Editorial

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Ageing, ACE2 deficiency and bad outcome in COVID-19

The rapid spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) across the world and the evidence of critical or fatal disease among hospitalized patients induced a lot of basic and clinical research on the potential prognostic factors for adverse outcome [1]. The United States Centers for Disease Control and Prevention (CDC) created a list of established risk factors that have been associated with severe disease [1]. It includes specific epidemiologic, clinical, and laboratory features (age, cancer, cerebrovascular and cardiovascular diseases, chronic kidney disease, diabetes mellitus, obesity, D-dimer, troponin, C reactive protein, and lymphocyte count) as established by observational studies, systematic reviews and meta-analyses [1]. Among these, ageing is strongly associated with mortality to Coronavirus Disease 2019 (COVID-19), and it has been recognized as an independent marker of severe illness and death [1, 2]. In a report from the Chinese Center for Disease Control and Prevention, the overall case-fatality rate of COVID-19 was 2.3% (1,023 deaths among 44,672 confirmed cases). No deaths occurred in the group aged 9 years or younger, but rates of death were 8 and 15% among patients aged 70–79 and 80 years or older, respectively [2].

In this issue of the *Journal*, the opinion paper by Mohaghegh and coworkers [3] offers relevant "food for thought" on the prognostic impact of age in the evolving scenario of COVID-19.

First, they highlighted the different infection rates between young and older adults. Available evidence supports the notion of a lower susceptibility to infection in children aged under 10 years compared to adults at the same level of exposure. Furthermore, adults aged over 60 years show higher susceptibility to infection when compared to younger/middle aged adults [4].

Second, they discussed the hypothesis that androgen sensitivity (which shows significant variations with gender and age) may concur to the development of severe forms of SARS-CoV-2 infection [3]. More specifically, the hyper-androgenic phenotype seems to be associated with COVID-19 increased viral load, increased viral dissemination, and severity of lung involvement [5].

Finally, Mohaghegh and coworkers [3] argued that the influence of angiotensin-converting enzyme 2 (ACE2) polymorphism on the severity of COVID-19 pneumonia may differ based on the age of patients (i.e. increased susceptibility to COVID-19 pneumonia among older patients).

These observations are relevant because they support a direct pathogenetic implication of age in the severe forms of COVID-19. There are several plausible explanations for these observations, but the most likely is the effect of ageing on ACE2 expression.

ACE2 is a trans-membrane type I glycoprotein belonging to the ACE family of dipeptidyl carboxydipeptidases composed by 805 amino acids [6]. ACE2 receptors are expressed in various human organs, including the heart (endothelium of coronary arteries, fibroblasts, cardiomyocytes, epicardial adipocites), vessels (vascular endothelial and smooth cells), gut (enterocytes of the small intestine and colon), lung (macrophages, tracheal and bronchial epithelial cells, type 2 pneumocytes), kidney (luminal surface of tubular epithelial cells), testis (spermatogonia, Leydig cells, and Sertoli cells), and brain (neurons and glial cells) [6]. This organ- and cell-specific expression of ACE2 supports its role in the regulation of cardiovascular and renal function [6, 7]. Indeed, ACE2 plays a key role in the renin-angiotensin-aldosterone-system (RAAS). It catalyzes the cleavage of angiotensin I into angiotensin$_{1-9}$, and angiotensin II into the

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vasodilator angiotensin$_{1-7}$. Notably, Angiotensin II serves as a potent vasoconstrictor and stimulant of aldosterone release and it triggers adverse reactions including endothelial dysfunction, hypercoagulability, enhanced inflammation, and oxidative stress. On the contrary, angiotensin$_{1,7}$ exerts some protective cardiovascular actions including vasodilatation, inhibition of angiotensin II-induced signaling, sympathetic modulation, increase of cardiac output and restoration of endothelial function [6, 7].

Unfortunately, ACE2 is also a functional receptor for the Spike glycoprotein of the SARS-CoV-2 [6]. It provides the entry point for the SARS-CoV-2 to hook into and infect human cells [6].

