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Covid-19, induced activation of hemostasis, and immune reactions: Can an auto-immune reaction contribute to the delayed severe complications observed in some patients?

Jean Amiral[a,*], Anne Marie Vissac[b], Jerard Seghatchian[c,*]

[a] SH/Scientific-Hemostasis, Scientific Director and Consultant in Hemostasis and Thrombosis Diagnostics, Franconville, France
[b] Hyphen BioMed, Neuville sur Oise, France
[c] International Consultancy in Strategic Safety Improvements of Blood-Derived Bioproducts and Suppliers Quality Audit / Inspection, London, UK

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ABSTRACT

Covid-19 is characterized by weak symptoms in most affected patients whilst severe clinical complications, with frequent fatal issues, occur in others. Disease severity is associated with age and comorbidities. Understanding of viral infectious mechanisms, and antibody immune response, can help to better control disease progression. SARS-CoV-2 has a major impact on the Renin Angiotensin Aldosterone System (RAAS), through its binding to the membrane cellular glycoprotein, Angiotensin Converting Enzyme-2 (ACE-2), then infecting cells for replication. This report hypothesizes the possible implication of an autoimmune response, induced by generation of allo- or autoantibodies to ACE-2, or to its complexes with viral spike protein. This could contribute to some delayed severe complications occurring in affected patients. We also propose a strategy for investigating this eventuality.

1. Introduction

Fast and extended knowledge is becoming available on the new viral disease that emerged in December 2019, causing enormous challenges at international levels. The pathology of this obscure virus, named Severe Acute Respiratory Syndrome - Coronavirus-2 (SARS-CoV-2), is characterized by many patients remaining asymptomatic or with only benign symptoms, but this disease becomes life-threatening in some patients and requires hospitalization in intensive care units or resuscitation [1,2], and is often with a fatal outcome, yet to be fully established. Activation of the hemostasis system has been observed in many patients with severe complications, with occurrence of disseminated intravascular coagulopathy (DIC) or pulmonary embolism (PE), and multiorgan failure [3]. DDimer is frequently elevated, and the disease prognosis worsens with its increasing concentration [2]. Sepsis can also be present in some cases. Anticoagulant therapy, especially with LMWH, can improve the disease evolution and reduce the lethality incidence [4]. In addition, many patients with severe complications face a sudden worsening, starting 7 to > 14 days after the preliminary symptoms, although the immune response is effective with the presence of IgG and/or IgMs and is expected to fight the disease by controlling its pathological evolution [5,6]. This worsening is associated with an exacerbated immunological activity, a strong inflammatory response, and a cytokine storm [7]. New therapeutic approaches rely on controlling the pro-inflammatory cytokines, mainly IL-6, IL-10, and TNF-α. Lastly, there is a strong association of disease severity with age and presence of comorbidities, mainly hypertension, diabetes, obesity, chronic obstructive pulmonary disease and cardiovascular diseases (CVD). However, complications can also occur in younger persons without any known risk factors [1,2].

2. Disease development

Understanding how SARS-Cov-2 infects patients, how disease develops, and why some patients have this delayed exacerbated immune response, is of major importance for better disease management and control, and for implementing promising therapeutic strategies. Association with age and existing pathologies is now well-documented, but the causes explaining the disease course in patients with severe or lethal complications are not completely understood. Viral load (increased tendency with age), infection development in affected patients, age, presence of comorbidities, and extent of tissue injuries contribute to this evolution [8]. However, the paradoxal delayed cytokine storm associated with the amplified immune reaction deserves attention. Analyzing the disease development and infection mechanisms can help to elaborate an hypothesis to understand this complication.

* Corresponding authors.
E-mail addresses: jean.amiral@scientific-hemostasis.com (J. Amiral), jseghatchian@btopenworld.com (J. Seghatchian).

