The differential expression of the ACE2 receptor across ages and gender explains the differential lethality of SARS-Cov-2 and suggests possible therapy.

Ugo Bastolla[(1)]

[(1)] Centro de Biologia Molecular "Severo Ochoa"

CSIC-UAM Cantoblanco, 28049 Madrid, Spain. email: ubastolla@cbm.csic.es

Abstract

There are striking differences in the lethality of the Covid-19 disease, from more than 90% estimated infected persons that experience only very weak or no symptoms to cases that require ICU assistance and result in death. The fatality rate escalates dramatically with age and is much larger in males than in females. I show here these dramatic variations in fatality rates across age and gender are impressively strongly correlated ($r^2 = 0.91$ with only one free parameter) with levels of the ACE2 protein in rat lungs, which are in turn qualitatively similar to expression in humans. This behaviour is predicted by a model that assumes that deaths are caused by the degradation of the ACE2 receptor by the virus, which uses it as entry point in the cells. The insufficient level of ACE2 exacerbates the inflammatory response eventually leading to death, but if the initial levels of ACE2 are high the immune system has enough time to resolve the infection before it gets to extreme consequences. These results are consistent with previous hypothesis on the protective role of ACE2, and suggest that, counterintuitively, drugs that act synergistically with ACE2 and enhance its expression, such as ACE inhibitors and angiotensin receptor blockers used to treat high blood pressure, may offer a promising therapy against the most adverse effects of the Covid-19 disease. ACE2 is also candidate to be a prognostic factor and a risk factor for detecting population that needs stronger protection.

The Covid-19 pandemics [1] is causing thousands of fatalities worldwide [2], creating a tremendous threat to global health [3]. This disease presents a striking gradient of fatalities across age and a marked gender bias that determines a much higher severity for males than for females. The first observation was generally attributed to the insurgence of comorbidities and to the weakening of the immune system with age. The second observation was initially attributed to the fact that men tend to smoke more than women [4]. This gender difference is very marked in China where Covid-19 emerged but it is

not so strong in other countries where it spread later, raising doubts on this explanation. Indeed many experts wondered whether the gender difference should be attributed to some more fundamental biological difference yet to be discovered [5, 6].

To make this issue even more puzzling, the infection fatality rate of the covid-19 disease was estimated to be around 1% [7, 8]. This implies that, except in countries that applied extremely intensive tests, at least 80-90% of the infections have not been detected [8, 9], presumably because the symptoms were mild enough to be confused with a common cold. So, how does Covid-19 escalate from extremely weak symptoms in the youngest age cohorts, where the fatality rate vanishes and hospital care is not needed, to 41% fatalities in male patients over 90 that had to be hospitalized, as reported in Italy [10]?

Here I show that all these seemingly puzzling and disparate observations on the influence of age, gender and viral load on the fatality rate can be rationalized assuming that the deaths caused by SARS-Cov2 occur if the virus degrades below a critical threshold the Angiotensin converting enzyme 2 (ACE2) receptors that it uses to entry into the cells. This model predicts the fatality rates across six classes of age and gender that span 3 orders of magnitude with a goodness-of-fit $r^2 = 0.90$, and it also predicts the qualitative effect of viral load, although the available data do not allow to test it quantitatively.

Together with the homologous enzyme ACE, whose action it counteracts, the membrane-bound enzyme ACE2 [11–13] is a key component of the Renin Angiotensin System (RAS) that regulates blood pressure and electrolyte homeostasis in blood [14]. While ACE cleaves angiotensin I to produce angiotensin II, a peptide that binds to the AT1R receptor producing vasoconstriction and increasing blood pressure, ACE2 cleaves angiotensin II to angiotensin 1-7, a peptide with vasodilator effect. ACE2 protects the lungs from severe injury induced by acid aspiration or sepsis [15, 16]. Decrease of ACE2 activity through SARS infection [17] or related to aging [18] exacerbate the severity of lung injuries and inflammatory lung diseases [19].

