COVID-19: Risk Groups, Mechanistic Insights, and Challenges

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Abstract

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As Dr. Thomson eloquently notes in his valuable letter [1], underlying respiratory diseases appear to be less of a risk factor for poor outcome in COVID-19 patients than either underlying cardiovascular disease or diabetes. This intriguing finding emerged from several studies that examined underlying medical conditions in COVID-19 patients.

In a single-center retrospective analysis of critically ill adults admitted to the intensive care unit of a hospital from China between late December 2019 and January 26, 2020, 22% of the non-survivors had cerebrovascular disease, 22% had diabetes, and 6% had chronic respiratory disease [2]. The analysis of data from patients with laboratory-confirmed COVID-19 from hospitals in China through January 29, 2020 found that 16.2% of those with serious disease had diabetes, 23.7% had hypertension, and 3.5% had chronic obstructive pulmonary disease [3]. A study of electronical medical records of COVID-19 patients admitted between January 16 and February 3, 2020 to a hospital from Wuhan found that hypertension and diabetes mellitus, the most common comorbidities, were present in 37.9%, 13.8%, of the patients with severe disease, respectively, but only in 3.4% of the patients with chronic obstructive pulmonary disease [4]. Finally, an analysis of all COVID-19 cases reported through February 11, 2020, extracted from the Infectious Disease Information System in China, found that case fatality rates in individuals with cardiovascular disease, chronic respiratory disease, and diabetes were 10.5%, 6.3%, and 7.3% respectively, as compared to 0.9% among patients with no comorbidities [5]. In a case series of COVID-19 patients hospitalized in Wuhan, China, ICU patients were more likely to have underlying diabetes than patients that did not receive ICU care (22.2% vs 5.9%) [6].

The studies mentioned above did not stratify patients by therapies they were receiving. However, one commonality between cardiovascular disease and diabetes is that they are often treated with angiotensin-converting enzyme (ACE) inhibitors and angiotensin II type-I receptor blockers (ARBs), widely used to inhibit the formation and action of angiotensin II.

ACE shares 42% amino acid identity with ACE2 [7], a membrane-bound aminopeptidase [8] extensively expressed on type II human alveolar cells [9]. The genes encoding these two proteins are thought to have emerged by duplication [10]. ACE2 is distributed on many tissues and shows highest expression levels in the heart, kidney, lung, small intestine, and testis [11]. On the apical surface of polarized respiratory epithelial cells, ACE2 is a crucial and primary receptor for the cellular entry of SARS-CoV, the virus that caused the 2002-2003 SARS outbreak [12-16]. SARS-CoV binding to ACE2 mediates entry into human or animal cells [17]. ACE2 is also the receptor for SARS-CoV-2, the etiologic agent of COVID-19 [18]. Structural analyses indicate that SARS-CoV-2 binds the ACE2 receptor with a 10-20-fold higher affinity than SARS-CoV [19, 20].

The entry of SARS-CoV and SARS-CoV-2 into their target cells is mediated by the viral spike (S) glycoprotein, which is located on the outer envelope of the virion [21]. The S glycoprotein has two functional subunits,

S1, which binds the cellular receptor, and S2, which contains domains required for the fusion between viral and cellular membranes [22, 23]. Viral binding and membrane fusion represent the initial and critical steps during the infection cycle of the coronavirus [24] and the first step in establishing the infection [25, 26]. Binding is followed by internalization of ACE2 and down-regulation of its activity on the cell surface [27-29].

SARS-CoV binds ACE2 through a region of the viral S1 subunit called the minimal receptor-binding domain (RBD) [17]. RBD is the most important determinant of the SARS-CoV host range, and studies about the "species jump" during the 2002-2003 SARS outbreak revealed that changes of only one or two amino acids in this region were sufficient to make the virus "jump" to a new host [26, 30, 31].

ACE and ACE2 are two members of the renin angiotensin system that negatively regulate each other [32, 33] and are distinct in their substrate specificity and function [34]. ACE converts angiotensin I to angiotensin II and mediates aldosterone release, vasoconstriction, sodium retention, cell proliferation, and organ hypertrophy [35]. ACE2 cleaves a single residue from angiotensin I to form angiotensin-(1-9), and a single residue from angiotensin II to form angiotensin-(1-7). In humans, ACE2 has a 400-fold higher catalytic efficiency when it uses angiotensin II as a substrate as compared to when it uses angiotensin I [36]. ACE2 and angiotensin-(1-7), through the Mas receptors, oppose ACE and mediate vasodilation and anti-proliferative, anti-hypertrophic, cardioprotective, and reno-protective effects [8, 35, 37]. ACE2 has physiological and pathological importance [25] and its dysregulation was implicated in heart disease, hypertension, and diabetes [36, 38-40]. ACE2 is not inhibited by ACE inhibitors [32] and several studies indicate that the ACE2/Angiotensin-(1-7)/Mas axis has anti-inflammatory effects [41, 42].

