

Renin-Angiotensin System, SARS-CoV-2 and Hypotheses about Adverse Effects Following Vaccination

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Abstract

Coronavirus disease 2019 (COVID-19) affects the lungs but also the cardiovascular system, especially since the spike protein of the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) uses the angiotensin-converting enzyme 2 (ACE2), existing on the membrane of many cell species, as a receptor. This connection has consequences on the renin-angiotensin system, arterial pressure and coagulation. An imbalance of blood pressure control, of coagulation and platelet functions, and of the kinin-kallikrein system may occur in natural disease but is also partly reflected in some possible negative consequences following vaccination. Knowledge of these mechanisms could help to identify the subjects most at risk of complications and to correctly carry out the causality assessment of adverse events.

Keywords: COVID-19; Renin-Angiotensin System; ACE2; Vaccination; Spike Protein; Arterial Pressure; Kinine System; Immunopharmacology; Thrombosis

Introduction

The severity of COVID-19 and other viral respiratory infections is known to be related to many different parameters (age, gender, nutritional status, comorbidities, etc.), as well as the virulence of the strain. People with pre-existing conditions such as diabetes, high blood pressure, chronic obstructive lung disease, heart and kidney disease, metabolic syndrome are at increased risk of developing severe consequences. The cardiovascular system is heavily involved in the pathogenesis of the disease, particularly in its severe forms and in elderly people, so much so that the prevalence of diabetes and arterial hypertension is particularly high in severe cases [1-3]. These issues characterize the COVID-19 disease in a special way compared to other respiratory infectious diseases and one can therefore ask whether similar complications may also affect a person who is inoculated with vaccines made of inactivated virus or inducing the synthesis of its spike proteins. In this work we try to examine the main aspects of the pathophysiology of COVID-19 that could also have implications in pharmacovigilance and in the causality assessment of adverse events following immunization (AEFI). The latter kind of analysis is particularly difficult and provoking in the presence of pathological phenomena that can be attributed to multiple causes, interacting in a

complex way [4-6].

A central aspect of circulatory pathophysiology concerns the renin-angiotensin system (RAS), which controls blood pressure and hydro-electrolytic balances, but also some inflammatory mechanisms and coagulation. Blood pressure is controlled by a very complex set

