Journal of Clinical Trials
ISSN: 2167-0870
OPEN ACCESS Freely available online
Research Article

Management of A COVID-19 Spike Protein based Vaccine which Inducing Blood Clots and Damage to ACE2 Expressing Cells-A Double-Edged Sword

Mahmoud Elkazzaz[1*], Yousry Abo-amer[2], Tamer Haydara[3]

[1]Department of Chemistry and Biochemistry, Damietta University, Damietta El-Gadeeda, Egypt; [2]Department of Hepatology, Gastroenterology and Infectious Diseases, Mahala Hepatology Teaching Hospital, Gharbiya, Egypt; [3]Department of Internal Medicine, Kafrelsheikh University, Kafr Elsheikh, Egypt

ABSTRACT

The interaction between various COVID-19 vaccines and our immune system's response to mediate protection or susceptibility to SARS-CoV-2 is in the very initial stages of understanding. Hundreds of 2019 Corona virus disease targeting vaccines are currently in progress, but success is unknown. Most of the vaccine candidates use a protein-based subunit (spike protein-based vaccine)-so, instead of using a complete pathogenic virus, they are built on a small component of it, such as a protein found in its outer shell. That protein is administered to patients in a high dose, with the aim of inducing a fast and strong reaction by the human immune system. Spike protein-based vaccines were granted emergency approval within a limited period of time and are now being rolled out. This type of vaccine provides our cells with signals to express a component of what is called the "COVID-19 spike protein." Here, we use existing and emerging evidence to propose a testable hypothesis that Spike protein-based vaccines may initiate blood clots as the same as the action of COVID-19 spike protein by the strong interaction between Angiotensin-converting enzyme 2 (ACE2) expressed on platelets and the receptor binding domain of the spike protein generated by vaccination leading to initiating autoantibodies to platelets that mistakenly react and target human platelets leading to serious complication presented in platelet aggregation and blood clots.

Keywords: Angiotensin-converting enzyme; COVID-19; Spike protein; Vaccine

INTRODUCTION

In addition to generating autoantibodies and memory T cells to ACE2 expressed on the epithelial cells in lungs and resulted in an auto-immune response to Angiotensin-converting enzyme 2 (ACE2). These autoantibodies may generated by enforced presentation of the Angiotensin-converting enzyme 2 (ACE2) protein in a complex with vaccine Spike protein in fragment crystallizable (FC) Receptor positive Antigen Presenting Cells in the lung. The development of autoantibodies might make injury and damage to the host epithelial cells and hamper their ACE2 dependent function in lungs, intestine and testes which express ACE2. In addition to inducing platelet aggregation, furthermore the efficacy and safety of various COVID-19 vaccines like spike protein based vaccine and life attenuated vaccine can be hampered by several factors like the Antibody Dependent Enhancement process (ADE). ADE is a phenomenon in which antiviral antibodies help target immune cells to become virally infected. We reported studies with up to date literature guidance to indicate that therapeutic molecules that potentially inhibit and downregulate ACE2 may be an effective treatment and promising adjuvant option for effective spike protein based vaccine of SARS-CoV-2 owing to their ability to reduce the risk of autoantibodies generation to ACE2 by downregulating the expression of ACE2 receptors to be less prone to attachment of spike protein based vaccine and immune system presentation. Noteworthy mentioning that a phase II clinical trial will be started soon to assess The longterm side effects of spike protein based vaccine in the era of COVID-19 (ClinicalTrials.gov Identifier: NCT04730895; First posted: January 29, 2021). The spike protein of COVID-19 attaches atrongly and directly with the host cell surface Angiotensin-converting enzyme 2 (ACE2) facilitating virus cell entry and replication [1-7]. Further investigations also suggested that COVID-19 more effectively recognizes and binds human ACE2 receptors than SARS-CoV, increasing COVID-19's ability to transmit among humans [8,9].

