THE COMBINATION OF INHALED BUDESONIDE AND FORMOTEROL AS AN EARLY TREATMENT FOR THE COVID-19 DISEASE

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ABSTRACT

The covid-19 pandemic caused by the SARS-cov2 coronavirus has sparked an unprecedented effort in the global scientific community. In a race against the clock, we are all on a mission to find a way to halt the so far unstoppable spread of the disease across the globe. Covid-19 would be one more viral disease among many others, if only it was not for the severe respiratory complications that occur in people with risk factors such as old age, high blood pressure, cardiovascular diseases or diabetes. The lethality of the disease is linked to the progression, towards a Severe Acute Respiratory Syndrome (SARS) in patients at risk. Here we propose a model that places the interplay of the renin-angiotensin system and the response of the lung's innate immune system against the virus at the origin of SARS. Accordingly, we put forward a treatment plan for the consideration of the scientific and medical community. This treatment is, with small modifications, basically the same as in the so-called SMART asthma protocol that uses a combination of an inhaled glucocorticoid (preferentially budesonide) and an inhaled long acting beta-2 adrenergic agonist (LABA) (preferentially formoterol). We recommend to begin treating the patients at risk early on in the disease (at the time of presentation of the first flu-like symptoms), instead of waiting for the first signs of SARS (dyspnea). This will allow us to gain time and prevent deaths before we are able to deploy an effective vaccine against the SARS-Cov2 coronavirus.

BACKGROUND

In december 2019, pneumonia cases caused by a new coronavirus (SARS-cov2) were starting to be diagnosed in Wuhan, China[1] [2] [3] . Since then, the virus has spread to mainland China and all over the world, reaching pandemic proportions.

The SARS-Cov2 virus is a close relative of the SARS-cov1 virus that caused the SARS epidemic in 2003. It belongs to the family of beta-coronavirus, single stranded +RNA viruses, that includes the SARS-Cov1 and the NL63, the latter being a frequent cause of seasonal common cold. Among the viral proteins, the spike (S1) protein is essential for host recognition and viral entry (infection) into the cells. It has been recognized that the cell surface receptor for the S1 protein of SARS-Cov2 is the same as for the SARS-cov1, the virus that caused the 2003 SARS pandemic [4].

In the majority of infected people, the SARS-cov2 virus causes a flu-like disease that resolves spontaneously (the so-called covid-19 disease). In a percentage of cases, however (between 10-20%, the subgroup of patients at risk of developing lethal complications), after the flu like syndrome, the disease can progress into a Severe Respiratory Distress Syndrome (SARS), which is the cause of hospitalization and eventually of death. Patients at risk of developing SARS after infection with the SARS-Cov2 virus include older patients (> 65 years old), people with one or more comorbidities (such as cardiovascular diseases, high blood pressure, diabetes, COPD, or active cancer) and people institutionalized in nursing homes[5].

VIRAL LOAD IS NOT CORRELATED WITH SARS OR DEATH. Much has been said about the relationship between the viral infectivity and the disease outcome. The most widely accepted opinion at the time of writing this paper is that there is a positive correlación between the quantity of virus isolated at a given time in a patient (the so-called viral load) and the severity of the disease. So, the race has started worldwide to find an anti-viral drug (new or old) in the hope that it will stop the SARS-Cov2 viral replication in the body tissues and therefore reduce the severity of the disease and the death rate.
There is however a growing current of thought against this view. In fact, as counterintuitive as it could seem, there are strong reasons to believe that the burden of viral infection by SARS-Cov2 (as well as its measurable surrogates, viral load and viremia) is not relevant to the appearance