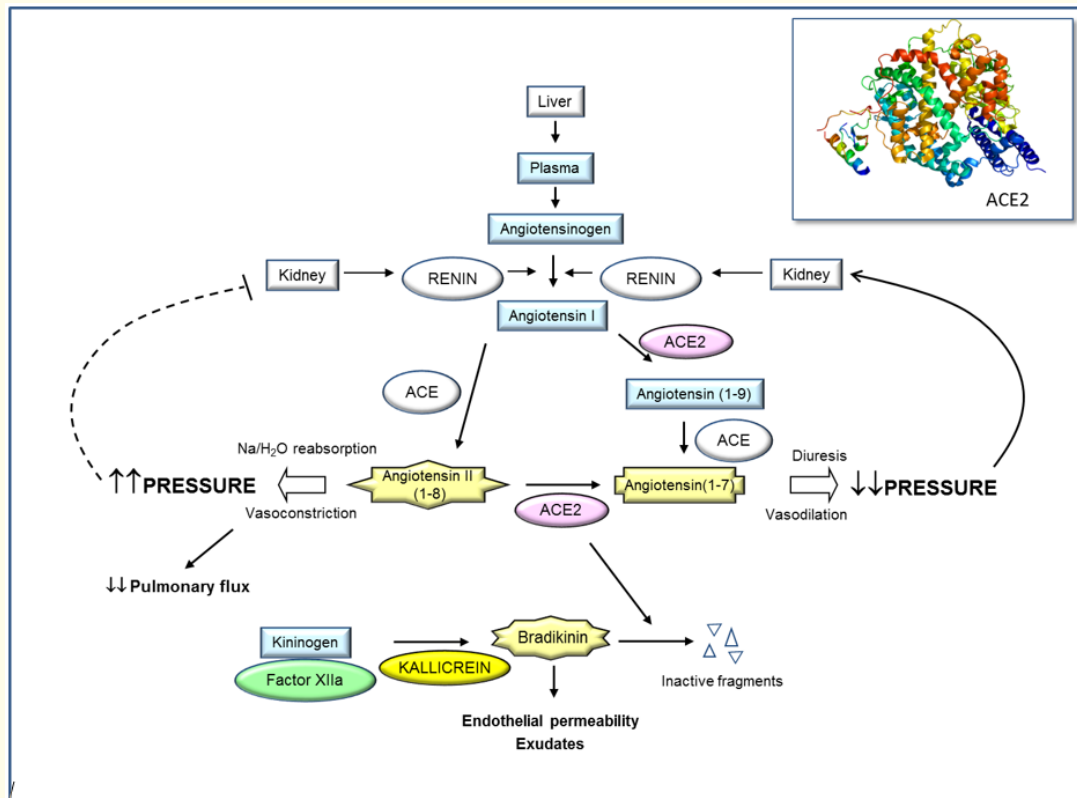


Figure 1: Diagram of RAS and its relationship with kinine system.

of functional centres, receptors and mediator molecules that generate a dynamic equilibrium, endowed with feedback loops and amplification systems (Figure 1).

A key control for this balance is given by the relationship between the various molecules of the angiotensin family and above all by the relationship between angiotensin II (a peptide of 8 amino acids that increases pressure, resultant from angiotensin I) and its derived angiotensin 1-7 formed by the enzymatic action of ACE2 (angiotensin-converting enzyme 2). The two molecular forms, which differ by a single amino acid, have almost antagonistic actions: angiotensin II increases pressure, induces water retention through aldosterone and promotes, in extreme conditions, vasoconstriction and oxidative stress, up to the consequences of cell damage and triggering of inflammatory and coagulant mechanisms through contact activation (intrinsic pathway), increased production of tissue factor and plasminogen-activator inhibitor-1 by endothelial cells [7].

These functions are moderated by the action of ACE2, which transforms angiotensin II into angiotensin 1-7. As a carboxypeptidase, ACE2 cleaves many biological substrates in addition to angiotensin II to control vasodilation and vascular permeability [8]. In fact, ACE2

splits bradykinin into inactive peptides and therefore reduces the actions of the kinin system such as vasodilation, increase in endothelial permeability and exudation. ACE2 is bound to the membrane of many cell types (called “mACE2”), but there is also a soluble form, free in plasma, called “sACE2”.

Understanding the pathophysiology of RAS and its relationships with various immune pathways, including complement, acute inflammation and coagulation, is fundamental for therapeutic and public health strategies. A retrospective observational study in COVID-19 patients found that complement activation and coagulation disorders are risk factors independent of age, sex or smoking history [9]. Furthermore, in a genetic association study these authors identified some coagulation and complement loci as possible genetic markers of susceptibility and clinical outcome.

RAS and COVID-19

The cardiovascular system is often involved early in COVID-19, as evidenced by the release of highly sensitive troponin and natriuretic peptides, particularly in patients showing increased cytokines such as interleukin-6 [7,10]. It is well known that the SARS-CoV-2 virus uses ACE2 as a cellular entry receptor, with the priming by the transmembrane serine protease isoform 2 (TMPRSS2), but it can also bind sACE2 and all this cannot fail to have consequences in the course of COVID-19 disease. ACE2 plays a role in the regulation of systems that could potentially be involved also in the pathogenesis of COVID-19: the kinine-kallikrein system, resulting in acute pulmonary inflammatory oedema; RAS, promoting cardiovascular instability and the coagulation system, leading to thromboembolism [11].