ACE2 was found to be the entry point in the cell for the SARS coronavirus, closely related to SARS-Cov2 [20]. SARS-Cov-2 uses the same protein to infect human cells [1]. The structure of the receptor [21] and of the viral spike protein that interacts with it [22] have been recently determined. Molecular evolution studies show that the viral spike protein has been subject to positive selection to increase its affinity for ACE2 [24, 25], which is larger than the one of the related SARS coronavirus [22, 39].

The dual role of ACE2 as a protective factor against lung injuries and inflammation and at the same time entry point for the virus has sparked intense debate on the overall effect of ARB and the drugs that enhance its expression (ACE-inhibitors ACE-I, angiotensin receptor blockers ARB [26] and ibuprofen). Does this effect consist in amplifying [27, 28] or mitigating [29, 30] the severity of Covid-19? At the present time, despite the clinical evidence supports a protective role of ACE2 and the drugs that enhance it [31–33], the consensus is that present data are insufficient to level the balance on one or the other side [34, 35]. Notably the debate existed also at the time of the 2003 SARS outbreak, and also at that time it was not solved [36], despite clinical evidences supported a protective effect [37]. Here I contribute to this debate in three ways: (1) I show that, contrary to the

naive expectation, the lethality of covid-19 is strongly anti-correlated with the expression of ACE2 in rat lungs, explaining striking variations with age and gender. Expression data and other arguments support that a similar correlation also exist with human ACE2; (2) I rationalize this finding with a mathematical model in which depletion of ACE2 through viral entry has a causative effect in covid-19 lethality; (3) Analysing a previous mathematical model of virus progression, I show that there are parameter regimes in which the increase of the receptor does not enhance, and even hinders, the rate of viral expansion, and that this model may rationalize differences with other coronavirus species (SARS-CoV and NL63) that present different affinity for the receptor.

Results

Age and gender specific lethality is negatively correlated with ACE2 levels

Due to its relevance as the entry point for SARS, the expression of the ACE2 protein was quantified in rat lung by Xie et al. across three age classes of the two genders [38]. These authors found that the expression of the ACE2 protein in the lungs strongly decays with age and it is generally larger for female than for male rats, with largest differences in the oldest cohort where the expression is almost double for female than for male rats. I obtained expression data from Fig.2 of Ref. [38].

Strikingly, the expression profile of ACE2 is very strongly anti-correlated with the lethality profile of SARS-CoV-2, as shown in Fig.1. In this figure, I plot on the horizontal axis the expression of the ACE2 protein in lung rats measured by Xie et al. [38] versus the case fatality ratio (CFR) of CoVid-19 registered in Italy [10] grouped into three age classes (young, 0-29, middle-age 30-59 and old > 60; vertical axis) of the two genders. The data strongly support the exponential decrease of mortality with ACE2 concentration,

$$\mathrm{CFR}(a) = \exp(-\alpha \mathrm{ACE2}(a)) \tag{1}$$

where a labels the age and gender class. The fit parameter is $\alpha = 7.07 \pm 0.18$. Note that there is only one free parameter, since I assume that the CFR is one at zero expression of ACE2, and nevertheless the fit is excellent, $r^2 = 0.91$ in linear scale, indicating that the ACE2 expression describes 91% of the variation of the CFR over three orders of magnitude with only one free parameter.

Although the data of ACE2 protein levels were obtained in rats, a recent preprint that analysed the GTEx database found the same qualitative trends in human mRNA expression across several tissues: ACE2 expression tends to be higher in women than in men and tends to decrease with age [40]. Protein data in rat lungs show larger differences than human mRNA data, and protein levels are more relevant for COVID-19 infection, but the trends are the same. Additional arguments that support the validity of rat data also for humans will be discussed later.

