Asian Journal of Biomedical and Pharmaceutical Sciences *ISSN 2249-622X*

SARS-Cov-2 infection as an ideal context for inducing autoantibodies to ACE2: Autoimmune clinical complications as possible causes for disease presentation, severity, and duration.

Jean Amiral*

Hemostasis and Thrombosis Diagnostics, Franconville, France

Abstract

A subset of patients affected by the recent Covid-19 pandemic develop critical or fatal clinical outcomes, which causes are not always fully understood. A delayed exaggerated immune and inflammatory response develops, inducing severe clinical complications which are associated with induced hemostasis activation, thrombosis, disseminated intra vascular coagulation, vasculitis, and multiorgan failure. This SARS-Cov-2 infection induces a dysregulation of the Renin Angiotensin Aldosterone system by shedding the protective cell surface receptor ACE2, and this can be a major contributor to disease evolution. In addition, the viral cell entry mechanism requires the formation of an intimate and high affinity binding complex between virus spike protein RBD and ACE2. An alloimmune response could then develop with generation of autoantibodies to ACE2, which could be responsible for an acute autoimmune response, and for the deleterious exacerbated inflammatory reaction. This report discusses the rationale for that hypothesis and describes how it could impact the disease course.

Keywords: Covid-19, SARS-Cov-2, Cell surface receptor, Antibodies.

Accepted on June19, 2020

Introduction

Covid-19, induced by SARS Cov-2 infection, has unexpected clinical presentations, and evolves to critical illness in some patients, with often fatal outcomes, mainly in elderly people or patients presenting with comorbidities [1-3]. This context is associated with hemostasis activation [4-6], a strong inflammatory and an exacerbated immune response, along with a cytokine storm: this exaggerated reaction induces the severe disease complications in affected patients [5,7]. There is not a full understanding yet on what could provoke this unexpected evolution. Surprisingly, complications can occur in a delayed manner from the original disease symptoms when the immune response is already present and expected to efficiently fight the infection [8]. In addition, the viral load is frequently low or undetectable when this severe evolution occurs. In a few subsets of patients Covid-19 presents with a biphasic evolution: clinical complications, possibly critical, develop 4 to more than 8 weeks after the onset of the first symptoms, following a state of apparent healing. This rebound effect suggests that non-identified pathological mechanisms, consequent and additional to the viral infection, could be involved in the disease course. Recent studies have reported that the antibody serological response tends to be higher or much higher in critically ill patients. The intensity of the immune response, with high levels of neutralizing antibodies [9,10], looks to be inversely correlated to the disease severity. The consequences of the immune response are then paradoxal, as they contribute to worsen the disease evolution, and not to heal the patient. This immune response is associated with an exacerbated inflammatory reaction, and the reported cytokine storm, involving especially IL-1, IL-6, IL-10 and TNF-α, which can reach very important levels [7,11]. The analysis of the virus infection mechanisms, through its binding to Angiotensin Converting Enzyme 2 (ACE2), of the disease course and of laboratory data, lead us to hypothesize that an autoimmune response could occur in some patients, and this can contribute to the severe disease outcome [12].

SARS-Coc-2 Cell Infection Strategy

Understanding of viral attack mechanisms has shown that the entry receptor for SARS Cov-2 cell infection is through its binding to Angiotensin Converting Enzyme 2 (ACE2) [13-15]. This cell surface receptor is a major component of the Renin Angiotensin Aldosterone System (RAAS), and it balances the effects of Angiotensin Converting Enzyme (ACE), the major actor for provoking the increase of blood volume, hypertension, and fibrosis [16-19]. A right equilibrium is then requested between ACE and ACE2 for keeping healthy the physiological state. When SARS-Cov-2 infects cells, it induces shedding of ACE2, as it was demonstrated for the 2002 SARS-Cov, with whom the same cell entry receptor is shared [13,14]. Practically, SARS-Cov-2 has an external spike protein (with 2 subunits: S1 and S2), containing a Receptor Binding Domain (RBD) sequence, which interacts strongly with ACE2, and this