Collectively, the homeostatic regulation of the cardiovascular system controlled by ACE2 would be disturbed in SARS-CoV-2 infection due to the binding of the virus to these cellular receptors [8]. The dysregulation could cause imbalance in both directions: on the one hand, the binding of the virus to the receptors causes its shedding and release in the blood, with increased sACE2 activity (Figure 2). When viruses attach to the cells, a certain amount of mACE2 is cleaved by the metalloproteinase domain 17 (ADAM17) and the transmembrane protease serine 2 (TMPRSS2), but [12] and pass into the plasma, in which case they can decrease angiotensin II leading to hypotension. In patients with severe COVID-19 there is, in fact, an increase in sACE2, but at the same time this does not seem sufficient to stop the systemic

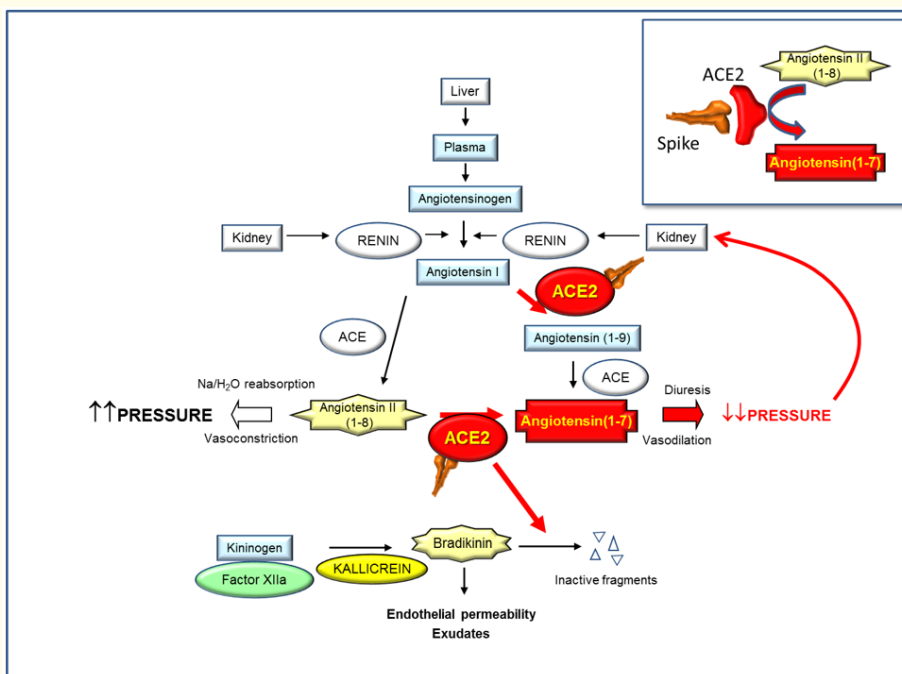


Figure 2: RAS imbalance due to shedding and activation of ACE2.

inflammatory processes triggered in the most clinically severe cases. To the pathophysiological picture described we must add that the trimeric spike protein SARS-CoV-2 increases ACE2 proteolytic activity by 3 - 10 times against a bradykinin analogue [8]. Enhancement of the ACE2 enzyme function, mediated by binding of the spike RBD domain, highlights the potential for SARS-CoV-2 infection to be relevant for COVID-19-associated cardiovascular symptoms.

On the other hand, the internalization of the virus, with sACE2 and mACE2 molecules linked on the spike, leads to a global depletion of enzymatic activity. In the first case, a tendency to decrease blood pressure is to be expected, in the second case a tendency to increase pressure.

The different situations may differ in different patients and alternate in the clinical course, so much so that the therapeutic approach