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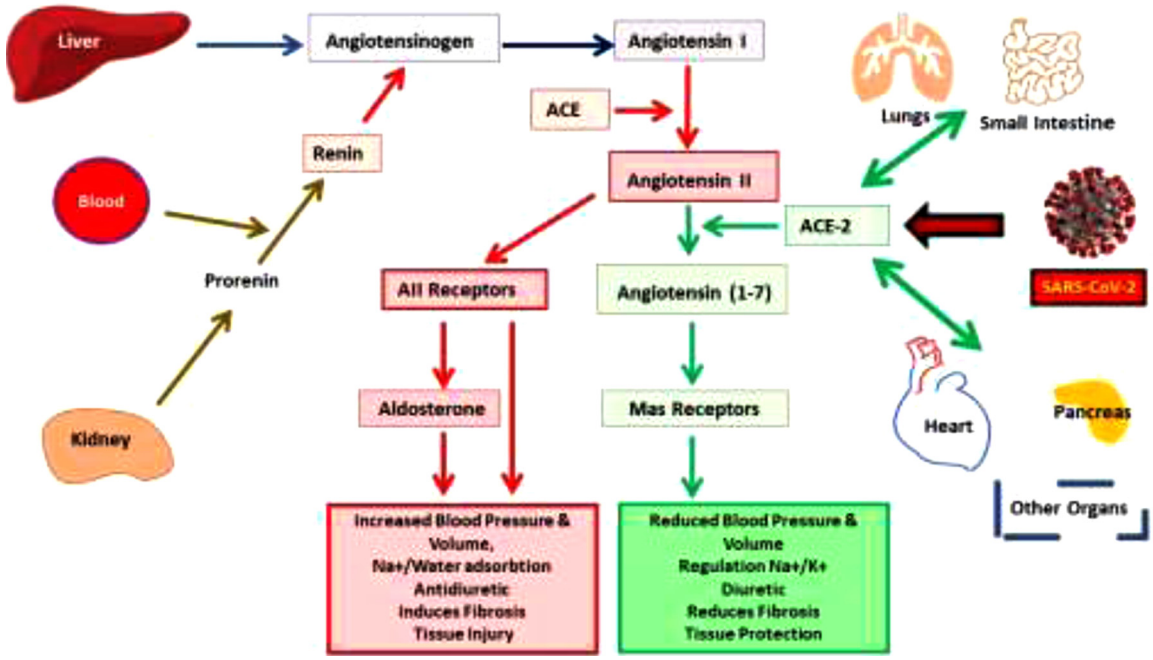

Fig. 1. ACE-2 regulates blood pressure and volume, inflammation, diuresis, N+/K + balance, and protects organs (mainly lung, heart and liver) from fibrosis. It is a key cell transmembrane glycoprotein which prevents hypertension and protects from tissue injury by opposing the activation of the Renin Angiotensin Aldosterone System (RAAS), and the deleterious effects of Angiotensin II (AII) and Aldosterone. Through its binding to ACE2, SARS-CoV-2 infects cells, such as lung alveolar epithelial cells (where ACE-2 is highly expressed), and interferes in the RAAS, by impacting ACE-2 beneficial action. We hypothesize that binding of SARS-CoV-2 S Protein to ACE-2 could induce generation of allo- or/and autoantibodies.

SARS-CoV-2 enters human cells through Angiotensin Converting Enzyme-2 (ACE-2), a membrane surface protein [9], which is a major regulator of blood pressure and of the Renin Angiotensin Aldosterone System (RAAS). This enzyme has an opposite effect to that of Angiotensin I Converting Enzyme (ACE), which generates Angiotensin II (AII: a nonapeptide) by cleaving angiotensin I (a decapeptide). Angiotensin II has many pleiotropic effects by acting on its receptors, leading to aldosterone production and, finally, inducing vasoconstriction, increased blood pressure and volume (through Na + and water reabsorption), fibrosis and tissue injury. ACE-2 opposes and regulates these effects, by cleaving AII to Angiotensin 1–7 [A (1–7)], which acts through its binding to Mas Receptor [10–13]. This induces vasodilatation, reduces blood pressure, regulates blood volume, and protects from tissue damages and fibrosis in liver and lungs [13]. RAAS function and implication in SARS-CoV-2 are summarized in Fig. 1. ACE-2 is highly distributed in lungs (epithelial alveolar cells), but also in some other tissues such as the small intestine, pancreas, heart, liver and brain. Both ACE and ACE-2 soluble forms can be present in blood circulation at low concentrations (< 10 ng/mL for ACE-2 in normals). The ACE/ACE-2 ratio is then of essence for maintaining the right balance of the RAAS, and it is impaired in patients with hypertension, obesity, cardiovascular diseases (CVD), and diabetes for example. Interestingly, the presence of these pathologies highly increases the risk for severe complications in Covid-19 patients [1,2]. Noteworthy, a dysfunction of RAAS can favor development of type 2 diabetes. Through its binding to ACE-2, SARS-CoV-2 interferes in the RAAS, and can impact its function. In addition to destruction of infected cells, this binding probably impacts RAAS balance and contributes to the disease course.