Correspondence to: Mahmoud Elkazzaz, Department of Chemistry and Biochemistry, Damietta University, Damietta El-Gadeeda, Egypt, E-mail: mahmoudramadan2051@yahoo.com

Received: 01-Aug-2022, Manuscript No. JCTR-22-002-PreQc-22; **Editor assigned:** 04-Aug-2022, PreQC No. JCTR-22-002-PreQc-22 (PQ); **Reviewed:** 18-Aug-2022, QC No. JCTR-22-002-PreQc-22; **Revised:** 25-Aug-2022, Manuscript No. JCTR-22-002-PreQc-22 (R); **Published:** 01-Sep-2022, DOI: 10.35248/2167 0870.22.12.503.

Citation: Elkazzaz M, Abo-amer Y, Haydara T (2022) Management of A COVID-19 Spike Protein based Vaccine which Inducing Blood Clots and Damage to ACE2 Expressing Cells-A Double-Edged Sword. J Clin Trials. 12:503.

Upregulation in the expression of human ACE2 induced disease severity and lethality in a mouse model of SARS-CoV infection, suggesting that virus entry into host cells is a potential and critical step [10,11]. Among all SARS-CoV structural proteins, the primary antigenic component responsible for inducing protecting immunity, host immune responses, and/or neutralizing antibodies to viral infection it is an expressed protein called Spike protein. Unfortunately, it is a possible that receiving spike protein based Vaccine may act the same action of COVID-19 spike protein and increase the risk of autoimmunity to cellular ACE2 by generating anti-ACE2 antibodies that target ACE2 receptors expressed in lungs and found on the surface of platelets leading to inflammation and damage of those sites in addition to inducing human platelets activation and aggregation leading to blood clots as a study showed that viral Spike protein potentially and dose-dependently induces platelet activation and aggregation a serious complication which may lead to blood clots [12]. Several studies demonstrated that anti-ACE2 antibodies (autoimmune response) may generated thorough compulsory presentation of the Angiotensin-converting enzyme 2 (ACE2) protein in a complex with vaccine spike protein in fragment crystallizable (FC) Receptor positive antigen presenting cells in the lung [13,14]. A COVID-19 infection is followed by inflammatory pneumonia in approximately ~14% of patients [15], and damage and injury of organ [16]. With certain predisposing conditions, the risk increases and also increases with age. High blood pressure of 6.3% highlights the risk of death among these, [17] by attaching to the ACE2 protein, COVID-19 enters cells. In hypertensive patients, angiotensin-converting enzyme 2 (ACE2) expression may be upregilated and this could improve virus uptake into cells that express ACE2 in the heart, blood vessels kidneys and, lungs [18]. There is no known pathological scientific role for the onset and occurrence of inflammatory pneumonia after clearance of viral infection and initial recovery. A similar pneumonia with inflammation linked with SARS vaccination (Severe Acute Respiratory Syndrome) or renewed exposure was expected to be due to T lymphocyte Hypergrowth [19,20], and It could be transferred by SARS-Specific Antibodies Spike protein in a Non-Human Primate (NHP) model [21]. Vaccine donation with full-length spike protein predisposed to the inflammatory pulmonary disease complication in multiple animal models [19]. Pneumonia resulting in pulmonary inflammation was associated with an early high titer neutralizing antibody response in patients with COVID-19 for Severe Acute Respiratory Syndrome (SARS) [22], and higher antibody titers are also associated with severe Covid-19 inflammatory disease [23]. In pathogenesis, the function of antibodies may be concentrate the Spike protein in fragment crystallizable (Fc) Receptor found on surface of antigen presenting cells in the lung. But why such a destructive and damaging immune response is initiated by the Spike protein (SP) is not clarified. The specificity of the lung-damaging T lymphocyte is also not clarified.