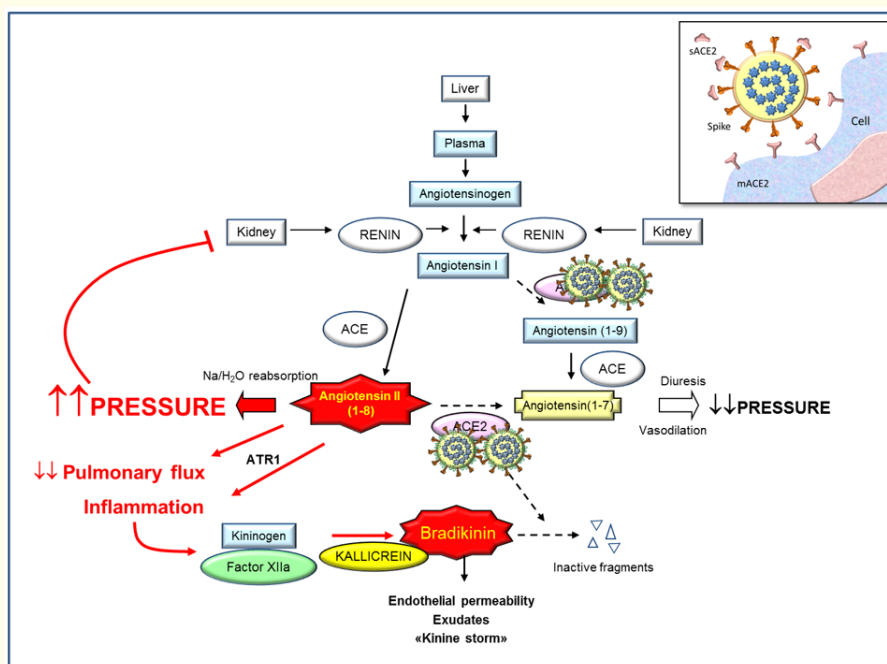


Figure 3: RAS imbalance due to internalization of SARS-CoV-2 with bound ACE2.

based on the control of blood pressure is difficult. The fact that there is no unanimous consensus on the therapeutic or aggravating role of ACE inhibitors is consistent with this view [13,14]. The imbalance of ACE2 activity could be particularly severe in patients with a low baseline level of this receptor [2]. Downregulation of ACE2 can result in lung injury and vasoconstriction because conversion of angiotensin II to angiotensin 1-7 fails [15,16] (Figure 3). Furthermore, increased angiotensin II can have more extensive effects on tissues because it triggers multiple inflammatory responses through the ATR1 receptors, also in the lungs [17].

Another important pathophysiological connection is established between the RAS and the kinin system. When activated factor XII (factor XIIa) is formed, it converts prekallikrein (PK) to kallikrein and kallikrein cleaves high molecular weight kininogen (HK) to release bradykinin. Thus, endothelial cell-dependent activation can be initiated by activation of factor XII or activation of PK-HK [18]. A dysregulation of bradykinin explains several mechanisms of inflammatory diseases and coagulation disorders. Various papers have highlighted the importance of the kinine system in COVID-19, even speaking of a “kinine storm” that would be responsible for uncontrolled phenomena of

vasodilation, vascular permeability and hypotension [19,20]. While entering the cell, the virus carries with it the receptors to which it has attached itself and also the ACE2 molecules eventually bound on its surface to the spikes. If this process involves many viruses and many cells, the balance of the RAS is disrupted with several consequences: increase in blood pressure, decrease of pulmonary flux, inflammatory reactions, oxidative stress, coagulation and thrombosis tendency. Another potential mechanism leading to the same outcome could be the onset of immunity after a few days of infection, which could accelerate the “clearance” of viruses and if they have bound sACE2, this could cause a rapid decrease in circulating ACE2 activity.

It has been shown that elements of the bradykinin, angiotensin and coagulation systems are co-expressed with ACE2 in the alveolar cells of the lung, which could explain how changes in ACE2 promoted by the entry of SARS-CoV-2 cells determine the most severe clinical forms of COVID-19 [21]. Furthermore, an excess of bradykinin can lead to hypokalemia, which is associated with arrhythmia and sudden cardiac death, both of which have been reported in COVID-19 [20] patients. Recent reports confirm that hypokalaemia occurs in severe cases of COVID-19 [22] and that hypokalemia correlates with increased D-dimer, a fibrin degradation product [23]. Bradykinin-mediated inflammation likely precipitates life-threatening respiratory complications in COVID-19 [24].

Recent reviews highlighted a possible beneficial effect of recombinant ACE2 [2,25] and reported clinical improvements after treatments with recombinant ACE2 in SARS-CoV-2, associated with reduction of inflammatory markers [26]. However, sure evidence from meta-analyses are still lacking, probably depending on the fact that each patient has his own dynamics that can change even quickly.

Recent studies suggest that SARS-CoV-2 can also infect endothelial cells by binding to ACE2 and that endothelial dysfunction during COVID-19 can exacerbate these events by inducing deleterious pulmonary and extra pulmonary thrombotic complications in severe COVID-19 [27]. The most common pathological cause of myocardial necrosis in COVID-19-positive patients are micro thrombi, independently of severe coronary artery disease [28].