More specifically, the N-terminal portion of the viral protein unit S1 binds to a pocket of the ACE2 receptor. After the binding, the receptor transmembrane protease serine 2 (TMPRSS2, structurally contiguous to ACE2) operates the protein cleavage between the S1 and S2 units of the viral Spike protein with a consequent conformational rearrangement of the S2 unit driving the fusion between the virus and cellular membrane. After the fusion, SARS-CoV-2 enters into the cell releasing its content and promoting replication, and infection of other cells [6].

Of note, the interaction between ACE2 and SARS-CoV-2 Spike proteins is associated with the internalization of ACE2 receptors into cell (downregulation) with substantial loss of ACE2 receptor activity from the external site of the cellular membrane [6].

Taken together, these phenomena lead to less angiotensin II inactivation and less generation of antiotensin$_{1-7}$. Of note, the imbalance between angiotensin II overactivity and angiotensin$_{1-7}$ deficiency may ultimately trigger inflammation, thrombosis, and other severe adverse reactions influencing the outcome of COVID-19 [7].

Some phenotypes associated with severe forms of COVID-19 share a variable degree of ACE2 deficiency [6]. It has been suggested that the loss of ACE2 enzymatic activity (mediated by the interaction between ACE2 and SARS-CoV-2 Spike proteins) is particularly detrimental among patients with baseline ACE2 deficiency for the consequent marked imbalance between angiotensin II and angiotensin$_{1,7}$ [6]. Specifically, phenotypes of lower ACE2 expression are identified by the presence of hypertension, diabetes, cardiovascular disease and, notably, older age [6].

Ageing has been associated with decline in levels of ACE2 expression in experimental and human models [8–10]. Ageing-related decrease in ACE2 expression levels was observed in lung epithelial cells of aged rats compared to young rats [9]. Xie and co-workers [9] determined the characteristic of ACE2 expression in lung and the effect of

ageing on its expression among rats at three distinct ages (young-adult, 3 months; middle-aged, 12 months; old, 24 months). ACE2 was predominantly expressed in alveolar epithelium, bronchiolar epithelium, endothelium and smooth muscle cells of pulmonary vessels with similar content [9]. The decrease of ACE2 content was relatively slight between young-adult and middle-aged groups [9]. Conversely, ACE2 expression was markedly reduced among older group (young-adult vs. old: by 78% in male rats and 67% in female rats, p<0.001; middle-aged vs. old: by 71% in male rats and 59% in female rats, p<0.001) [9].

Similarly, a study by Yoon and coworkers [8] evaluated the association between the change in the expression of ACE2 and arterial ageing in mice. Levels of ACE2 were measured in the thoracic aortas from 2-month-old, 12-month-old, and 24-month-old C57/BL6 mice. Results demonstrated that the expressions of ACE2 decreased with age [8].

A bioinformatic analysis of publicly available human genomics and transcriptomics gene expression data by Chen and coworkers [10] demonstrated that ACE2 expression decreases during ageing in many tissues. Specifically, the Authors documented a decrease in ACE2 expression with age in blood, adrenal gland, colon, nervous system, adipose tissues, and salivary gland [10]. They also documented a significant association of age, sex, ethnic groups, and body mass index (BMI) with ACE2 expression. However, the association with age was the strongest followed by sex, ethnicity, and BMI [10].

In conclusion, while it has been coopted as the entry point for the SARS-CoV-2 on host cells, the ACE2 enzyme also modulates the balance between vasoconstrictors and vasodilators within the heart and kidney, and plays a significant role in regulating cardiovascular and renal function [6, 7]. The loss of ACE2 receptor activity from the external site of the cellular membrane, as mediated by the interaction between ACE2 and SARS-CoV-2 Spike proteins, leads to less angiotensin II inactivation and less generation of antiotensin$_{1-7}$. The imbalance between angiotensin II overactivity and angiotensin$_{1-7}$ deficiency may trigger inflammation, thrombosis, and other adverse reactions [6, 7].