METHODS

Our hypothesis

The strong interaction between Angiotensin-converting enzyme 2 (ACE2) and the Receptor Binding Domain (RBD) of the Spike protein (SP) of COVID-19 is with affinity (~10 nM), and this binding affinity is equivalent to many monoclonal antibodies (MAbs) [17]. The same binding affinity and strong interaction are expected to be found between ACE2 receptors and the Receptor Binding Domain of the Spike protein based vaccine. As such, association of Angiotensin-converting enzyme 2 (ACE2) with the binding domain of the Spike protein is likely to be strong and long lived interaction, and is expected to result in Angiotensin-converting enzyme 2 (ACE2) entering antigen presenting cells associated with the Spike protein produced by vaccine or the Spike protein found on the surface of COVID-19. This may be enhanced by fragment crystallizable (Fc) mediated uptake *via* fragment crystallizable (Fc) Receptors once an antibody response to the spike has occurred, and may set up conditions for extreme presentation of Angiotensin-converting enzyme 2 (ACE2) epitopes to B and T cells, aided by strong T cells help from epitopes derived from Spike protein attachment or other viral expressed proteins. ACE2 expression in heart, kidney and lung would lead to inflammatory action at those sites. Furthermore, loss the activity of respiratory ACE2 may be connected with increased activity of angiotensin 2 *via* the AT2 type I receptors in the lung, that are thought to be involved in initiating inflammatory response and its complication [24]. Autoantibodies to ACE2 have been showed [25] linked with vasculopathies including respiratory hypertension. The role of antibodies in pathogenesis may be to concentrate the Spike protein in fragment crystallizable (Fc) Receptor found on surface of Antigen Presenting Cells in the lung. Therefore, we believe that spike protein based vaccine may potentially bind to the elevated levels of the soluble enzyme ACE2 and it is very possible that generated antibodies will target the human angiotensin converting enzyme 2 receptors. Therefore we hypothesize that vaccines based on the spike protein might initiate Autoantibodies and T cells to ACE2. The development of autoantibodies to ACE2 might make damage to the host epithelial cell in lungs and the other different organs which express ACE2. This pattern of lung injury also occurs in Pulmonary Hypertension secondary to Scleroderma with elevated levels of anti ACE2 antibodies [26,27]. These autoimmune process may also explain why myocarditis and other forms of inflammatory responses show up weeks or months after a patient has ostensibly recovered from COVID-19 infection. In addition, we assume that any medication that upregulats ACE2 as ACE2 blocker in case of diabetic and hypertensive patients may increase the risk of autoantibodies in the event of receiving of spike protein based vaccine as showed in Figure 1. The COVID-19 vaccine may be altered each year to counter changes to circulating strains Therefore, with vaccination; the potential risk of cellular ACE2 damage by autoantibodies developed by the COVID-19 vaccine could be increased.

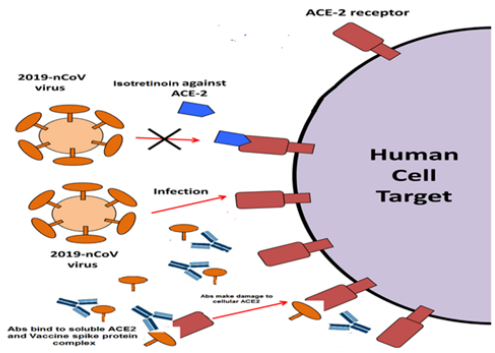

Figure 1: Autoantibodies generated by antigenic presentation of soluble ACE2 or cellular ACE2 cmbined with spike protein based vaccine or COVID-19 spike protein.

Note: Where autoantibodies target cellular ACE2 and isotretinoin binds to ACE2 receptors leading to its genetic expression down regulation, preventing autoantibodies formation and spike protein-ACE2 attachment.