It was recently hypothesized that treatment with ACE inhibitors and/or ARBs may lead to ACE2 overexpression and this could increase the risk of severe COVID-19 [43], possibly by increasing the internalization of SARS-CoV-2. Several lines of evidence indicate that pharmacological manipulation of the renin-angiotensin-aldosterone pathway could affect ACE2 receptor levels. In animal studies, the selective blockade of angiotensin II synthesis or activity increased cardiac *Ace2* gene expression and activity [44, 45], and treatment with ARBs increased the levels of cardiovascular ACE2 receptors [46-49]. While this link is thought-provoking as a possibility, there isn't currently sufficient evidence to contemplate changing patients' existing therapeutic regimens in order to minimize their risk of COVID-19 complications. The first clinical evidence exploring this link indicated that the use of ACEI and ARBs appear to improve the clinical outcome of COVID-19 patients with hypertension [50]. We will only learn about any possible associations, along with their magnitude and direction, from carefully conducted and adequately powered clinical trials.

It is also important to consider that an increase in ACE2 levels does not necessarily entail a negative impact for the course of COVID-19. ACE2, by forming angiotensin-(1-7) from angiotensin II, could diminish the deleterious effects of angiotensin II and, consequently, it is also possible that ACE inhibitors or ARBs could, in fact, lower the risk of complications [51]. However, increased ACE2 and the formation of angiotensin-(1-7), by inhibiting COX-2, could exert anti-inflammatory effects [52, 53], underscoring the multitude of possible effects and the need to conduct studies to interrogate these connections. Finally, it is not known whether an increase in the expression of ACE2 would also lead to an increased shedding and increased levels of soluble ACE2, which could act as a decoy receptor and lower viral entry into cells [54]. In support of this, recombinant human ACE2 ameliorated the lung injury induced by the avian influenza H5N1 virus in mice [55]. It is also important to consider that from the relatively limited amount of human data, plasma ACE2 activity does not appear to be statistically different between individuals taking ACE inhibitors or ARBs and those not taking these medications, but these results do not reflect the levels of cellular receptors [56]. Structural analyses indicate that the binding of the SARS-CoV spike protein to ACE2 does not occlude the catalytically active site of the receptor [26, 57], and it was hypothesized that angiotensin II binding to ACE2 could induce a conformational change in the receptor, which will no longer be favorable for SARS-CoV-2 binding [54]. The mining of existing datasets, preclinical studies, and clinical trials will help shed light on these complex and sometimes conflicting scenarios.

A decrease in the number of ACE2 receptors appears to be involved in acute lung injury and cardiovascular pathology [58, 59], and may be detrimental during coronavirus infection. A mouse *Ace2* knockout developed

severe cardiac contractility defects and increased angiotensin II levels, and the additional deletion of *Ace* rescued this phenotype [60]. In acute lung injury models, the loss of *Ace2*precipitated severe acute lung failure, and this was attenuated by the exogenous recombinant human ACE2 in both *Ace2* knock-out and in wild-type mice [59]. Attenuation of the Ace2 catalytic function perturbed the pulmonary renin-angiotensin-aldosterone system and increased inflammation and vascular permeability [61], and Ace2 overexpression decreased lung inflammation in an animal model of acute lung injury [62]. *In vitro* and in experimental animals, SARS-CoV and the SARS-CoV spike protein downregulated ACE2 expression [12, 28]. In mice with lung injury, injection of the SARS-CoV spike protein worsened the acute lung failure and caused lung edema, increased vascular permeability, and decreased lung function, and this pathology was attenuated by blocking the renin-angiotensin-aldosterone pathway [12]. Thus, animals infected with SARS-CoV or treated with the spike protein resemble *Ace2* knockout animals [12]. It is relevant that a pilot study of patients with acute respiratory distress syndrome reported the accumulation of angiotensin I and the decrease of angiotensin-(1-9), indicating decreased ACE2 activity, among non-survivors [63]. Thus, SARS-CoV and SARS-CoV-2 might contribute to severe respiratory symptomatology partly because the viruses, by binding the ACE2 receptors, also deregulate protective pathways in the lungs.