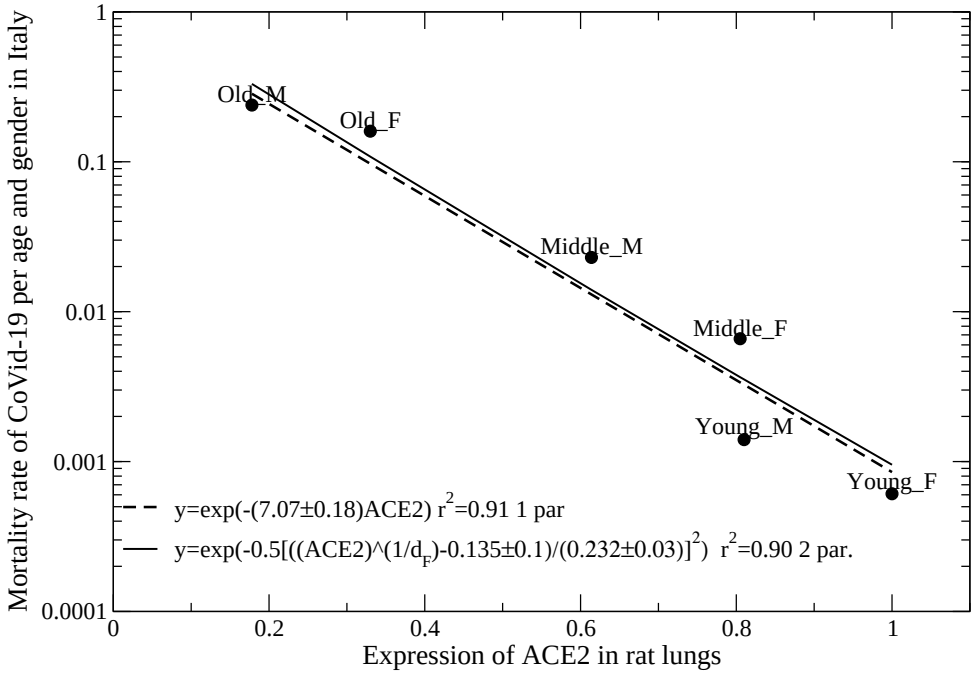

Figure 1: Expression of the ACE2 protein in lung rats (horizontal axis) versus case fatality ratio of CoVid-19 registered in Italy (vertical axis) in three age classes (young 0-29, middle-age 30-59 and old > 59) and two genders (male and female).

The GTEx database also shows that, despite being the organ that is more severely damaged by COVID-19, the lungs only rank 19 over 54 among the tissues with the highest expression of ACE2 mRNA [41]. The expression is much higher in tissues from reproductive organs, intestine, adipose tissue, kidney, hearth, thyroid, esophagus, breast, salivary glands and pancreas, and nevertheless these organs do not suffer serious damage, consistent with the negative correlation between ACE2 levels in lungs and lethality.

ACE2 degradation may play a key role in lethality

To explain the very strong influence of ACE2 on lethality, I developed a mathematical model that assumes that death arises when the ACE2 receptor is degraded below a critical threshold. This hypothesis is supported by studies conducted on mice, which have shown that the interaction of the spike protein of SARS-CoV-2 with ACE2 leads to internalization and degradation of the protein that critically contributes to lung damage [16, 19].

I denote by $t_d(A_0)$ the time needed by the virus to deplete the ACE2 content $A(t)$ below the critical threshold A_c starting from the initial concentration equal to $A_0(a)$. The model assumes that death occurs if this time is smaller than the time t_i needed by the immune system for recognizing and neutralizing the virus. This time is modelled as a random variable. Since t_i is the sum of several intermediate steps in the maturation of

the immune response, it is natural to model it as a Gaussian variable, which is the limit distribution of the sum of independent random variables. An alternative hypothesis is that t_i has exponential distribution, which is the distribution with maximum entropy for given average value, but this hypothesis produces a worse fit to the data and it is not adopted here (not shown). Therefore, I express the death rate for age class a as

$$P_d(a) \equiv \mathrm{P}(t_i > t_d(a)) \approx \exp\left(-\frac{(t_d(a)-\mu)^2}{2\sigma^2}\right). \tag{2}$$

In the following, I shall not consider the dependence of the mean μ and variance σ^2 of the time t_i on the age and gender class a for two reasons. First, there are not enough data for obtaining age- and gender-specific parameters. Second and most important, I want to test the hypothesis that the levels of ACE2 alone are sufficient to determine the lethality profile without considering the weakening of the immune response with age.