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[1] "Clinical features of patients infected with 2019 novel coronavirus." 24 Jan. 2020, https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(20)30183-5/fulltext.
[2] "A novel coronavirus outbreak of global health concern - The ...." 24 Jan. 2020, https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(20)30185-9/fulltext.
[3] "A Novel Coronavirus from Patients with Pneumonia in China ...." 20 Feb. 2020, https://www.nejm.org/doi/full/10.1056/NEJMoa2001017.
[4] "The proximal origin of SARS-CoV-2 | Nature Medicine." 17 Mar. 2020, https://www.nature.com/articles/s41591-020-0820-9.
[5] "Brief Review on COVID-19: The 2020 Pandemic Caused by ...." 24 Mar. 2020, https://www.cureus.com/articles/29459-brief-review-on-covid-19-the-2020-pandemic-caused-by-sars-cov-2.

of the lethal complications of the covid-19 disease. First, a multicenter study published on 15 April 2020[6] concludes that "There was no obvious difference in viral loads across sex, age groups and disease severity". In another study on hospitalized patients, differences of viral load are not statistically significant when compared the mild vs severe cases or the people under or over age 65[7]. Moreover, people who are asymptomatic all through the infection have the same viral load as patients with covid-19 symptoms[8]. Furthermore, lung epithelium of wild type mice have much less affinity for the host recognition protein of the SARS-cov virus than its human counterpart. When older mice (surrogate of old humans) are infected with the the SARS-Cov1 virus, they present a mild viral infection, with low viral loads, but after a week of disease, however, they develop a form of lethal SARS similar to the human disease[9]. Finally, the lung viral replication kinetics is similar in AC70 and AC22 mouse lineages, one of them being susceptible (AC70) and the other resistant to the SARS[10].

To summarize, viral replication rates are unrelated to either the presence of covid-19 symptoms or to the severity of the disease.

AN EXCESSIVE INFLAMMATORY REACTION IN THE AIRWAYS IS THE CAUSE OF DEATH in COVID-19 PATIENTS. SARS therefore occurs in the subgroup of patients at risk, where a hyper-inflammatory reaction within the lung airway and in the lung interstitial tissue occurs during the course of the viral infection. This aberrant inflammatory response leads to tissue injury, leaking of fluid into the alveolar cavity, impaired gas exchange, hypoxia, and, eventually, death by respiratory failure.

Several factors are believed to contribute to the pathogenesis of SARS. Some of them are outlined here:

1. The presence of a local inflammatory niche in patients at risk. An excessive activation of the Renin Angiotensin System (RAS) has been postulated to be at the core of this exaggerated inflammatory response. Several observations strongly sustain this thesis:

[6] "Temporal dynamics in viral shedding and transmissibility of ...." 15 Apr. 2020, https://www.nature.com/articles/s41591-020-0869-5.
[7] "SARS-CoV-2: virus dynamics and host response - The Lancet ...." 23 Mar. 2020, https://www.thelancet.com/journals/laninf/article/PIIS1473-3099(20)30235-8/fulltext.
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[9] "Cellular immune responses to severe acute respiratory ...." 11 Nov. 2009, https://www.ncbi.nlm.nih.gov/pubmed/19906920.
[10] "Differential virological and immunological outcome of severe ...." 18 Mar. 2009, https://www.ncbi.nlm.nih.gov/pubmed/19297479.