highly reduces the cell surface density of this receptor. ACE2 function in RAAS is then impaired and cannot counterbalance ACE, which activity becomes in excess [13,20,21]. Therefore, in addition to the infected cell destruction, many pathological consequences of SARS-Cov-2 infection, which induces Covid-19, can be related to its interference in the RAAS, especially by altering the ACE2 protective function. Furthermore, SARS-Cov-2 strongly binds to ACE2, a cell surface receptor ubiquitously distributed among many organs, with a much higher affinity than SARS-Cov [13,14]. The adaptive immune response, which should be only targeted to viruses, could be deviated as the consequence of epitope spreading, and extended to the self-component ACE2 itself, when it is intimately complexed with RBD. This process could lead to generation of autoantibodies to ACE2, when this protein is complexed with SARS-Cov-2. The autoimmune response could nevertheless be targeted to any other self-component involved in the viral attack complex formed of RBD, ACE2 and other cell proteins. We recently hypothesized this possible complication, which can correspond to an acute autoimmune pathology, consistent with the exacerbated inflammatory and prothrombotic secondary clinical evolution [12].

The Renin Angiotensin Aldosterone System in SARS-Cov-2 Infection

ACE2 plays a major role for preventing from hypertension and regulating the intracellular Na+/K+ balance; it reduces blood volume and protects from fibrosis, whilst ACE has opposite effects and can produce microvascular lesions and fibrosis [16,17,19,22].

Table 1.*The balanced actions of the Renin-Angiotensin-Aldosterone System (RAAS) through the 2 major cell surface receptors which regulate its function: ACE and ACE2.*

ACE-Angiotensin II-Angiotensin II Receptors Axis	ACE2-Agiotensin (1-7)-Mas Receptor Axis
Increased Blood Pressure and Volume,	Reduced Blood Pressure and Volume
Na+/Water adsorption	Regulation Na+/K+
Antidiuretic	Diuretic
Induces Fibrosis	Reduces Fibrosis
Tissue Injury	Tissue Protection

ACE cleaves the decapeptide angiotensin, released from angiotensinogen by renin, and generates angiotensin II (AII), an octapeptide which activates its 2 corresponding cell receptors, which induces release of aldosterone among other activities [18]. ACE and ACE2 are both transmembrane receptors, and show some sequence identity and similarity, but they have opposite biological effects [19,23]. ACE2 converts AII to angiotensin 1-7 [A-(1-7)] and has beneficial and tissue protective effects through inactivation of AII and activation of the MAS receptor. The extracellular domains of ACE and ACE2 can be cleaved and are present in blood circulation, with a remaining activity. Table 1 shows the balanced effects of RAAS through the ACE-AII axis as opposed to those of the ACE2-A-(1-7)-MAS receptor axis.

ACE2 is involved in regulating the pathogenesis of respiratory distress syndrome, opposes liver and lung fibrosis, controls hypertension, as well as type II diabetes and obesity [16,18,19,22]. When its activity is decreased, progression of these pathologies occurs. A dysfunction of the RAAS system can then favor development of obesity and type II diabetes [24,25]. Interestingly, the here above pathologies are the major comorbidities reported as risk clinical situations for Covid-19 patients, those who develop the most critical complications [1,3]. Binding of SARS Cov-2 to ACE2 and decrease of this cell surface receptor density interfere with the RAAS and impact the protective functions of ACE2 [26]. Interestingly, recombinant ACE2 is tested as a candidate drug for treating patients with acute respiratory distress syndrome and evaluated for severe Covid-19 [27,28]. Therefore, the viral interference with RAAS could be a major mechanism for disease pathogenicity and could explain the increased incidence of complications in patients with diabetes type II, obesity, hypertension, and cardiovascular or respiratory diseases.

