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Research paper

Renin-angiotensin system at the heart of COVID-19 pandemic

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ABSTRACT

Significant aspects of COVID-19 pandemic remain obscure. Angiotensin converting enzyme 2 (ACE2), a component of the renin-angiotensin system, whose expression dominates on lung alveolar epithelial cells, is the human cell receptor of SARS-CoV-2, the causative agent of COVID-19. We strongly encourage the concept that thorough considerations of receptor-ligand interactions should be kept at the heart of scientific debate on infection. In this idea, the whole renin-angiotensin system has to be evaluated. We hypothesize that factors related to ethnicity, environment, behaviors, associated illness, and medications involving this complex system are probably responsible for situations regarded as anomalous from both an epidemiological and a clinical point of view, but, taken together, such factors may explain most of the aspects of current outbreak. We decided to use the analogy of a play and speculate about the possible impact in this tragedy of 1) air pollution via the interference of nitrogen dioxide on ACE2 expression; 2) the dual role of nicotine; 3) the hypothetical involvement of ACE2 polymorphisms, the relationships of which with ethnic factors and susceptibility to cardiovascular disease seems intriguing; 4) the impact on the severity of infection of hypertension and related medications acting on the renin/angiotensin system, and, finally, 5) the possible helpful role of chloroquine, thanks to its capacity of modifying ACE2 affinity to the viral spike protein by altering glycosylation. This hypothesis paper is an urgent call for the development of research programs that aim at questioning whether the putative protagonists of this tragedy are real-life actors in COVID-19.

1. The play

Significant aspects of COVID-19 pandemic progression remain obscure. After its initial spread in China, the pandemic is now progressing at an accelerating rate in western Europe and the United States of America. Yet, in the other regions of the world, the kinetics of diffusion and mortality seem less impressive, and this in spite of globalization of exchanges and travels. With respect to mortality, some comorbidities, namely diabetes and hypertension, usually having a modest impact on the course of severe diseases, seems to play a major role in the course of Severe Acute Respiratory Syndrome (SARS) COVID-19 [1].

2. The cast

The severe acute respiratory syndrome corona virus −2 (SARS-CoV-2), the causative agent of COVID-19, is a RNA virus with an envelope constituted by spike projections used as a key to enter the cell. SARS-CoV-2 and SARS-CoV (responsible for the 2003 global outbreak) share very high phylogenetic similarities, with 99% identity between their spikes [2]. Therefore it is not surprising that both viruses exploit the same human cell receptor namely angiotensin converting enzyme 2 (ACE2), whose expression dominates on lung alveolar epithelial cells [3].

ACE2 is an 805-amino acids captopril-insensitive carboxypeptidase with a 17-amino acids N-terminal signal peptide and a C-terminal membrane anchor [4]. ACE2 catalyzes the cleavage of angiotensin I into angiotensin 1-9, and of angiotensin II into the vasodilator angiotensin 1-7, thus playing a key role in systemic blood pressure homeostasis, counterbalancing the vasoconstrictive

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action of angiotensin II, which is generated by cleavage of angiotensin I catalyzed by ACE [5]. Although ACE2 mRNA is present in all organs, its protein expression dominates on lung alveolar epithelial cells, enterocytes, arterial and venous endothelial cells, and arterial smooth muscle cells [6]. Although it seems evident that lung is a primary target of COVID-19, it is not counterintuitive that associated cardiovascular conditions or related management could interact with susceptibility to infection and/or severity of the clinical course.

3. The scenario

The concept of the modulation of the receptor-ligand interaction (ACE2-COVID-19) and the broad consequences on the host should be kept at the heart of debate. As function and regulation of ACE2 are interrelated to ACE inside the renin-angiotensin system, the whole system has to be taken into account because of its possible impact on probability of COVID-19 infection and severity of clinical course. Thus, molecular, ethnical, environmental, behavioral, and pharmacological factors involving this complex system are probably responsible for situations regarded as anomalous from both an epidemiological and a clinical point of view, but can be, at last in part, explained at light of available knowledge.