a. RAS is overactive in old mice[11] as well as in patients at risk of developing SARS[12] . During hypertension, dysregulation of the Renin-Angiotensin System is associated with increased expression of pro-inflammatory cytokines and reactive oxygen species causing tissue damage, endothelial dysfunction, and increase in sympathetic activity, among other damages, eventually leading to decline in organ function. Ang II increases, whereas RAS blockade decreased AT gene expression of inflammatory markers, thereby affecting glucose homeostasis in rodents[13]. Also, the Angiotensin Receptor Blocker (ARB) valsartan reduced abdominal subcutaneous adipocyte size compared with placebo in subjects with impaired glucose metabolism[14] . Also hyperglycemia-induced Angiotensin II Receptor 1 (AT1R) activation impairs insulin secretion, and thus contributes to diabetes[15].
b. Angiotensin II, the pro-inflammatory hormone of the RAS, appears to be activated in hospitalized patients with SARS (both the serum levels and the indirect signs of activation)[16].
c. ACE2, the receptor of the S1 viral protein, is at the core of the anti-inflammatory/anti-hypertensive branch of the RAS system. ACE2 cleavage of Angiotensin II results in, on the one hand, the decrease of AT II activity and, on the other hand, the generation of AT(1-7) which, by through its main functions (anti-inflammatory, vasodilation, and reduction of oxidative damage) is the natural counterweight of angiotensin II[17]. Interestingly, ACE2 baseline expression in the lungs of patients at risk is generally lower than in patients that have a milder covid-19 disease[18]. In contrast, hyipertensive patients under treatment with ACE blockers (ATII inhibitors) have a higher expression of ACE2. This observation has given support to the popular argument that considers that the higher expression of ACE2 could be an independent risk factor for developing the lethal complications of the covid-19 disease. This thesis has sparked a huge controversy as to whether HTA patients on inhibitors of the AT II system should suspend their treatment[19]. Fortunately this controversy has now been put to good rest. Now we believe that this group of hypertensive patients on inhibitors are

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[11] "Age-Associated Changes in the Vascular Renin-Angiotensin ...." https://www.hindawi.com/journals/omcl/2016/6731093/.
[12] "The impact of age-related dysregulation of the angiotensin ...." 24 Nov. 2014, https://www.ncbi.nlm.nih.gov/pubmed/25505418.
[13] "The renin-angiotensin system in the pathophysiology of type 2 ...." 5 Sep. 2012, https://www.ncbi.nlm.nih.gov/pubmed/22986649.
[14] "Valsartan improves adipose tissue function in humans ... - NCBI." 29 Jun. 2012, https://www.ncbi.nlm.nih.gov/pubmed/22768174.
[15] "Effects of hyperglycemia on angiotensin II receptor type ... - NCBI." 8 May. 2008, https://www.ncbi.nlm.nih.gov/pubmed/18469441.
[16] "Coronavirus Disease 2019 (COVID-19) and Cardiovascular ...." 1 Apr. 2020, https://www.ahajournals.org/doi/10.1161/JAHA.120.016219.
[17] "The Anti-Inflammatory Potential of ACE2/Angiotensin ... - NCBI." https://www.ncbi.nlm.nih.gov/pubmed/27469342.
[18] "COVID-19 induced Renin–Angiotensin System (RAS) - The BMJ." 31 Jan. 2020, https://www.bmj.com/content/368/bmj.m406/rr-19.
[19] "Coronavirus debate: Could blood pressure meds make ...." 31 Mar. 2020, https://www.inquirer.com/health/coronavirus/coronavirus-blood-pressure-drugs-beneficial-harmful-20200331.html.

probably partially protected from developing SARS, precisely because of the treatment and the high expression levels of ACE2 that this treatment causes.

2. An excessive reaction of the innate immune system to the viral infection. The innate immune system is the first line of defense at the surface of the airway epithelium. There, there is a group of molecules (both at the cell surface and inside the cells) that are in charge of recognizing foreing organisms and getting rid of them. Molecular recognition is achieved by a group of cell surface receptors (the so-called Toll Like Receptor or TLRs) and intracellular recognition molecules (the NOD like receptors). These receptors recognize molecular patterns (thus their name Pattern Recognition Receptors or PRR) on microorganisms such as virus, bacteria, fungus or parasites. Recognition of these patterns trigger a coordinated response that involves most cells of the (local) immune system, including macrophages, neutrophils, lymphocytes and others[20]. At least two discrete TLR signaling pathways are involved in SARS-CoV pathogenesis. For instance, mice that are deficient in some TLRs (TLR3(-/-), TLR4(-/-), and TRAM(-/-)) are more prone to develop SARS, suggesting that this receptors could make a contribution to forbid the development of SARS[21]. Moreover, the Membrane (M) protein of SARS-CoV can directly promote the activation of both beta interferon (IFN-β) and NF-κB through a TLR-related signaling pathway independent of TRAF3. Apparently this pathway involves the recognition of the viral protein by a novel mechanism mediated by an intracellular PRR[22] .