SARS-Cov-2 Infection and Body's Defense

SARS Cov-2 binds to ACE2 with high affinity, through the S1 subunit RBD. It has recently been shown that complexes between 2 viral S spike protein trimers with an ACE2 dimer are formed. Binding occurs through the S1 subunit RBD [13,29]. Subsequently, the S2 subunit is cleaved by TMPRSS2 (a membrane serine esterase encoded by TMPRSS2 gene) and contributes to the cell virus infection and replication. The immune response to the viral infection is first targeted to the spike protein, including the S1 and S2 subunits and the RBD, and to the nucleocapsid protein. Immunoassays for detecting the presence of antibodies generated in Covid-19 infection are currently designed with the spike protein S1 subunit, or better the RBD peptide, and with the nucleocapsid protein as capture antigens [9]. Specific antibodies are present 2 to 3 weeks after the onset of disease symptoms and become rapidly neutralizing. Whether the ACE2 cell surface density or its distribution among tissues and organs is a key factor for infection is still debated. However, we can consider that only few ACE2 receptors are required for virus entry into cells, and only few copies are necessary for viral replication. In body, ACE2 is widely distributed, and is present on cell lung epithelial type II cells, but also on many other cells from heart, pancreas, small intestine, brain, kidney, bone marrow, vascular endothelium, oral mucosa and lymphoid tissues, this list being not restrictive [15]. Tissue expression of ACE2 is especially relevant in lungs and the small intestine. All these tissues and organs are then potential targets for viral infection, although there is no evidence that all are directly and effectively attacked by virus but they are concerned by the exacerbated immune and inflammatory reaction with the cytokine storm and macrophage activation [7,30]. Multiorgan failure can occur in severely ill patients. Delayed clinical complications, which

concern various organs, such as heart, liver, kidney, and also endothelium/vasculitis, neurological disorders, or gastrointestinal symptoms are frequently reported in addition to dyspnea and to the acute respiratory syndrome. The frequent presence of blood activation and thrombosis can be the consequence of viral cell destruction, which releases procoagulant and pro-inflammatory products into blood circulation, but also of the strong inflammatory response, with hyperfibrinogenemia, vasoconstriction, and organs ' dysfunction [5]. The clinical course of this disease, with this exaggerated and late immune response, suggests that a pathological trigger can still be present, even when the viral load is low or undetectable, while the antibody response is high and expected to efficiently fight infection. Our hypothesis on the involvement of an immune response, which can be deviated and targeted to some self-components such as ACE2, or proteins involved in the virus entry mechanisms, is supported by these observations: at least in some patients, and acute autoimmune event could occur [12].

Hypothesis on Pathogenicity of SARS-Cov-2 Induced Autoantibodies

RBD binds to ACE2 with high affinity, which could induce the immune response turning to an alloimmune one and be targeted to ACE2 itself. In presence of a persistent immune stimulation, the immune system is deceived and progressively targets all components of the viral pathological molecular complex containing non-self and self-components. The immune response becomes then allo-immune and could extend the illness damages to the organs with cells exposing ACE2. Possibly, the immune response could become autoimmune in some survivor patients when the viral material is eliminated. This mechanism results from the epitope spreading and has already been reported in some pathological complications [31]. For example, when bovine thrombin was used for fibrin glue, some patients developed first antibodies to bovine thrombin, rapidly extending to human thrombin [32]. The discriminant immune response was first targeted to only epitopes present on bovine thrombin, but it lost rapidly its specificity by targeting the whole bovine thrombin molecule. Generated antibodies then cross-reacted with human thrombin, provoking an acute disseminated thrombotic complication, often fatal [32]. The proposed mechanism also resembles the development of Heparin Induced Thrombocytopenia (HIT), when stoichiometric heparin and platelet factor 4 complexes induce generation of antibodies responsible for platelet activation and destruction, and thrombosis [33]. Many other examples could be cited. Especially, allo- or auto-antibodies can be generated during some viral infections and provoke severe clinical complications. In varicella, some rare cases of autoantibodies to coagulation protein S have been reported, with occurrence of thrombosis in the macro- or micro-circulation [34]. Idiopathic Thrombocytopenic Purpura (ITP) can be the consequence of platelet viral infection by Epstein Bar or Cytomegalovirus (CMV), and the immune response can extend to the generation of autoantibodies to platelet surface glycoproteins. Autoimmune disease can become chronic in presence of induced platelet autoantibodies [35]. Many other examples can be found in literature.