The most serious consequences of our hypothesis

Since December 2019, the COVID-19 pandemic worldwide has become a severe public health crisis [28]. A variety of important cardiovascular complications have been associated with COVID-19 [12] and even people without a history of cardiovascular disease are at risk of cardiovascular complications [29]. Thrombotic disorders, sepsis, and disseminated intravascular coagulation (DIC) are usually encountered in patients with extreme COVID-19 and these conditions have been closely related to higher mortality rates [30,31]. Large-scale trials found that 18.8% to 36.2% of patients [32,33] present with thrombocytopenia on admission. In addition, the total incidence of thrombotic complications was 31% for COVID-19 patients in the ICU, while thrombotic complications were encountered by just 1.3% of non-COVID-19 ICU patients experience thrombotic complications [34]. Although the evidence supports a link between COVID-19 and the development of a hypercoagulable state, the underlying mechanisms for this association remain elusive A large recent study conducted on 201 healthy volunteers and 589 patients suspected of having COVID-19 found that there is a relationship among COVID-19 spike protein, ACE2 expressed on platelets, the platelet hyper activation and coagulation parameters in COVID-19 patients [35]. In addition this study found that human platelets exhibit robust expression of ACE2 at both the RNA and protein levels as detected by RT-PCR [138] [36]. Moreover, it found that SARS-CoV-2 Spike protein directly and dose-dependently potentiates platelet activation, enhanced platelet aggregation and ATP release. These data indicate that S1, but not S2, binds ACE2 to regulate platelet function, which corroborates the finding that the receptor-binding domain (RBD) of the spike protein is found in the S1 subunit [17]. After incubation with Spike or S1 protein, platelets also displayed markedly accelerated spreading and clot retraction. The effect of COVID-19 spike protein on platelet function could be explained by The strong interaction between Angiotensin-converting enzyme 2 (ACE2) expressed on platelets and the Receptor Binding Domain (RBD) of the Spike protein (SP) of COVID-19 is with affinity (~10 nM), and this binding affinity is equivalent to many monoclonal antibodies (MAbs). Furthermore, attaching COVID-19 spike protein to ACE2 receptor on platelet could induce autoantibodies against human platelets and cause blood clot and this could explain Continuous thrombocytopenia after SARS-CoV-2 nucleic acid negative in a non-severe COVID-19 patient for several months [37]. Could spike protein dependent vaccine cause thrombosis in vaccine candidates? The critical issue listed here, we assume that the Spike protein-based vaccine may induce platelet activation pathways through the induction of autoantibodies against human platelets and strong interaction with ACE2 on human platelets because vaccination against corona virus is an important and focal point to eliminate the virus and limit its spread. Here, we must look for a means that can reduce the incidence of autoantibodies to ACE2 and improve the efficacy of COVID-19 vaccine which is represented in molecules that could inhibit ACE2 potentially and making these receptors less prone to spike protein attachment of both COVID-19 and spike protein based vaccine.

RESULTS AND DISCUSSION

Suitable treatment according to our hypothesis

13 cis retinoic acid could be an effective treatment and spike protein based vaccine adjuvant by inhibiting both platelet aggregation and thrombin: More than one-third of patients seriously ill with COVID-19 worldwide are afflicted with dangerously high levels of blood clotting [38]. Indeed, of the very first 99 patients hospitalized in Wuhan, China, 36% presented with elevated blood levels of the so-called D-dimer, a dimeric fragment of fibrin that is the most widely used diagnostic blood marker of recent and/or ongoing coagulopathy [39]. Thrombin is an important and key enzyme in hemostasis and thrombosis, regulating pro-and anticoagulant reactions by interacting with other receptors and coagulation proteins [40]. Thrombin is linked to other complex biological processes such as inflammation [41]. A study reported that RA is found to possess *in-vitro* anti-platelet, anti-inflammatory, and fibrinolytic prosperities. In this study they have tested the *in-vitro* thrombin inhibitory and platelet aggregation activities of vit A and its derivatives. Retinaldehyde, retinoic acid, and retinol showed potent inhibitory effect on thrombin, Retinoic acid showed the highest inhibition of both the forms of thrombin. Vitamin A and its derivatives retinaldehyde, retinoic acid, and retinol also displayed remarkable inhibition of platelet aggregation. This is the first report of vitamin A and its derivatives showing inhibition of thrombin and platelet aggregation *in-vitro* [42]. Co-incubation with 13-cis-RA and IL-1 α resulted in a synergic increase in the release of Prostacyclin Synthase (PGI2) [43]. PGI is a powerful vasodilator that inhibits platelet aggregation through activation of adenylate cyclase. Consistently [44], 13-cis-RA increased the ability of HUVEC to inhibit Arachidonic acid-induced platelet aggregation.