4. The tragedy

It has been pointed out that in patients with severe form of COVID-19 disease, comorbidities are frequent. In the Report of the WHO-China Joint Mission on COVID-19, severe disease (dyspnea, respiratory frequency $\geq$30/minute, blood oxygen saturation $\leq$93%, PaO2/FiO2 ratio <300, and/or lung infiltrates >50% of the lung field within 24–48 h) accounted for 13.8% of cases, and overall mortality rate was 3.8% (4.7% in men vs. 2.8% in women) [7]. Individuals at highest risk for severe disease and death were those aged over 60 years, and with underlying illnesses including hypertension, diabetes, cardiovascular disease, chronic respiratory disease and cancer. Crude fatality rate was 1.4% in the absence of comorbid conditions, but increased to 7.6, 8.0%, 8.4%, 9.2% and 13.2% in case of cancer, chronic respiratory disease, hypertension, diabetes, and cardiovascular disease, respectively [7]. In the recent study on 1099 infected patients by Guan et al., hypertension, diabetes mellitus, coronary heart and cerebrovascular diseases were found in 23.7%, 16.2%, 5.8%, and 2.3% of 173 patients with severe disease [8]. The 20th March report from Italian Health authorities on deceased patients showed that 74% of them had arterial hypertension, 63% underlying cardiovascular conditions (ischemic heart disease, 30%, atrial fibrillation, 22%, previous stroke, 11.2%), and 34% diabetes [9].

Although concurrent cardiovascular disease might explain increased mortality in a severe infection responsible for respiratory failure and deterioration of cardiac function, the observations on hypertension warrant urgent speculation and reflection, while waiting for results of large-scale studies evaluating the independent value of each risk factor.

5. The co-protagonists

ACE2 polymorphisms: *imbrication with associated illness and possible different susceptibility to infection*. The ACE2 gene (which is located on X chromosome) is characterized by a large polymorphism, and significant associations between polymorphisms and occurrence of arterial hypertension have been reported for women of different race and ethnicity and in Han Chinese men [10,11]. More recently, the combination of ACE1 and ACE2 polymorphisms has been associated with susceptibility to hypertension in Brazilian patients [12]. Within the Chinese population, the polymorphism distribution seems different between regions; polymorphisms of ACE2 are associated with different blood pressure response to the cold pressor test. An enhanced response is associated with polymorphisms more frequently observed in northern regions of China, and lowered response with polymorphisms more frequently observed in southern regions. This observation leads the authors to speculate that this was a consequence of exposure to different climatic conditions, possibly because of adaptive selection over generations [10,13]. Furthermore, ACE2 polymorphism is correlated to diabetes mellitus, cerebral stroke, septal wall thickness, ventricular hypertrophy, and coronary artery disease in Asian populations [14]. However, whether ACE2 polymorphism is associated with a different susceptibility to Covid-19 infection or its severity is still speculative.

The different geographic and ethnic distribution of polymorphisms could partly explain the currently observed differences in incidence between countries (for example very low in Africa), and despite globalization of exchanges and travels.

Environmental factors: air pollution factors should be taken into account. In animal models a 100-fold increase in ACE activity was observed after exposure to NO2, which also induces an increase in Angiotensin II binding to its receptor [15,16]. Of note, ACE2 expression is associated with Angiotensin II binding to type-1 receptor (AT1R). In human cardiofibroblasts Angiotensin II induces overexpression of both ACE2 mRNA and protein in a dose-dependent manner, through regulatory factor(s) interacting with a regulatory sequence located at −516/-481 of human ACE2 gene [17]. NO2 is a major air pollutant. Increased concentration exposure to NO2 was found to be associated to an increased risk of hypertension in a population-based, cross-sectional study including 15,477 Chinese adults [18]. Furthermore, in the 2003 SARS epidemic, a positive association between air pollution (defined by the concentrations of nitrogen dioxide, particulate matters, sulfur dioxide, carbon monoxide and ground-level ozone) and case fatality was observed in Chinese population [19]. Public opinion is currently impressed by the reported spectacular decrease in NO2 pollution worldwide, thanks to widespread lockdown measures. However, if we look at baseline data, NO2 pollution was remarkable in regions where COVID-19 epidemic is now particularly lethal: the cases of Lombardy for Italy, Paris and eastern France are markedly impressive [20].