Each time the virus infects a cell a receptor is degraded and a number Y (yield) of new virus are synthetized by the infected cell. Therefore, I shall assume that the number of degraded receptors is proportional to the viral population times a proportionality factor X. The lethality condition is $V(t_d) = X(A_0 - A_c)$. If the growth rate of the virus does not depend on the initial level of ACE2 A_0, the time t_d is an increasing function of A_0 and the death probability is a decreasing function of A_0, as observed in the data.

However, since ACE2 is the receptor used by the virus to enter the cell, the naive expectation is that a raise in ACE2 level will enhance the rate at which SARS-CoV2 propagates in the organism and make the outcome of the infection worse. This reasoning lead to propose that drugs that stimulate the expression of ACE2, such as ACE inhibitors (ACE-i) and angiotensin receptor blockers (ARB) that are taken by million persons as a therapy against high blood pressure, may increase the risk of severe COVID-19 [27, 28].

So, what do mathematical models have to say in this context? The simplest dynamical models of viral progression consider three populations: Uninfected cells $U(t)$, free virus $V(t)$ that enter the cells with rate $k_A U(t)V(t)$ (adsorption), and infected cells $I(t)$ that, after a delay τ called eclipse time, produce new virus at rate $k_V Y I(t)$ until they ultimately die [42]. The parameters of the model have been fitted from the time courses of influenza infections [43]. In the simple mean-field version in which spatial structure is not considered the model predicts that the initial growth rate of the virus is proportional to the adsorption constant k_A. From chemical kinetics, k_A for COVID19 is proportional to the amount of ACE2 expressed by susceptible cells, implying that ACE2 levels accelerate the virus progression in the organism.

Nevertheless, considering spatial diffusion in the respiratory tract can modify important predictions and parameters of the mathematical model [44]. Luckily, the analytical solution of a mathematical model of viral infection in space is already available [45]. Despite this model has been tested with experiments on the spread of bacteriophages in lysis plaques, the mathematical formulation can be readily applied to the present setting and in fact it is conceptually equivalent to the models presented above. The spatial model predicts that the virus travels in space as a wave. The authors provide approximate so-

lutions for the viral velocity in the regime in which adsorption is slow compared with the production of new virus, $k_A U_0 \ll k_V$. In this regime, when the adsorption is slow with respect to the eclipse time τ, $k_A U_0 < 1/(\tau Y)$, the viral velocity increases with k_A as $c = 2\sqrt{D\frac{k_A U_0 Y}{1+k_A U_0 Y}}$, where D is the effective diffusion constant that depends on cell shape and density. However, when the adsorption overcomes the critical rate $k_A U_0 \geq 1/\tau Y$, the viral progression saturates and stays constant at the limit value $c = \sqrt{2D/\tau}$ [45]. In this regime, the model predicts that the viral progression is not enhanced by an increase in the number of receptors.

Interestingly, the formulas presented in Ref. [45] also allow considering the regime in which adsorption is very fast ($k_A U_0 \gg k_V$), although this regime is not explicitly discussed in the paper. Counter-intuitively, in this regime the virus speed does decrease with k_A as $c = \sqrt{k_V/k_A U_0}$. This result is surprising: how can the virus progression be slowed down by increasing the adsorption rate? Since this is a mathematical model, the answer is readily found: in the model, viral particles are consumed when they enter a cell but the viral yield per infected cell does not increase when the cell is infected by multiple viruses. Indeed, it was even proposed that multiple viral entries in the same cell interfere with the viral replication. This seems to be a real and important biological phenomenon, since several viruses evolved active mechanisms for downregulating their own receptors, thereby reducing the probability of co-infection of the same cell, such as HIV [46, 47], measles virus [48], influenza virus [49] and hepatitis B [50]. Although the biological mechanisms behind this phenomenon are not fully understood, these observations lend support to the idea that, after the infection is established, very fast adsorption does not accelerate the propagation of the virus.