3. Role of ACE-2 in viral infection

The S Protein of SRAS Cov-2 (S Protein) presents a sequence, named Receptor Binding Domain (RBD), which has a high affinity for ACE-2. SARS-CoV-2 is first primed by another membrane protein, TMPRSS2 (a membrane serine protease encoded by the TMPRSS2 gene), which cleaves S Protein and favors its binding to ACE-2. This S Protein is then separated in 2 subunits, S1 bound to ACE-2, and S2, which helps the viral envelope to merge with the infected cell membrane, permitting the viral RNA entrance and replication [9].

The binding of viral S Protein to ACE-2 is a critical phenomenon for infection and disease progression. Interestingly, ACE-2, which is a transmembrane cell surface expressed enzyme, can form a dimer with a high affinity for S1 trimers, which increases binding of viruses and their cell entry [9]. In addition, ACE-2 is present in a soluble form in the blood. In infected patients, it could circulate bound to SARS-CoV-2 or to the S1 subunit of viral S Protein, and virus has been detected in blood from some patients. As ACE-2 plays a major role in patients with hypertension, CVD, obesity, or type 2 diabetes, by opposing the harmful effects of the RAAS, mainly through the ACE-AI [1–7] axis and Mas Receptor, the ACE/ACE-2 ratio could be a good indicator of disease progression [10–13]. Lastly, there are polymorphisms of ACE and ACE-2 which increase the risk to develop these diseases. From these considerations, the role of ACE-2 (and impairment of its function when complexed with S1) can be important for Covid-19 clinical complications, especially those associated with comorbidities, and not only because it is the entrance receptor for SARS-CoV-2.

4. Activation of hemostasis

Activation of hemostasis is a major event occurring in Covid-19 patients with severe complications and a need for intensive care or resuscitation. From the Yang report, DIC develops in most patients with a fatal outcome, whilst it remains rare in those who survive [1]. The extensive tissue damage, especially in the lung, and the multiorgan failure can be causes for this extensive blood coagulation activation. Use of an anticoagulant therapy, as, for example with low molecular weight heparin, improves the patients' outcome and reduces lethality [3,4]. Many coagulation assays are impacted with prolonged prothrombine time (PT) or activated partial thromboplastin time (APTT), reduced antithrombin (AT), elevated DDimer, thrombocytopenia, associated with leucopenia, and low lymphocytes [14]. Mechanisms that cause this direct blood activation can be multiple, and involve the

damaged tissues' debris or released procoagulants, or exposure of activating surfaces to blood circulation in damaged organs. The amplified immune response itself, with inflammation and cytokines release and the cross talks with other response modifiers, can contribute to hypercoagulability. The entry mode for SARS-CoV-2 in infected cells leads one to suspect the involvement of all side effects of a RAAS dysfunction.

5. Immune response

For virus cell entry, a strong complex is first formed between the viral S protein, primed and cleaved by TMPRSS2, and ACE-2, at the cell surface which exposes this protein [9]. In addition, ACE-2 is also present in the blood at low concentration where it could be complexed with S1 or with the virus itself (detected in the blood of some patients). IgM and IgG antibodies to the various viral proteins, including S Protein RBD and nucleoprotein can be detected in patients several days after the onset of symptoms [5,6,15]. With the present data available, this serological positivity sometimes develops concomitantly with a virus load detectable by PCR, or some days later. One to 2 weeks after the onset of symptoms, patients have IgMs and/or IgGs, with little delay between generation of IgGs and IgMs. IgMs tend to decrease after 2 weeks, whilst IgGs continue increasing or stabilize [5,6,15]. The presence of these antibodies is expected to control, reverse and stop infection progression. Surprisingly, patients with the highest antibody concentration were the most critical [5].