Potential Laboratory Investigations

Complementary laboratory investigations to those currently practiced for Covid-19 could be very informative and could possibly contribute to a better control of the disease course and of its harmful consequences. These investigations should focus on the viral infection impact on RAAS, which has a key function in body defense regulation and control. SARS-Cov-2 impairs the ACE/ACE2 balance, reducing the beneficial and protective role of ACE2, and enhancing the harmful uncontrolled one of ACE and AII. This balance is however difficult to evaluate, as most of ACE and ACE2 activities occur on the cell surface, although soluble forms of ACE and ACE2 can be measured in plasma [18]. There is no evidence that these plasma ACE or ACE2 activities reflect the corresponding cell surface activities. The soluble forms are cleaved from the cell surface exposed ACE or ACE2 receptors, and they could be mainly markers of abnormal activities. For example, plasma soluble ACE2 has been reported to be elevated in some diseases.

Exploring the various markers and activities of the RAAS could provide a useful information on the pathological state and risk of Covid-19 patients. Therefore, measurement of AII, A-(1-7), ACE and ACE2 activities in plasma should be considered in this disease. In addition, measuring the ACE2/ACE activity ratio could be highly informative. In our hypothesis, the possible presence of antibodies to ACE2, or to its complexes with the S1 viral protein, or to any other complex involving a self-component combined with viral material, should be explored. Testing for those antibodies is envisageable by Elisa. Recombinant human ACE2 and viral proteins (Spike protein, S1, RBD, S2) are currently available from various suppliers, although at a very high cost. Conventional capture immunoassay principles, designed for the testing of autoantibodies with these recombinant proteins, could be used. In practice, the ACE2 recombinant protein, alone or in combination with S1 or RBD, could be coated onto an Elisa plate and used for capturing possible antibodies, then detected with a labelled conjugate specific for IgM, IgG or IgA isotypes. If present, monitoring these antibody kinetics during the disease course could contribute to a better knowledge and management of this infectious pathology.

Discussion

The specificity of SARS-Cov-2 infection mechanism, through its strong binding to ACE2, matches with the clinical contexts which can induce an allo-immunization, and an acute autoimmune response. ACE2, directly or through the exposure of cryptic epitopes or denatured structures when bound to viral proteins, could be the target for this autoimmune reactivity. This hypothesis needs to be demonstrated by developing the appropriate assays and testing patients, especially those with a delayed disease rebound occurring several weeks after the onset of the first symptoms. Some delayed clinical

complications, such as the strong inflammatory and cytokine response, the chemoattraction of monocytes and macrophages to multiple pathological sites, the disease targeting various organs and microcirculation, the disseminated pathogenic effects, the development of vasculitis in some patients, are clinical observations consistent with an autoimmune (maybe better alloimmune) disease evolution, which can reverse when patients recover, but which could be fatal in some of them.

Among the SARS-Cov-2 deleterious effects, its impact on the RAAS could be of high relevance, especially because comorbidities are those associated with a dysfunction of that system, and because the virus targets ACE2, the protective side of this RAAS, which prevents from hypertension, fibrosis and tissue damage.

Obviously, the pathogenic mechanisms proposed in this review need to be investigated before any confirmation. However, as demonstrated in this article, this possible autoimmune reaction deserves to be investigated. Binding of SARS-Cov-1 to ACE2 for cell entry was already identified in the SARS-Cov ARDS from 2002 [13,26]. However, the binding of SARS-Cov-2 to ACE2 has a much higher affinity, and this could favor the immune system extended reactivity to the whole pathogenic complex, involving viral and self-components [29]. This affinity is of essence for supporting our autoimmune hypothesis [12]. This strong interaction could participate to the alteration or the ternary structure of ACE2, favoring the exposition of cryptic or hindered epitopes, and contribute to autoantibody generation [36,37].