Inspired by the present data, I shall consider that the virus travels in space with constant velocity c that does not depend on the number of receptors that are present on the cell membrane. The number of infected cells increases with time as $I(t) \propto t^{d_F}$, where d_F is the fractal dimension of the organ where the virus propagate. For the lungs, which are one of the classical examples of a fractal organ [51], I use here the value $d_F = 2.35$ [52,53]. The critical time t_d when the ACE2 level in the organ is reduced below the critical level A_c is given by the equation $I(t_d) \propto t_d^{d_F} \propto A_0 - A_c$, which leads to

$$t_d \propto (A_0 - A_c)^{1/d_F} \tag{3}$$

For simplicity, in order to reduce the number of fit parameters I shall consider that $A_c \approx 0$, which is in good agreement with the data. From the above equation, using Eq.(2), the model predicts that the lethality depends on the ACE2 level A_0 as

$$P_d(A_0) \approx \exp\left(-\frac{\left(A_0^{1/d_F} - \mu\right)^2}{2\sigma^2}\right). \tag{4}$$

The above equation has two free parameters, since I set $d_F = 2.35$ and $A_c = 0$. Fitted against the lethality rate per age and gender class, it yields a very accurate fit that

accounts for 90% of the variation of the mortality rate (Fig.1). The fit parameters, in units of the normalized ACE2 levels (setting the maximum equal to 1) are $\mu = 0.135 \pm 0.10$ and $\sigma = 0.232 \pm 0.027$. In other words, if one has normalized ACE2 levels below 0.15 in the lungs and gets infected, he/she needs rather good luck to survive. On the other hand, if the ACE2 level is above 0.5, the extreme outcome of the infection is unlikely.

The present model also predicts that the lethality of the virus increases for larger initial viral load, since the time necessary for the critical viral spreading will be reduced. Health workers are subject to high viral load. There have been frequent but anecdotic report in the press claiming that the lethality is higher among health workers than in the general population. Data from Italy [10] suggest that this effect may exist, after taking into account the different incidence of undetected cases among health workers and I the general population, but they are not sufficient to demonstrate it.

Finally, it is tempting to speculate that the differences between the infections of different coronavirus that use the ACE2 as entry point in the cells may originate from the differential affinity of their spike proteins for ACE2. The SARS-Cov-2 spike protein has a very high affinity for ACE2, four (table 1 of Ref. [23]) or 10-20 times [22] higher than SARS-Cov. The results presented here suggest that its adsorption rate is high enough to make the viral progression independent of the levels of ACE2 in the lungs for the whole range of ACE2 expression, otherwise the correlation between ACE2 and lethality would not be so strongly negative. On the other hand of the spectrum, the coronavirus NL63 binds to ACE2 [54] but it is not generally associated with acute respiratory distress syndrome (ARDS). Its affinity for ACE2 is thought to be smaller than for SARS-Cov [54], and it is possible that its low adsorption rate makes it unable to progress in the lungs, an organ that presents relatively low ACE2 abundance. Consistently, NL63 infection of humans is usually acquired during childhood [54], when the concentration of the ACE2 receptor is highest. Finally, SARS-Cov has a spike protein that binds ACE2 with intermediate affinity and it is capable of infecting the lungs. Compared to SARS-Cov-2, the increase of lethality with age is not so pronounced. For 1775 Hong-Kong patients of SARS-CoV, the case fatality rate was 3% (≤ 39 years), 13.4% (40-59) and 54.5% (> 59) [55], while for SARS-Cov-2 in Italy the CFR for the same age classes was 0.022%, 1.7% and 27.5% [10], with a much larger increase from young to middle age (77 compared to 4.5) and from middle to old (16 compared to 4). This weaker incidence of age might be explained if the affinity of the spike protein is in a regime in which the propagation of the virus still marginally increases with the expression of the receptor.