Smoking habit: cigarette smoking could also interact with susceptibility to SARS-CoV-2 infection. Although chronic lung damage induced by smoking would increase susceptibility to severe forms of viral pneumonia, available literature strongly suggests that nicotine upregulates the detrimental angiotensin-converting enzyme (ACE) but downregulates the compensatory ACE2/ANG-(1–7) receptor axis [21]. Thus, the hypothesis that cigarette smoking is either detrimental or provides a counterintuitive protective effect, warrants specific epidemiologic and clinical studies. A preliminary meta-analysis indicates that active smoking is not associated with severity of coronavirus disease [22]. The possible downregulation of ACE2 associated with a possible decrease of the virus-binding sites may counteract the general increased susceptibility to lung diseases usually observed in smokers.

ACE2 expression regulation: we speculate that the binding of SARS-CoV-2 to ACE2 is likely to alter the function of the latter, and massive loss of ACE2 function could participate in the pathophysiology of cardio-respiratory failure. In animal models, the loss of ACE2 resulted in increased production of reactive oxygen species (ROS) via NADPH oxidase 2 activation, and administration of recombinant ACE2 was shown to inhibit the angiotensin II effects on TGF-β1 activation and collagen production [14]. Recombinant ACE2 pulmonary overexpression attenuates whole aspects of pulmonary artery hypertension pathophysiology [23], suggesting that its loss

of function (as could occur in case of massive binding by viral particles, with subsequent endocytosis and activation of proteolytic cleavage [24]) could participate in the severity of respiratory distress. More importantly, loss of function of ACE2 cannot counterbalance the deleterious effects of Angiotensin II in promoting degradation of cardiac and lung structures and function [24]. Thus, angiotensin II receptor blockers could also potentially exert a protective action in the late phases of SARS COVID-19 by counteracting the negative effects of Angiotensin II.

Arterial hypertension medications: The urgent need of reflection should take into account the effect of medications on ACE2 synthesis, expression, and action whose theoretical impact on COVID-19 prevention, infection, severity, and management would be not negligible. Hypertension involves 1.39 billion people worldwide, 349 million in high-income and 1.04 billion in low- and middle-income countries. However, large discrepancies in exposure to antihypertensive drugs worldwide exist: the awareness, treatment, and control concern 67%, 56%, and 28% of patients in high income, and 37%, 29%, and 7.7% of patients in low- and middle-income countries [25]. Most patients with arterial hypertension and/or cardiac failure are treated by ACE1 inhibitors or angiotensin II receptor blockers (sartans). Prescription of sartans increases each year in high-income countries, and are probably the most frequently prescribed drugs in diabetic patients with hypertension. These medications increase gene expression of ACE2 in rats [26]. Although there are no indications to date that the same situations occurs in humans, this could result in increased binding site for COVID-19 in non-infected cases and in the diffusion phases of viral infection, but, as stated above, could represent part of a therapeutic armamentarium in severely ill patients with compromised cardiorespiratory function. This therapeutic opportunity has been suggested by the march 19, 2020 joint statement of HFSA/ACC/AHA [27]. In this statement, discontinuation of ACE inhibitors or sartans was discouraged for those patients currently prescribed, but the idea of updating these recommendations in the case of evolution in further research and developments was set. A warning signal on the possible impact of these drugs has been published in an authoritative journal [1].

6. Act V (conclusion)

The fact that the renin/angiotensin system ACE and ACE2 is at the center of the discussion is probably due to its potential dual effect in different phases of COVID-19 (Fig. 1). It should be emphasized that chloroquine has been advocated as a potentially effective drug to be administered in CIVID-19 associated pneumonia [28]. *In vitro* models showed that inhibitory effects are observed either before or after exposure to the SARS-CoV, suggesting both prophylactic and therapeutic advantages [29]. Chloroquine acts by elevating the endosomal pH, and interferes with terminal glycosylation of ACE2, thus negatively influencing the SARS virus-receptor binding as indicated in 2005 by in vitro studies [29]. In the early phase of infection with SARS-Cov-2, chloroquine could reduce the binding of the virus to ACE2 on alveolar lung cells membrane and possibly without interfering with ACE2 function. This would result in a reduction of direct viral cytotoxicity without unbalancing the complex equilibrium of the ACE system especially in the setting of ACE2 overexpression. Ongoing randomized trials will answer the question of usefulness of this medication.