Conclusion

Interestingly, in few children a possible association of Kawasaki disease induced by Covid-19 has been reported, with endothelial complications, especially in microcirculation. This Kawasaki disease has been strongly suspected to result from an autoimmune pathology. Interestingly, anti-ACE2 autoantibodies were described by Takahashi et al., in 2010, in connective tissue disease, and pathologies induced by a low ACE2 activity favoring the harmful effects of AII. This study indirectly supports our hypothesis.

References

1. Yang J, Zheng Y, Gou X, et al. Prevalence of comorbidities and its effects in patients infected with SARS-CoV-2: A systematic review and meta-analysis. Int J Infect Dis. 2020; 94:91 - 95.
2. Terpos E, Ntanasis-Stathopoulos I, Elalamy I, et al. Hematological findings and complications of COVID-19. Am J Hematol. 2020.
3. Wang D, Hu B, Hu C, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA. 2020; 323: 1061 - 69.
4. Connors JM, Levy JH. Thromboinflammation and the hypercoagulability of COVID-19, J ThrombHaemost. 2020.
5. Joly BS, Siguret V, Veyradier A. Understanding pathophysiology of hemostasis disorders in critically ill patients with COVID-19, Intensive Care Med. 2020;1 - 4.
6. Tang N, Bai H, Chen X, et al. Anticoagulant treatment is associated with decreased mortality in severe coronavirus disease 2019 patients with coagulopathy. J ThrombHaemost. 2020;18:1094 - 99.
7. Gupta E, Carey, McDermott K, et al. Role of Serum Cytokine Levels in Acute Respiratory Distress Syndrome Patients on Extracorporeal Membrane Oxygenation Support. World J Cardiovascul Surg. 2020; 10: 1-10.
8. Jin Y, Wang M, Zuo Z, et al. Diagnostic value and dynamic variance of serum antibody in coronavirus disease 2019. Int J Infect Dis. 2020;94:49 - 52.
9. Fafi-Kremer S, Bruel T, Madec Y, et al. Serologic responses to SARS-CoV-2 infection among hospital staff with mild disease in eastern France. 2020.
10. Shen C, Wang Z, Zhao F, et al. Treatment of 5 Critically Ill Patients With COVID-19 With Convalescent Plasma, JAMA. 2020; 323:1582 - 89.
11. Qu R, Ling Y, Zhang YH, et al. Platelet-to-lymphocyte ratio is associated with prognosis in patients with coronavirus disease-19. J Med Virol. 2020.
12. Amiral J, Vissac AM, Seghatchian J. Covid-19, induced activation of hemostasis, and immune reactions: Can an auto-immune reaction contribute to the delayed severe complications observed in some patients? TransfusApher Sci. 2020;102804.
13. Lan J, Ge J, Yu J, et al. Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. Nature. 2020; 581:215 - 20.
14. Cagno V. SARS-CoV-2 cellular tropism. The Lancet Microbe. 2020; 1: e2-e3.
15. Sarzi-Puttini P, Giorgi V, Sirotti S, et al. COVID-19, cytokines and immunosuppression: what can we learn from severe acute respiratory syndrome? ClinExpRheumatol. 2020; 38:337 - 42.
16. Ferreira AJ, Shenoy V, Qi Y, et al. Angiotensin-converting enzyme 2 activation protects against hypertension-induced cardiac fibrosis involving extracellular signal-regulated kinases. Exp Physiol. 2011; 96:287 - 94.
17. Turner AJ. ACE2 Cell Biology, Regulation, and Physiological Functions. The Protective Arm of the Renin Angiotensin System (RAS). 2015; 185-89.
18. Muñoz DN, Fuentes CA, Castillo AE, et al. Role of the Renin-Angiotensin-Aldosterone System beyond Blood Pressure Regulation: Molecular and Cellular Mechanisms Involved in End-Organ Damage during Arterial Hypertension. Int J Mol Sci. 2016; 17:797.
19. Patel VB, Zhong JC, Grant MB, et al. Role of the ACE2/ Angiotensin 1-7 Axis of the Renin-Angiotensin System in Heart Failure. Circ Res. 2016; 118:1313 - 26.
20. Yan R, Zhang Y, Li Y, et al. Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2. Science. 2020; 367:1444 - 48.