13 cis retinoic acid could be an effective treatment and spike protein based vaccine adjuvant by decreasing the risk of autoantibodies generation to ACE2: A large study analyzed a broad set of 672 clinically approved drugs for treatment in cell lines demonstrated that isotretinoin was the potent and strongest downregulator of Angiotensin-converting enzyme 2 (ACE2) receptors [45] and further, studies reported that it may prevent the cellular entry of SARS-CoV-2 and can be a taken as a targeted therapy in COVID-19 [46-48]. therefore, we suggest iotretinoin treatment and co administration of isotretinoin as adjuvant vaccine with spike protein based vaccine will reduce the expected risk of platelet aggregation and blood clots *via* blocking ACE2 receptors, Furthermore, its ability to induce mucosal IgA antibodies that are less prone to ADE phenomenon and responsible for passive mucosal immunity in the respiratory tract. Furthermore, in addition its impact on Memory T cells, $CD4^+$/$CD8^+$ ratio, Neutrophil Chetnotaxis, Interferon Type1, Thrombin, Transmembrane serine protease 2 (TMPRSS2), toll-like receptor 3 (TLR3), mitochondrial antiviral-signaling protein (MAVS), papain-like protease (PLpro), and Interleukin 6 (Il-6). The primary isomers of RA formed *In-vivo* are 9-cis-retinoic acid (9cRA) and All-trans-Retinoic Acid (atRA) and; each binds separate RA receptor types, thus acting upon a select subset of genes [49]. 13cRA is a synthetic form that may function similar to the other produced isoforms, or by isomerization to atRA and 9cRA. Although the exact mechanism of action is unclear; in other words, Therefore, we hypothesize that isotretinoin binds directly to ACE2 receptors and contributes to their expression downregulation by blocking the binding capacity of ACE2 and this mechanism may lead to blocking the binding of spike protein based vaccine or COVID-19 to cellular and soluble ace2 receptors as showed in Figure 1.

13 cis retinoic acid could be effective treatment better than more re-purposed drugs against COVID-19: 13 cis retinoic acid increased CD4 cells and markedly inhibited viremia in HIV (highly mutated virus) positive patients suffering from acne vulgaris [50]. Males infected with COVID-19 have higher rates of mortality

and hospitalization than females [51] and among severe cases of disease, males have more severe lymphopenia [52]. There may also be a bias to effective and stronger $CD4^+$ and $CD8^+$ T cell activation in females with COVID-19 [53]. In addition administration of 13 cis retinoic acid improved $CD4^+/CD8^+$ ratio in advanced ovarian cancer [54,55]. Furthermore, retenoids (13 cis retinoic acid) inhibited Epstein-Barr virus related lymphoproliferative disorders of immunosuppressed patients [56]. In addition retinoic acid induces homing of protective T and B cells to the gut after subcutaneous immunization in mice [57]. Moreover, the high neutrophil to lymphocyte ratio observed in critically ill patients infected with COVID-19 is associated with excessive levels of reactive oxygen species (ROS), which promote a cascade of biological events that drive pathological host responses. ROS induce tissue damage, thrombosis and red blood cell dysfunction, which contribute to COVID-19 disease severity [26,58]. Isotretinoin produces significant inhibition of neutrophil chetnotaxis and monocyte and *In-vivo* patients with cystic acne [59]. In addition, 13 cis retinoic acid have more therapeutic features which may make it promising treatment against COVID-19. A recent study demonstrated that HDL-scavenger receptor B type 1 (SR-B1) facilitates SARS-CoV-2 entry [19]. SR-B1 is coexpressed with angiotensin-converting enzyme 2 (ACE2) in multiple extrapulmonary tissues including testis and the retina. The existing expression profiles of ACE2 and SR-B1 also show their coexpression in multiple metabolic organs [60-65] which could indicate an enhanced degree of trophism for extrapulmonary tissues, thereby contributing to the multiple-organ pathologies of COVID-19 [66-70]. Retinoic acid-induced down-regulation of HDL-scavenger receptor B type 1 (SR-B1) *via* retinoic acid receptors induced expression of the intestinal transcription factor (ISX). ISX then inhibited the expression of SR-B1 [71]. Furthermore, a study demonstrated that isotretinoin is a potential repressor and inhibitor of papain-like protease (PLpro), which is a lethal protein, expressed by COVID-19 genes [72] and is a deubiquitinating enzyme, which facilitates and induces the host cell ubiquitination process to the advantage of COVID-19 [73]. Interleukin-6 (IL-6) is one of the main mediators of inflammatory and immune response initiated by COVID-19 infection or injury and increased levels of Interleukin-6 are found in more than one half of patients with COVID-19 [74-77]. Levels of Interleukin-6 seem to be associated with respiratory failure, inflammatory response, needing for mechanical ventilation and mortality in patients with COVID-19 [78,79]. Many studies found that 13 cis retinoic acid is an effective treatment for inhibition of IL-6 [80-84]. Therefore, we first hypothesized in our large clinical trial (ClinicalTrials.gov; NCT04353180) conducted on 1000 patients with COVID-19 that 13 cis retinoic acid (isotretinoin) may be potential treatment for il-6 inhibition in case of COVID-19 and what confirms our hypothesis is that a large study published in nature after our clinical trial demonstrated that depletion of retinoic acid causes excessive cytokine release, is called "retinoic acid depletion syndrome." COVID-19 and previously defined sepsis, SIRS and ARDS are each retinoic acid depletion syndrome [85]. Various studies demonstrated that SARS-CoV-2 uses the SARS-CoV receptor ACE2 for entry and the serine protease TMPRSS2 for S protein priming [86-91]. In COVID-19, it has been hypothesized that higher androgen levels and hence sustained the serine protease TMPRSS2 expression among men might explain their predominance in numbers of deaths from the disease versus women [92-96]. The serine protease TMPRSS2 expression is androgen-regulated TMPRSS2 gene as in Figure 2 [97-99].