7. The epilogue: from fiction to reality of science

Further data should be urgently gathered to clarify the mechanisms underlying pathophysiology of COVID-19 infection, although

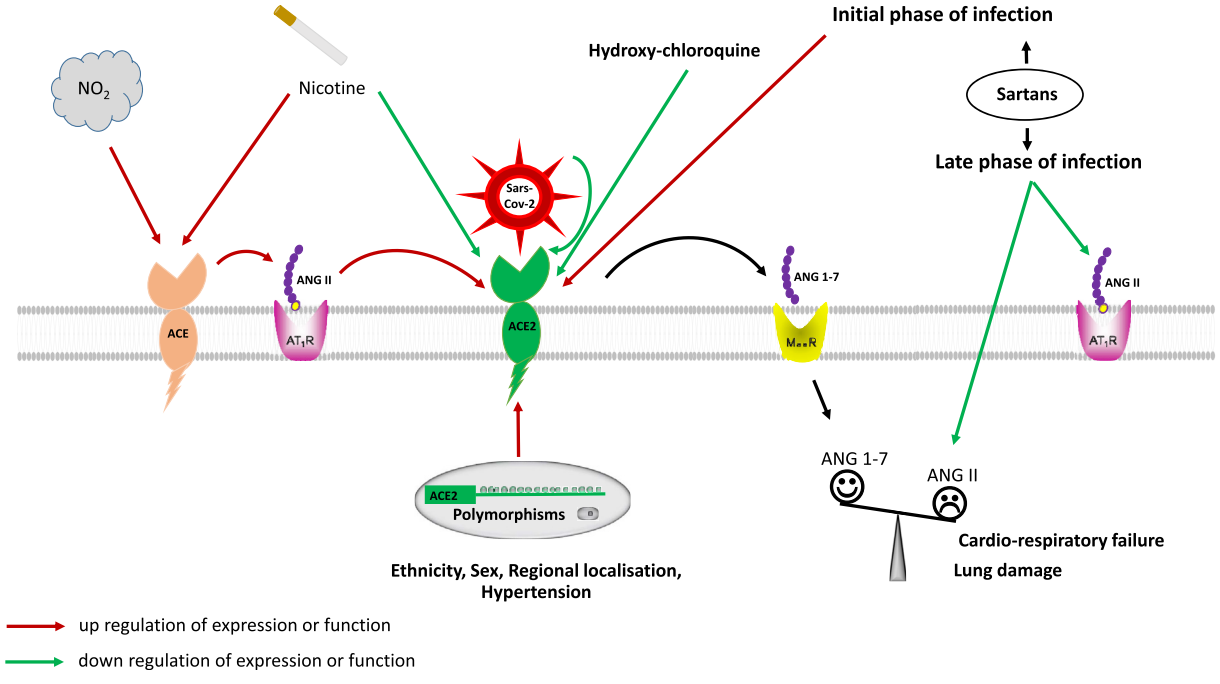

Fig. 1. The stage. Scene 1: In the renin angiotensin system, angiotensin-converting enzyme (ACE) cleaves angiotensin I (Ang I) to produce angiotensin II (Ang II). Ang II action is mediated by the angiotensin II receptor type 1 (AT1R). ACE, Ang II, and AT1R appears to promote lung injury. Angiotensin converting enzyme 2 (ACE2) removes a single residue from Ang II to generate the vasodilator Ang-(1–7), counterbalancing the effects of Ang II. The main actions described for Ang-(1–7) are associated with Mas receptor activation. The ACE2/Ang-(1–7)/Mas arm high activation is effective in reducing lung inflammation, fibrosis, and pulmonary artery hypertension. Scene 2: SARS-CoV-2 uses ACE2 as a receptor for entry into the cell, once the S protein has been primed by a serine protease. Scene 3: The expression of ACE2 and the balance of ANG II/ANG 1–7 influence the course of the disease. Accordingly, ACE 2 may be upregulated due to the individual polymorphism, NO2 associated with air pollution, and ACE inhibitors/Sartans. ACE2 affinity for spike protein may be lowered by hydroxy-chloroquine. Sartans may inhibit the deleterious effects of Ang II during the late phase of the infection.
Footnote: Nicotine may have dual action by enhancing ACE and down-regulating ACE2.

available data on viral receptor strongly support the concept that some obscure aspects could have a theoretical explanation; these conjectures need to be proven by specifically designed studies.

Authors contribution

Marco Alifano: Conception, designing and writing.
Pietro Alifano: Conception, designing and writing.
Patricia Forgez: Conception, designing and writing.
Antonio Iannelli: Conception, designing and writing.
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Declaration of competing interest

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