Possible implications for COVID-19 vaccines

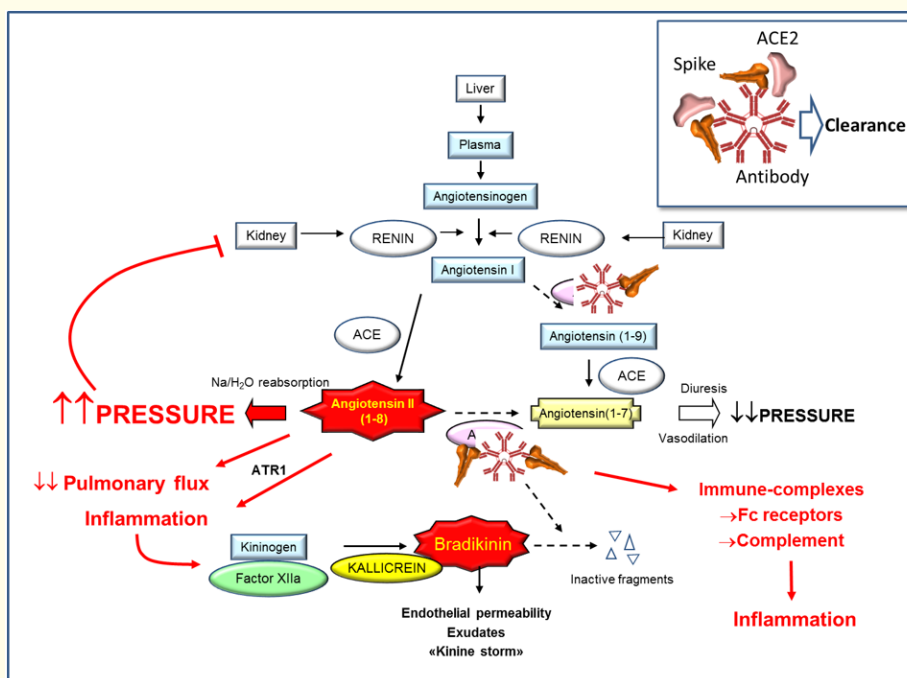


Figure 4: Hypothesis of RAS imbalance due to clearance of the spike protein with bound ACE2 by antibodies elicited by the vaccine.

Based on the above for natural infection, it is possible to formulate the hypothesis that similar mechanisms could complicate the effect of vaccination with inactivated SARS-CoV-2 virus or its isolated spike proteins produced by cells. Most COVID-19 vaccines contain inactivated virus, or Adenovirus vectors for spike gene delivery, or mRNA for spike protein, or virus-free protein S as the final derivative to elicit the immune response within the body. Spike proteins, either alone or expressed by the action of the vector virus, can bind and internalize mACE2 upon binding [16] and this internalization can reduce the overall availability of ACE2. This phenomenon could be heightened when the vaccine-induced antibodies begin to form, because this leads to the formation of immune complexes that intercept antigens (Figure 4).

If the interaction between the spike produced by the vaccine and the endogenous ACE2 would lead to an increase in enzymatic activity, a biphasic trend on cardiovascular function would be expected: in the first days a decrease in angiotensin II and hypotension would be observed, while in the following days, when the anti-spike antibodies begin to form, there could be a reversal trend. In fact, in the hypotensive phase the homeostatic response of the organism would lead to a compensatory increase in angiotensin II production, without specific consequences due to the presence of increased ACE2.

However, as soon as antibodies start to form, these would bind the spike proteins formed in the meantime and could also bind the ACE2 molecules with them. Consequently, the immune complexes would be eliminated by the action of the monocyte-macrophage system and the concentration of ACE2 would end up decreasing rapidly. This would lead to a rapid increase in angiotensin II, no longer opposed by ACE2, with increased pressure and pro-inflammatory consequences related to the activation of ATR1 receptors. It goes without saying that a sudden increase in pressure in a previously hypotensive person could have serious consequences on the heart and vessels, especially if an anatomic (e.g. arterial aneurysm) or pathological (e.g. atherosclerosis) degree of vulnerability is present.

