both an enzyme in the renin-angiotensin system and as the internalizing receptor for SARS-CoV-2 virus. Proteases such as TMPRSS2 typically act as the co-receptor. However, in dilated cardiomyopathy (DCM), there is 2-fold up-regulation of ACE2 and potential co-receptor integrin α-5 (Iα-5, which is a product of the ITGA5 gene). In patients who respond to β-blockade, there is a reduction of ACE2 gene expression to normal levels, together with ITGA5 and integrin expression and reverse remodeling. LVEF = left ventricular ejection fraction; MRA = mineralocorticoid receptor antagonist.

remodeling. Thus, integrins like ITA5 may function as an alternative co-receptor to ACE2 in facilitating SARS-CoV-2 infection intracellularly (**Figure 1**). The study by Bristow et al. (3) thus helps to illuminate the following questions.

IS ACE2 EXPRESSION ELEVATED IN THE HEARTS OF PATIENTS WITH CARDIOVASCULAR DISEASE?

Although it is known that RAS is highly activated in patients with cardiovascular diseases, the direct demonstration of increased tissue ACE2 expression in the diseased human heart is actually rare and often sampled at end stage. There have been reports from Chinese and German investigators, indicating that ACE2 expression is particularly up-regulated in the pericytes of the heart in contrast to low levels of expression in myocytes (5). Interestingly, ACE2 can be cleaved by the disintegrin ADAM17, shed into the circulation, and function as a biomarker. Circulating ACE2 levels have been found to be increased in patients with heart failure, with a suggestion that this is accompanied by reduced myocardial expression.

The Bristow et al. study (3) challenged the latter scenario and clearly demonstrated there was a significant up-regulation of ACE2 in the myocardium in patients with DCM. Using the cohort in the Bristow et al. study was particularly advantageous because these patients were relatively young and were at earlier stages of their disease. This avoided the typical confounding effects of taking tissue samples

from patients with end-stage heart failure with multiple comorbidities and complex drug regimens. The increased ACE2 expression most likely represents a compensatory mechanism to counterbalance the increase in RAS activity in progressive heart failure. This would also support, although not demonstrate directly, that the shedding of ACE2 in the circulation is a consequence of increased receptor turnover and not the exhaustion of ACE2 in the myocardium. Thus, increased circulating ACE2 levels likely reflect increased tissue ACE2 levels.

IS THE UP-REGULATED ACE2 IN DCM MODIFIABLE BY THERAPEUTIC TREATMENT?

Major controversy has arisen regarding ACE2 regulation by drug treatment because of pre-clinical studies that have suggested exposure to ACE inhibitors may up-regulate the expression of ACE2. This has resulted in confusion with respect to RAS inhibitor continuation or termination in COVID-19. The Bristow et al. (3) study did not find any changes in ACE2 gene expression levels with RAS inhibitor exposure or dosages because of a relatively small sample size. This question ultimately can only be addressed by large global prospective randomized trials. We are coordinating one—COVID-RASi (Coronavirus Disease-Renin-Angiotensin System Inhibitors) trial. Other investigator-initiated trials are ongoing around the world.

However, Bristow et al. (3) found an intriguing dynamic pattern of ACE expression with β-blockade. Of the 30 responders, there was actual normalization of ACE2 expression compared with baseline, in contrast to the lack of significant change in non-responders, This underscored 2 important concepts. The first is that ACE2 expression levels, even in established conditions such as DCM, are dynamic and can be potentially modified. Second, therapeutic agents that can modify the natural history of the underlying disease and reverse remodel the cardiomyopathy are also most effective in normalizing ACE2 expression. This also indicated that ACE2 up-regulation is not inherent to DCM, but is a response to its secondary RAS activation.

BECAUSE ACE2 EXISTS IN THE MYOCARDIUM, WHERE IS THE SARS-CoV-2 VIRUS IN THE HEART?

Patients with severe COVID-19 infection often also experience release of cardiac enzymes (e.g., troponins), which suggests ongoing cardiac injury. This injury signal is also highly prognostic. The question remains whether SARS-CoV-2 can directly infect the myocardium and cause myocyte damage. To date, there have been only a few case reports of cardiac pathology in COVID-19, and most have not shown evidence of viral genetic material in the myocyte. This is in contrast to evidence of virus in the lungs, in the immune cell (e.g., macrophages), or in the vascular system. The question is how readily SARS-CoV-2 virus can infect myocytes or could the myocardial injury be indirect through perturbations in the immune or vascular system? Although the Bristow et al. study (3) does not directly address this question, it does provide insight into the permissiveness of viral entry. The focus is particularly on the dynamics of potential co-receptors in the heart, such as the membrane-associated proteases, including TMPRSS2. The presence of membrane proteases such as TMPRSS2 help to induce membrane fusion following virus–ACE2 engagement, and significantly increases the efficiency of infection.

According to the Human Tissue Atlas Database, proteases such as TMPRSS2 are highly expressed in the lung, gastrointestinal, and kidney tissues. The levels in the myocardium are relatively low, and according to the data in the Bristow et al. study (3), there is no dynamic up-regulation of the proteases either with DCM or down-regulation with β-blockade, despite significant changes with ACE2. This might suggest that although ACE2 is expressed in significant quantities in the myocardium, but without the significant dynamic presence of the proteases as co-receptors, such as TMPRSS2, in vivo access of the SARS-CoV-2 virus to the myocardium may be limited.

ARE THERE ALTERNATE CO-RECEPTORS FOR SARS-CoV-2 VIRUS FOR THE HEART?

Interesting gene expression association studies done in this paper showed that alternate co-receptors for SARS-CoV-2, such as integrins binding to the RGD motif in the spike protein, may be particularly relevant for the cardiovascular system (4). Integrin α-5, which was encoded by the gene *ITGA5*, was increased in the heart of patients with DCM. *ITGA5* also showed co-regulation with β-blockade. Integrin α-5 is an important component of fibronectin, which is an essential matrix protein, especially in the vasculature, and the maturation of adipocytes. This may in part contribute to the propensity for COVID-19 to be present in those with hypertension and obesity and its involvement of the vasculature. However, these are hypotheses generated by the intriguing new data, which will require validation with properly designed experiments.

Therefore, the overall implication of the study by Bristow et al. (3) is important. The first is that ACE2 expression levels are increased in the heart early in conditions such as DCM, leading to heart failure. Furthermore, the increased ACE2 levels are not coupled to the underlying condition, but appear to be dynamic, and can be normalized by effective treatment to reverse the disease process. This implies that in the COVID-19 era, more aggressive treatment for patients with cardiovascular disease or risk factors is even more important, to mitigate their COVID-19 risk and to decrease activation of the RAS system. Finally, the elucidation of potential additional SARS-CoV-2 co-receptors may help to identify additional tools for the prevention or treatment of COVID-19.

COVID-19 has exposed many of the weaknesses of our cardiovascular patients and also issues within our health system. Careful rethinking of our strategies of prevention and mitigation of these cardiovascular conditions will not only help our patients but our health system and society at large.

ADDRESS FOR CORRESPONDENCE: Dr. Peter P. Liu, University of Ottawa Heart Institute, 40 Ruskin Street, Room H2238, Ottawa, Ontario K1Y 4W7, Canada. E-mail: peter.liu@utoronto.ca OR pliu@ottawaheart.ca.

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