Discussion

This work suggests that the variation of the lethality of the CoVid-19 disease over six age and gender classes that span 3 decades is in very large measure explained by the variation of the expression of the protein ACE2 in the lungs. This membrane protein is used by the SARS-Cov-2 virus and the related SARS-Cov virus as entry point in the

cells, so that it has been proposed that its high concentration has an adverse effect, and that drugs that stimulate ACE2 should be avoided in the context of the Covid-19 pandemics [27, 28]. Quite oppositely, we found that an increasing concentration of ACE2 decreases very strongly (exponentially) the lethality of the infection. This result can be rationalized by a mathematical model of viral progression in space [45] that analytically showed that the speed of viral propagation in the infected organ is independent of the adsorption rate once this overcomes a critical threshold, implying that the increase of the receptor level does not always enhance the viral progression. Interestingly, the model predicts that a very high adsorption rate even slows down viral progression, since viral particles are consumed when they enter a cell multiple times but the viral yield does not increase. Indeed, it has been proposed that multiple viral entries in the same cell hinder the maturation of viral particles, and this effect is thought to underlay the evolution of mechanisms that limit the co-infection of the same cell by multiple viral particles by downregulating their own receptor after successful cell entry [46–50]. This tendency of some virus to downregulate their receptor was used by Gurwitz to argue against the idea that ACE2 expression always favours SARS-CoV-2 infection [30].

Consistent with these results, the lungs are the organ where SARS-CoV-2 produces the largest damage but they rank only 19 among 54 human tissues for the expression level of ACE2, according to the GTEx database [41].

One limitation of the present work is that I used data of ACE2 protein expression obtained in rat lungs [38]. Nevertheless, a recent preprint that analysed the GTEx database [41] found the same qualitative trends of ACE2 mRNA expression in several human organs: ACE2 is more expressed in female than in male subjects, and its expression decays with age [40]. Furthermore, several arguments support the portability of expression data from rats to humans. First, there is an almost exact factor two between ACE2 expression in old female and male rats. This factor two is expected, since the ACE2 gene is coded in the X chromosome both in rats and in humans, and females have two copies of it while males have only one. However, the gender difference in expression is very small for young rats. It is possible that young males compensate the disadvantage of having a single copy of the ACE2 gene by overexpressing it as a protective mechanism against cardio-vascular diseases (CVD), since some of the sex differences in CVD have been attributed to ACE2 [56]. Expression data show that this hypothetical mechanism fades with age until the factor two gender-difference in ACE2 expression is reached at old age. These features (X chromosome location of the ACE2 gene, compensatory overexpression in males that decays with age) are likely to be general and shared also by humans. In contrast, a recent clinical study could not find significant differences in ACE2 expression between patients with acute respiratory distress syndrome (ARDS) of different age [57]. However, it is likely that the response to ARDS involves a complex dynamics of the RAS system to which ACE2 belongs. The clinical study measured the activity of RAS enzymes at only one time point for each patient, so that the dynamics may have obscured the individual differences.

It is known that the SARS infection leads to degradation of the ACE2 receptor, with

detrimental effects on the lungs [16, 19]. According to these studies, "the spike protein binds to ACE2 and subsequently down regulates ACE2 protein expression and results in worsened acid aspiration pneumonia". Furthermore, low levels of ACE2 worsen the inflammatory response to the infection that is thought to be the main responsible of the patient death.

The model presented here assumes that the degradation of ACE2 is the main causative factor of the lethality induced by the virus. The model assumes that death occurs if the ACE2 level decays below a critical threshold before the immune system is able to control the viral population. Together with the hypothesis that the ACE2 level does not enhance viral progression, this hypothesis implies that the death rate in a specific age and gender class is a decreasing function of the ACE2 level in that class. The resulting model provides an excellent fit to the observed age and gender specific mortality rates ($r^2 = 0.90$) with two free parameters. Admittedly, the number of data points (six) is very reduced, but the variation that they span is huge and the agreement between data and model is impressive.