3. The cross-road: Interaction of the RAS system with the innate immune system. Finally, activation of the AT1R by Angiotensin II leads to increased expression of TLR4 as well as enhanced inflammatory response after LPS stimulation of TLR4[23]. Furthermore, Angiotensin II induces microglial activation in the brain, an effect that is blunted in the absence of functional TLR4[24]. There is thus growing evidence that points to the existence of close cross-communication between the RAS and the innate immune system. The role of this mechanism in Covid-19, although still in need of confirmation, could prove to be key to the unfolding of SARS.

   Taken together, these data suggest that when the SARS-Cov2 virus infects patients at risk (i.e. with a high basal activación of the pro-inflammatory RAS) it will induce an exaggerated response of the innate immune system, which is therefore the molecular and cellular trigger of SARS.

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[20] "Innate Immune Recognition: An Issue More ... - Frontiers." 3 Jul. 2019, https://www.frontiersin.org/articles/464963.
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[24] "The interplay between Angiotensin II, TLR4 and hypertension.." https://www.ncbi.nlm.nih.gov/pubmed/28330785.

ASTHMA IS AN INDEPENDENT PROTECTIVE FACTOR FOR COVID-19

From the study of all the major multi-center patient series of covid-19 patients (from China[25] [26], Italy or the USA[27]), a few points stand out as interesting possible therapeutic lead points: 1) Among the patients with a severe clinical presentation, there is an over representation of older people as well as of a number of comorbidities (cardiovascular diseases, cardiac arrhythmias, high blood pressure, diabetes or COPD). These comorbidities have something in common: a hyperactivation of the Renin Angiotensin System (RAS) and a chronic inflammatory basal state 2) Viral replication rates are essentially the same across sex, age groups and disease severity, indicating that anti-viral drugs are probably not at the core of future anti-covid-19 therapies. 3) Blood indicators of inflammation as well as lung tissue histopathology evidence the presence of an overwhelming local inflammatory process originating in the bronchioalveolar space and expanding into the lung interstitial space. 4) Finally, among all the series of covid-19 (mild or severe presentations alike), there is a striking underrepresentation of patients with chronic asthma[28]. This raises the issue of whether suffering from asthma is in itself a protective factor against developing a symptomatic covid -19 disease. To solve this quandary, we should also take into account the fact that asthma patients are, at least on paper, on chronic anti-asthmatic medication (most of them on different combinations of inhaled Long Acting Beta adrenergic agonists and inhaled corticosteroids)[29]. There is however a caveat in that asthma patients are well known for their low adherence rate to therapy. At any rate, whether it is the fact of having asthma or the asthma therapy that protects from covid-19 disease is still an unsolved issue. What is certain is that asthma therapy, as specified by the worldwide used SMART protocol[30], is a potent airway immunosuppressant. Some reviewers have rightly pointed to us that the use of immunosuppressants, such as the corticosteroids in anti-asthma therapy, may foster viral replication and, therefore, worsen the clinical outcome. First, as we discussed above, viral replication seems to be irrelevant to the disease outcome, and, second, the jury is still out as of the effects of inhaled corticosteroids on viral proliferation. For instance, treatment of primary human airway cells in vitro with GCS prior to rhinovirus (RV) or influenza A virus (IAV) infection significantly increases viral replication[31]. However, closer to home, both in-vitro and in vivo models, using inhaled corticosteroids alone[33] or in combination with bronchodilators, have been

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[25] "Comorbidity and its impact on 1590 patients with Covid-19 in ...." https://erj.ersjournals.com/content/early/2020/03/17/13993003.00547-2020.