21. Hasan A, Paray BA, Hussain A, et al. A review on the cleavage priming of the spike protein on coronavirus by angiotensin-converting enzyme-2 and furin. J BiomolStructDyn. 2020;1‐9.
22. Zhang H, Baker A. Recombinant human ACE2: acing out angiotensin II in ARDS therapy. Crit Care. 2017; 21:305.
23. Yang CW, Lu LC, Chang CC, et al. Imbalanced plasma ACE and ACE2 level in the uremic patients with cardiovascular diseases and its change during a single hemodialysis session. Ren Fail. 2017; 39:719‐28.
24. Abdul-Hafez A, Mohamed T, Omar H, et al. The renin angiotensin system in liver and lung: impact and therapeutic potential in organ fibrosis. J Lung PulmRespir Res. 2018; 5:00160.
25. Batlle D, Jose Soler M, Ye M. ACE2 and diabetes: ACE of ACEs?Diabet. 2010; 59:2994‐96
26. Glowacka I, Bertram S, Herzog P, et al. Differential downregulation of ACE2 by the spike proteins of severe acute respiratory syndrome coronavirus and human coronavirus NL63. J Virol. 2010; 84:1198‐205
27. Khan A, Benthin C, Zeno B, et al. A pilot clinical trial of recombinant human angiotensin-converting enzyme 2 in acute respiratory distress syndrome. Crit Care. 2017; 21:234.
28. Zhang H, Penninger JM, Li Y, et al. Angiotensin-converting enzyme 2 (ACE2) as a SARS-CoV-2 receptor: molecular mechanisms and potential therapeutic target. Intensive Care Med. 2020; 46:586‐90.
29. Tai W, He L, Zhang X, et al. Characterization of the receptor-binding domain (RBD) of 2019 novel coronavirus: Implication for development of RBD protein as a viral attachment inhibitor and vaccine. Cell MolImmunol. 2020;17:613‐20.
30. Yilla M, Harcourt BH, Hickman CJ, et al. SARS-coronavirus replication in human peripheral monocytes/ macrophages. Virus Res. 2005; 107:93‐101.
31. Amiral J. Immunomodulation, autoimmunity and haemostatic dysfunctions. Transfus Sci. 1997; 18:361‐65.
32. Berruyer M, Amiral J, Ffrench P, et al. Immunization by bovine thrombin used with fibrin glue during cardiovascular operations. Development of thrombin and factor V inhibitors. J ThoracCardiovasc Surg. 1993;105:892‐97.
33. Amiral J, Meyer D. Heparin-induced thrombocytopenia: Diagnostic tests and biological mechanisms. Baillieres Clin Haematol. 1998; 11:447‐60.
34. Regnault V, Boehlen F, Ozsahin H, et al. Anti-protein S antibodies following a varicella infection: detection, characterization and influence on thrombin generation. J ThrombHaemost. 2005;3:1243‐49.
35. Rand ML, Wright JF. Virus-associated idiopathic thrombocytopenic purpura. Transfus Sci. 1998;19:253‐59.
36. Sakurai Y. Autoimmune Aspects of Kawasaki Disease. J InvestigAllergolClinImmunol. 2019; 29:251‐61.
37. Takahashi Y, Haga S, Ishizaka Y, et al. Autoantibodies to angiotensin-converting enzyme 2 in patients with connective tissue diseases. Arthritis Res Ther. 2010;12:R85.

*Correspondence to

Jean Amiral Scientific-Hemostasis

Hemostasis and Thrombosis Diagnostics,

Franconville, France

E-mail: jean.amirasl@scientific-hemostasis.com