If the spikes caught by the new antibodies are in turn bound to sACE2 molecules, it is to be expected that the net result is a decrease in the ACE2 enzymatic activity. If this is the case, this will raise the level of angiotensin II and an abrupt increase of pressure and activation of the kinin system could occur in some individuals a few days after vaccination and the phenomenon could have more significant consequences in the case of elderly persons or subjects with heart or vascular diseases. It is conceivable that healthy individuals can overcome this transient vaccine-induced increase in angiotensin II as they do in viral infections. However, with an already unbalanced RAS, elderly and comorbid individuals taking the vaccine may experience adverse effects mediated by sudden angiotensin II spikes that must be carefully predicted and monitored.

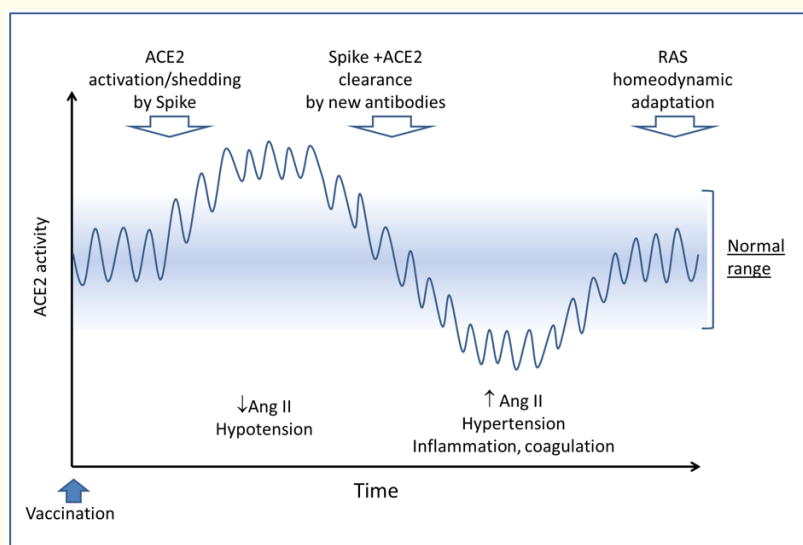


Figure 5: Conceptual model of the changes in the ACE2 plasma activity after mRNA anti-COVID-19 vaccine and its pathophysiological consequences. ↓ Ang II: decrease of angiotensin II; ↑ Ang II: increase of angiotensin II.

It should be noted that, in analogy with natural infection, this response could be preceded by a phase of increase in the enzymatic activity of ACE2, in the event that the interaction between ACE2 and the spike proteins of the vaccine had an activating function as in the case of the wild virus. Consequently, an imbalance in blood pressure could unexpectedly arise in two opposite directions: a) hypotension, in the event that the new spike proteins activate the enzymatic function of ACE2 in plasma or in the cell membrane, or b) rapid rise in blood pressure and systemic inflammation, in the event that the spike proteins bind to sACE2 and are then cleared by newly formed antibodies. A conceptual model of these dynamics is presented in figure 5.

This conceptual model does not exclude that, in some patients with predisposing conditions, thrombotic events may also occur in the first days after vaccination, when spike proteins are produced and in the presence of ACE2 activity. COVID-19 patients have an increase in mean platelet volume and platelet hyperactivity, correlated with a decrease in overall platelet count. This phenomenon has been attributed to the effect of the spike protein which binds to ACE2 present on their membrane through RBD and increases platelet aggregation and the secretion of dense granules induced by various agonists [29]. Furthermore, the spike protein potentiates thrombus formation *in vivo* on hACE2 transgenic mice, while it has no effect on wild-type mice that do not have this receptor. The isolated full-length subunits 1 of the spike proteins (without virion) were injected into laboratory mice and caused endothelial damage to brain micro vessels, mediated by ACE2 binding [30].