[26] "Clinical Characteristics of Coronavirus Disease 2019 in China ...." https://www.nejm.org/doi/full/10.1056/NEJMoa2002032.

[27] "Preliminary Estimates of the Prevalence of Selected Underlying." https://www.cdc.gov/mmwr/volumes/69/wr/mm6913e2.htm.

[28] "Clinical characteristics of 140 patients infected with SARS ...." 19 Feb. 2020, https://www.ncbi.nlm.nih.gov/pubmed/32077115.

[29] "Asthma may be the key for preventing and treating covid19 ...." 2 Apr. 2020, https://www.youtube.com/watch?v=IhBBhLf7PmI.

[30] "Single maintenance and reliever therapy (SMART) of asthma ...." https://thorax.bmj.com/content/65/8/747.

[31] "Glucocorticosteroids enhance replication of respiratory viruses ...." https://www.ncbi.nlm.nih.gov/pubmed/25417801.

shown to suppress coronavirus replication[32] and cytokine production[33]. In one of these papers[33], glucocorticoides exert their antiviral effect by targeting the nonstructural protein (NSP) 15 of the SARS-Cov2 coronavirus, suggesting that the effect of inhaled corticosteroids on Cov2 viral replication may be specific for this virus.

To conclude, we think it is worth to carefully consider the possibility of using a modified version of the SMART Asthma protocol in the early phases of the covid-19 viral disease, where a potent inflammatory response starts mounting in the lung's small bronchi and alveoli.

THE STANDARD ASTHMA TREATMENT IS A POTENT LOCAL IMMUNOSUPPRESSANT

The goal of treating asthma patients with a Chronic schedule is twofold: First, the use of LABA ensures a good control over bronchial smooth muscle activity on a sustained basis, and, second the glucocorticoids decrease the chronic inflammation that builds up within the bronchial wall over the years of asthma progression. But both in vivo and in vitro studies show that this treatment has an effect that goes beyond the individual actions of either of the two drugs separately. This phenomenon has been thoroughly studied, in particular the anti-inflammatory/immunosuppressive effects of this drug combination, which were well known already more than a decade ago[34]. Here we provide evidence that the most widely studied combination, both in vitro and in vivo (budesonide/formoterol) is the best suited as a candidate drug for Covid-19.

A well studied LABA, formoterol is more effective than salmeterol in suppressing neutrophil reactivity. β_2-agonists neither influenced the LTB_4 nor the IL-β secretion in either blood monocytes or alveolar macrophages with the exception of formoterol, which stimulated IL-1β secretion at the highest concentration (10^{-5}mol/L, $p < 0.05$)[35]. However, Formoterol and salmeterol both induced enhancement of IL-6 and IL-8 release and added to the effect of organic dust on A549 cells and primary bronchial epithelial cells (PBEC)[36]. Treatment with inhaled formoterol significantly reduced the number of eosinophils (EG2+) in the submucosa and epithelium, but this was not paralleled by changes in cytokine immunoreactivity. In contrast, treatment with budesonide significantly reduced both the number of eosinophils (EG2+) and immunoreactivity for IL-4 and IL-5 in the submucosa[37]. Additive anti-inflammatory effect of

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[32] "Inhibitory effects of glycopyrronium, formoterol, and ... - NCBI." https://www.ncbi.nlm.nih.gov/pubmed/32094077.
[33] "The inhaled corticosteroid ciclesonide blocks coronavirus ...." https://www.biorxiv.org/content/10.1101/2020.03.11.987016v1.
[34] "Budesonide/formoterol maintenance and reliever therapy: a ...." https://www.ncbi.nlm.nih.gov/pubmed/17605896.
[35] "Effects of beta2-agonists and budesonide on interleukin-1beta ...." https://www.ncbi.nlm.nih.gov/pubmed/9777883.
[36] "Effect of formoterol and salmeterol on IL-6 and IL-8 release in ...." https://www.ncbi.nlm.nih.gov/pubmed/17229563.
[37] "The effects of regular inhaled formoterol and budesonide on ...." https://www.ncbi.nlm.nih.gov/pubmed/12477218.