6. Hypothesis on the induction of an auto-immune complication

The formation of a strong complex between ACE-2, a self-component, and viral S1 (or S Protein), constitutes the basic context for developing allo-antibodies and generating a delayed autoimmune response, with antibodies first targeted to viral antigens, but which can extend to the associated self-component through epitope spreading. Then, antibodies to ACE-2 could develop, or eventually to another cell protein close to or complexed with ACE-2. We then hypothesize that an allo-immune response can follow the initial immune reaction to the viral infection, and that epitope spreading can induce antibodies to ACE-2, or to proteins with which it is complexed, thus targeting the immune system to cells exposing ACE-2 (abundant in lungs and some other organs). This delayed autoimmune response can contribute to the cytokine storm and generate tissue injury and destruction. This can activate hemostasis, beyond acute hypercoagulability, stimulate tissue injury, and totally impair the body's regulation of hypertension and physiological defense mechanisms such as existing pathways of haemostasis/ thrombosis and inflammatory processes. We do not know, at this stage, if interaction between ACE-2 and S Protein, or its S1 subunit or with another viral or cell protein, can induce structural modifications of ACE-2 and expose cryptic or neo-epitopes. This possibility needs to be considered as it could yet stimulate the immune reaction and generate autoantibodies.

7. Strategy for investigating allo/autoantibodies

We are convinced that this hypothesis could be easily explored by testing allo-antibodies to ACE-2, or to its complexes with SARS-CoV-2 S Protein or its cleaved subunits, S1, and eventually associated with S2 or nucleoprotein. ACE-2 is now available in the recombinant form, even if still very expensive, and recombinant SARS-CoV-2 proteins or peptide sequences, including S Protein and S1, are available from various suppliers. A capture ELISA, designed by coating recombinant ACE-2, or its complexes with S-Protein or its S1 subunit, or eventually the viral nucleoprotein, onto the plate could be designed for capturing possible antibodies present in Covid-19 patients, especially those with delayed severe complications. Binding of antibody to these components, in the presence or absence of ACE-2 must be compared. This approach can allow the measurement amount of the kinetic course of these antibodies during the pathological evolution, and eventually in patients following their recovery. If present, these antibodies are expected to be alloantibodies, as induced by the association of a viral protein with a body's self-component. However, if immuno stimulation is first induced by neo-epitopes exposed on ACE-2 when complexed with S Protein or S1, the response could be autoimmune. Laboratory investigations can clarify these considerations.

8. Conclusions

This concise manuscript is intended to spotlight some of the factors involved in the COVID-19 process focusing on our current efforts to shine some light by proving laboratory evidence to support our working hypothesis. People respond differently to COVID-19 some without major clinical problems, others, especially older population or with comorbidities, develop severe or fatal complications [1,2]. This virus does not recognize age or rank and where the individual variability appears to matter is how people respond to viral infectivity: the good responders overcome the virus by developing high affinity antibodies, whereas the poor responders are doomed to severe health Issues. It is nevertheless more than intriguing to note that the highest antibody concentrations are noted in the most severe patients, and this does not always correspond to the viral load detected by PCR [5,15]. We emphasize that, in addition to the viral deleterious effects of infection in lungs and organs, and stimulation of the immune system, a possible rebound trigger could be induced by an autoimmune response, especially when complications are delayed from the initial symptoms. The mechanisms of viral infection and entry into cells through the ACE-2 receptor create a unique context for development of allo- or autoantibodies, which can induce their harmful effect on the body's cells and tissues exposing ACE-2. This mode of action is similar to that of 2002 SARS-CoV [16], but SARS-CoV-2 has a higher affinity for ACE2. Therefore COVID-19-induced activation of hemostasis and immune reactions remains, for a while, in the spotlight to be fully proven without any doubt. Autoimmune complications, although rare, have been observed in many infectious diseases, as for example anti-coagulation Protein S in varicella, anti-prothrombine, anti-Platelet Factor 4, anti-FXIII, etc. Autoantibodies are investigated only because of the associated clinical syndromes induced (as Lupus Anticoagulant, thrombosis, bruises). Understanding the various routes of pathology development can help to design the most efficient diagnostic tools and therapeutic approaches as recently reviewed [9,12]. Recombinant ACE2 is being developed for treating the acute respiratory distress syndrome. Other strategies in the spirit of unities in these massive risky periods, in the real crisis time, requiring prompt action, are warranted. Specific recommendations for a standardized preparation, and an optimal use of convalescent plasma at a global level are greatly needed in COVID 19 patients. This will be helpful in designing future clinical trials in this area of investigation.

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