The model does not consider the decay of the efficiency of the immune system with age, which does not mean that this decay is not important, it certainly is, but the data can be explained even without considering it.

The model thus suggests the possibility to reduce the lethality in the old age classes by raising their ACE2 levels towards the levels found in younger individuals. Crucially, these results support a simple and promising therapy against the most adverse consequences of the Covid-19 infection, consisting in stimulating the production of ACE2 through ACE inhibitors (ACE-I) and angiotensin receptor blockers (ARB) that are used to treat high blood pressure and upregulate ACE2 expression [11, 26]. The same proposal has been recently advocated by Gurwitz [30].

Short after the discovery of ACE2, it was shown that this enzyme protects against lung damage in a mouse model of diffuse alveolar damage (DAD) [15], and that its downregulation in mice infected with SARS-CoV contributes to lung injury [17, 36]. A posterior meta-analysis [37] found that use of ACE-I provide a 34% reduction in risk of pneumonia compared with controls, in particular in patients with stroke and heart failure.

Recent clinical studies in China and Italy support the beneficial role of ACE2 in protecting the lungs and mitigating the severity of COVID-19 [29, 31, 33, 35].

However, this protective role of ACE2 and of ACE-I and ARB that enhance its expression has been out-weighted by its role as entry point of SARS-Cov and SARS-Cov-2 in the cells, and it was speculated that ARB and ACE-I may favour the viral progression and should be avoided [27, 28]. Several medical societies expressed firm statements opposing the suggestion to remove ACE-I and ARB treatments for patients that do need them [58–60]. For instance, the European Society of Cardiology stated that the speculation about the safety of ACE-i or ARB treatment in relation to COVID-19 does not have a sound scientific basis or evidence to support it. Indeed, there is evidence from studies in animals suggesting that these medications might be rather protective against serious lung complications in patients with COVID-19 infection, but to date there is no data in humans [58]. The current consensus is that data in humans are too limited to support

either hypothesis that ACE-I and ARB may be detrimental or beneficial in COVID19 infections, but withdrawal of anti-hypertensive drugs in patients that need them may be harmful [34]. Of note, a similar discussion already took place as a consequence of the 2003 SARS outbreak when it was proposed the anti-intuitive concept that ACE2 may have a protective effect despite being the virus receptor [36, 37].

The very strong negative correlation between the ACE2 expression and the lethality of the SARS-Cov-2 infection found in this work, and the mathematical prediction that an increased adsorption rate does not enhance viral progression, strongly adds to the clinical evidence to suggest that the effect of ACE2 on survival is positive. This holds true even if the mathematical model that I propose to rationalize the data were wrong. Thus, in the context of the debate on the COVID19 treatment with ARB and ACE-I, the results presented in this work strongly support the use of these drugs to counteract the most adverse consequences of the CoVid-19 disease.

Finally, the results presented here suggest a prognostic role for the measurements of ACE2 levels, which may predict the severity of the disease already at an early stage and may allow detecting risk groups besides the elder. Indeed, hypertension is an important comorbidity of COVID-19 [4] that can be explained by the fact that both diseases are made more severe by lower levels of ACE2, supporting the hypothesis that individuals with low levels of ACE2 have to be protected more by the consequences of SARS-CoV-2 infection.

Acknowledgements

I would like to thank medical doctors, nurses, police and other public employees that fight against the virus at the risk of their lives. This work is dedicated to my father, who passed away few days before the coronavirus outbreak became manifested in Italy, and my mother, who suffered of cardiovascular diseases. I acknowledge useful discussions with several colleagues, in particular Esther Lazaro, Alberto Rastrojo, Mario Mencia, Fatima Sanchez and Manolo Fresno.

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