In this context, it has been recently suggested that the loss of ACE2 activity induced by viral invasion may be especially detrimental in people with baseline ACE2 deficiency [6, 7]. In other words, among COVID-19 patients the prognosis may be worse due to a deficiency of ACE2 expression levels. On the basis of current knowledge, age exerts a significant influence on ACE2 expression and older age is associated with a marked decrease of ACE2 levels. Thus, advanced age may be considered a phenotype of ACE2 deficiency which identifies the critically ill patient with COVID-19 (Figure 1).
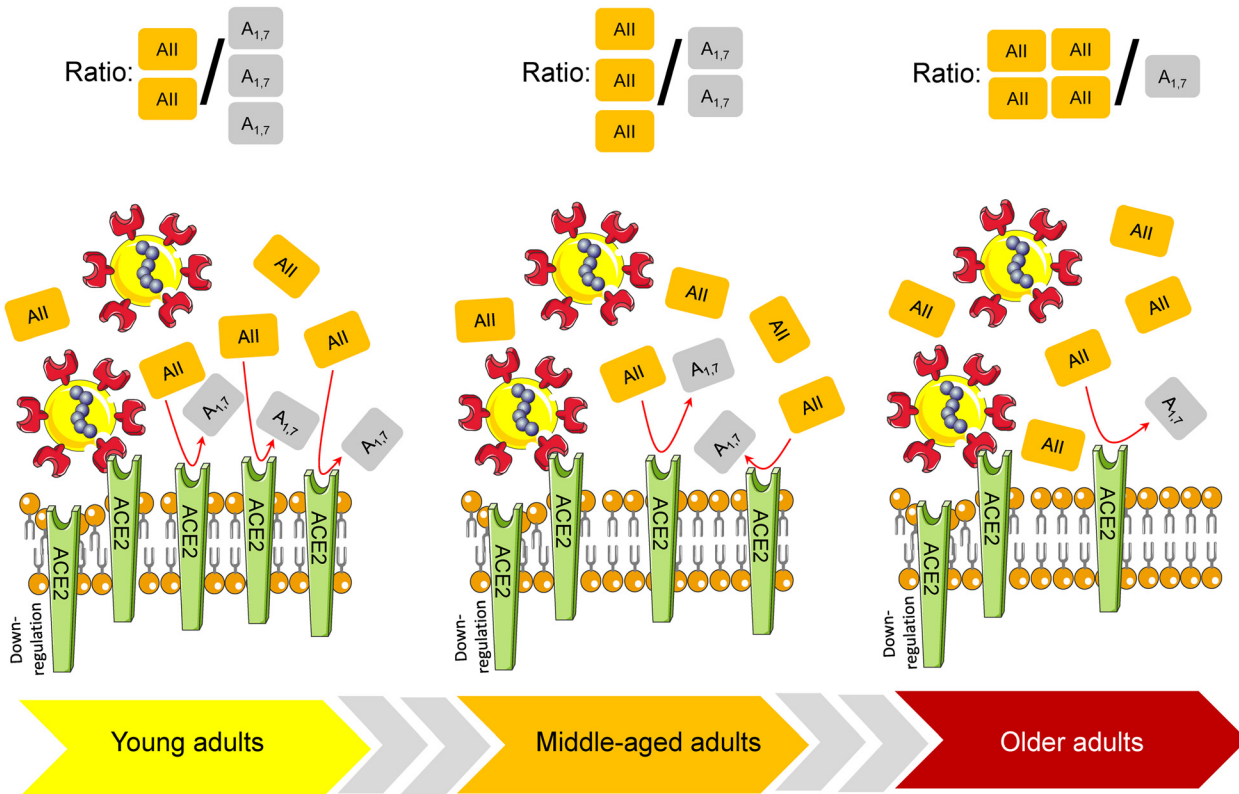
Figure 1: Effect of age on ACE2 expression.

Ageing is associated with decreased levels of ACE2 and with a progressive imbalance between angiotensin II inactivation and angiotensin$_{1-7}$ generation. Thus, the loss of ACE2 activity induced by viral invasion may be especially detrimental in older adults with baseline ACE2 deficiency. AII, angiotensin II; A$_{1,7}$, angiotensin$_{1,7}$; ACE2, angiotensin converting enzyme 2.

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