Thus, either increased or decreased numbers of pulmonary ACE2 receptors may be detrimental during SARS-CoV or SARS-CoV-2 infection, most likely for distinct reasons. An increased number of ACE2 receptors may lead to a higher viral load and more severe clinical disease. Diabetes increases ACE2 expression, as shown in several experimental models, and the resulting increased viral load might explain the more severe course of COVID-19 in diabetic patients [64, 65]. Interestingly, in a rodent model of diabetes, ibuprofen inhibited the ACE/angiotensin II/angiotensin type 1 receptor axis and enhanced the ACE2/angiotensin-(1-7)/Mas receptor axis [66]. Too few functional ACE2 receptors, which decrease even more as a result of high viral loads and enhanced receptor internalization [67], might exacerbate acute lung injury, increase angiotensin II levels, and alter the balance between pro- and anti-inflammatory responses. It is relevant that in a study on twelve COVID-19 patients from China, plasma angiotensin II levels were markedly elevated as compared to healthy control individuals, and linearly associated with the viral load and with the lung injury [68]. The animal studies that documented an age-dependent decrease in ACE2 expression in the lung and the aortic might also explain, at least in part, the age-dependent increase in the risk of serious COVID-19 complications [69, 70].

SARS-CoV can also bind cells through alternative receptors that include the C-type lectins DC-SIGN (dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin) and/or L-SIGN (liver/lymph node-SIGN) [14, 71-73]. It will be critical to understand the potential involvement of the same, or alternative receptors in the pathogenesis of COVID-19.

It has been less clear why SARS-CoV and SARS-CoV-2 lead to severe lung disease [57], in contrast to other, previously known coronaviruses, which usually result in mild upper respiratory infections and cause pneumonia only rarely, mostly in newborn, the elderly, and immunocompromised individuals [74-77]. One of the possibilities advanced for SARS is that the burden of viral replication and the immune status of the host may both shape the severity of the infection [57, 78, 79]. The same might be true for COVID-19, and further exploring the link between viral burden, chronic medical conditions, long-term medication usage, and the severity of the infection will be critical.

An important lesson from SARS and MERS is the association between the incubation period and disease severity. For any infectious disease, the incubation period varies among individuals, even for the same outbreak, and depends on the initial infective dose, the speed of pathogen replication within a host, and host defense mechanisms [80]. During the 2002-2003 SARS outbreak, a study in Hong Kong revealed that patients with shorter incubation times developed more severe disease [81]. The same was found in MERS patients from South Korea, where longer incubation times were associated with a lower risk of death [82]. Interestingly, during the SARS outbreak in Hong Kong, healthcare workers, who have a higher infecting dose, had 34% shorter median incubation times than non-healthcare workers [83]. It will be interesting to examine whether the same is true for SARS-CoV-2, and whether the incubation period is different in COVID-

19 patients when they are stratified by age, coexisting morbidities, and therapies they receive for chronic diseases. While the association between the incubation period and mortality might simply indicate that the disease was confirmed earlier in patients with longer incubations, and reflect earlier treatment opportunities [82], it is also plausible that high viral loads might mediate the link between the two.

Two factors decisive for the successful control of outbreaks are the ability to isolate asymptomatic individuals and the ability to trace and quarantine their contacts [84, 85]. Several studies reported asymptomatic shedding of SARS-CoV-2, indicating that asymptomatic carriers, or individuals with very mild symptoms, may sustain transmission [86-89]. For example, nearly 18% of the passengers who tested positive for SARS-CoV-2 on the Diamond Princess cruise ship were asymptomatic [88]. Another valuable finding that emerged from the COVID-19 outbreak analysis in Singapore, and has a strong impact on infection control, is that after becoming asymptomatic, some patients continued to shed the virus for up to several days. In one instance, a patient continued to have detectable respiratory shedding, as shown by PCR, for eight consecutive days after becoming asymptomatic [90]. Another study revealed that several children with COVID-19 persistently tested positive for viral RNA on fecal swabs after their nasopharyngeal cultures became negative. Even though replication-competent virus was not detected in the fecal swabs, this finding leaves open the possibility of SARS-CoV-2 fecal-oral transmission [91]. These findings illustrate the challenges in understanding SARS-CoV-2 transmission and in identifying infected individuals, tracing their contacts, and implementing preparedness plans. One of the absolute requirements, to clarify these questions and overcome these obstacles, is ensuring the prompt and large-scale testing of symptomatic individuals and of their asymptomatic contacts. This, together with the social distancing measures, are currently our only available assets in facing a pandemic that, even though it was preceded by multiple warnings in recent years, is unlike any other infectious disease that we experienced in modern history.

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