formoterol and budesonide on human lung fibroblasts[38]. At least some of the therapeutic efficacy of inhaled corticosteroids is mediated through inhibition of NF-kappaB-regulated gene expression, whereas the reduction in airway eosinophilia by long-acting beta(2)-agonists probably operates through alternative pathways[39]. The additive sometimes synergistic effect of combining budesonide and formoterol has been observed in human rhinovirus infection, where budesonide and formoterol can inhibit bronchial epithelial cell inflammatory responses *in vitro* without interfering with viral replication or production of interferons[40]. Furthermore, High concentration of budesonide alone could partially protect the lungs against cadmium exposure induced-acute neutrophilic pulmonary inflammation via the inhibition of MMP-9 activity. The combination with formoterol could enhance the protective effects of budesonide in a mouse model of lung inflammation after cadmium exposure[41]. Finally, iNOS together with proinflammatory cytokines produced by Alveolar Macrophages might play a pivotal role in the pathogenesis of acute lung injury[42] and NO production is inhibited after Budesonide/Formoterol inhalation[43].

To summarize, we believe the real problem to address in the covid-19 disease is the uncontrolled immunological reaction that underlies the progression to SARS. In order to implement an early treatment, there are a few points that we should take into consideration: 1) Ideally the treatment should be started before the whole inflammatory process is in full swing (i.e. during the early phase of flu-like disease), 2) It should be preferentially administered to the very well defined group of patients at risk, and finally, 3) it should be locally administered (i.e. inhaled) to, on the one hand, concentrate the drugs in the disease epicenter (the lung), and, on the other hand, to avoid the side effects associated with the systemic use of immunosuppressive drugs.

TREATMENT OF PATIENTS WITH EARLY SYMPTOMS OF COVID-19 WITH IMMUNOSUPPRESSIVE DOSES OF INHALED BUDESONIDE+FORMOTEROL According to the maintenance and reliever therapy protocol (SMART), the inhaled combination of budesonide+formoterol (B+F) is recommended either as maintenance dosing (low to moderate doses twice a day) or as a relieve dosing (up to eight inhalations per day are supported as safe in this protocol.

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[38] "Additive anti-inflammatory effect of formoterol and budesonide ...." https://www.ncbi.nlm.nih.gov/pubmed/11867828.
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[43] "Inhalation of budesonide/formoterol increases ... - NCBI." 25 Jul. 2012, https://www.ncbi.nlm.nih.gov/pubmed/22824973.

Accordingly, we suggest a starting schedule of a inhaled high dose (F/B:360/8) every 8 hours. The treatment should start in patients at risk, as soon as the first symptoms of a suspected covid-19 disease appear in a epidemiologically positive environment. Symptoms compatible with the early diagnosis of covid-19 could be headache, diarrhea, nausea/vomits, cough, fever, anosmia, chest tightness, astenia, exantema or any combination thereof. As of today, the PCR test for viral presence in upper or lower airway samples is not always available for suspected patients. In addition, there is a sizable percentage of false negativity associated with the test. Therefore, as of today, we shouldn't wait for a positive PCR to begin treating patients at risk of developing SARS (>65, cardiovascular diseases, high blood pressure, diabetes, COPD or active cancer). Finally, both dose and the daily frequency of inhalations could be individually adjusted according to patient's tolerance and treatment efficacy, and it should be continued for at least one month (the minimum period that we deem should be met to consider the patient as "out of risk")

To conclude, we believe that the very early treatment with inhaled immunosuppressants will be the key to prevent the lethal complications of the covid-19 disease and to drastically reduce mortality in these patients.

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