In addition, minimal doses of purified spike protein prompt the production of many types of cytokines in the whole blood of patients with COVID-19 but also the production of some cytokines such as RANTES (regulated on activation, expressed normal T cells and secreted), PDGF, Platelet-derived Growth Factor) and IL-9 in the blood of non-COVID people [31]. In view of these evidences, it is possible to hypothesize that cytokine disorders with a pro-inflammatory and pro-thrombotic tendency may also arise due to the action of spike proteins produced following vaccination. Considering platelets, it could also be hypothesized that their activation by the spike protein could lead to an unwanted release of serotonin in bloodstream and this could perhaps explain some neurological symptoms frequently reported in vaccine trials, such as fatigue and gastrointestinal symptoms. Moreover, as shown in figure 4, immune complexes formed by spikes and new antibodies could enhance the reactions of phagocytes through their Fc receptors or trigger C1q and subsequent complement cascade [32].

At the moment it is difficult to predict what might actually happen in different persons because the alterations of these delicate balances depend on many different factors, on the patient's condition, on the phases of the immune system response and on other drugs that impact on the same systems.

This hypothesis could be verified by carefully monitoring the cardiovascular function of vaccinated subjects, starting with the measurement of arterial pressure and ECG, and by assessing blood markers of inflammation, D-dimer and, above all, ACE2 quantity (by ELISA) or ACE2 activity (fluorimetric assay) in plasma. In particular, the angiotensin 1-7/angiotensin II ratio is particularly useful since it predicts improved survival in patients with heart failure [33]. Platelet responsiveness to agonists can be assessed by aggregometry or adhesion assay [34] and by plasma markers such as beta-thromboglobulin. Knowledge of models involving the role of RAS in the pathogenesis of COVID-19 and, perhaps, of adverse reactions to vaccines could lead to greater attention to the dynamics of blood pressure and plasma markers of the phenomena described. Other authors have highlighted how important it is to be aware that the spike protein produced by vaccines can directly affect host cells via receptor-mediated interaction [35].

Conclusion

It is important to keep these possibilities in mind in the clinical care of COVID-19 patients, but also of vaccinated persons presenting some symptoms of adverse effects. In fact, in the event that the diagnostic suspicion leads to the execution of appropriate laboratory tests that highlight disturbances of circulation or haemostasis/coagulation, it would be possible to intervene earlier with specific therapies. In

perspective, it would also be possible to investigate, for preventive purposes, suitable drugs or supplements with the ability to interfere with the link between spike and ACE2.

Finally, a related topic concerns the assessment of causal link of any unwanted adverse event following immunization (AEFI), involving cardiovascular system such as existence of thrombi or micro thrombi, cardiac arrest, stroke, haemorrhage, and shock. A sudden increase in pressure could also be fatal in people with brain aneurysms. When making a causality assessment of serious AEFI, WHO guidelines ask to consider all other possible causes that may have led to the event [36]. The guidelines then explain that a detailed medical history, clinical examinations and investigations, including laboratory tests on the patient, can help identify other conditions such as other diseases and congenital anomalies that may have caused the event. This procedure would appear logical and valid only if the “other condition” was independent of a possible interaction with the vaccine. This is why the biological plausibility of a causal (or concausal) relationship is so important [4,5]. The fact that vaccines made with spike proteins may have a dysregulatory influence on RAS means that, in case of people affected by diseases of cardiovascular nature, or by increased susceptibility to thrombotic events, an interaction between vaccine and underlying condition is more plausible than in the case of other vaccines. Although the benefits of vaccination in people who could be severely affected by COVID-19 are indisputable, ignoring the problem of possible vaccine interactions with RAS could lead to a systematic underestimation of the risk posed by these new vaccines, particularly in susceptible and frail people.

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Competing Interests

The author is free of competing interests. In the past three years he has been involved in some consultancy, conferences and publications in which he has supported the freedom of vaccination. The author has always carried out these activities for free and in none of these activities he has received any payment, direct or indirect. In particular, the author advised the Veneto Region on the occasion of the appeal to the Italian Constitutional Court against Law 119/2017 and wrote a document on invitation to the Senate Hygiene and Health Commission advocating the cause of vaccination freedom. The proceeds of PB's book “Vaccines yes, Obligations no” (Libreria Cortina Editions, Verona, 2017) is entirely donated to a charity association. Since October 2020, the author has been scientific consultant from Vanda Omeopatici s.r.l. (Frascati, Roma), a company that produces food supplements.

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