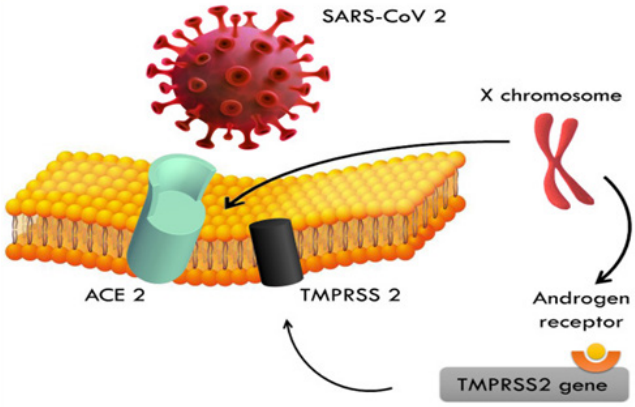

Figure 2: Pathway for COVID-19 infection initiated by androgenic hormones activity.

Isotretinoin alone can decrease androgen levels *via* attenuating its effect [60-62] Moreover, we hypothesize that any drug which downregulates the serine protease TMPRSS2 expression through targeting Androgenic receptors (AR), Androgenic receptors(AR) co-regulatory factors, or Androgenic receptors (AR) transcription factors might be clinically effective for investigation against COVID-19 and is worth investigating under a clinical trial. Noteworthy mentioning that a phase III clinical trial will be started soon to assess the efficacy of isotretinoin (13-cis-retinoic acid) -a retinoid used in severe acne due to hyperandrogenism—in the treatment of COVID-19(ClinicalTrials.gov; NCT04353180; First Posted: April 20, 2020 estimated study start date June 2020) [100-102].

13 cis retinoic acid could be an effective treatment and COVID-19 vaccine adjuvant by inducing mucosal IgA antibodies thus are the first line of defense: Since, antibody-dependent enhancement (ADE) process could hamper the action of several covid-19 vaccines thorough targeting IGA antibodies and vaccine can fail to protect against covid-19 infection. ADE is a mechanism based on IgG antibodies that COVID-19 uses to penetrate and invade cells.. The mechanisms of antibody-dependent enhancement (ADE) are different from those in other viruses, such as dengue virus, and are more closely related to the presence of different subtypes of virus strain, according to previous research on human coronaviruses MERS-CoV and SARS-CoV. Human coronavirus enter cells under the support of fragment crystallizable (FcR) receptors. SARS-CoV causes enhanced lung injury by inducing hyperimmunity through the interactions of FcR and antibody, which alters the functions of macrophages (Figure 3) [51].

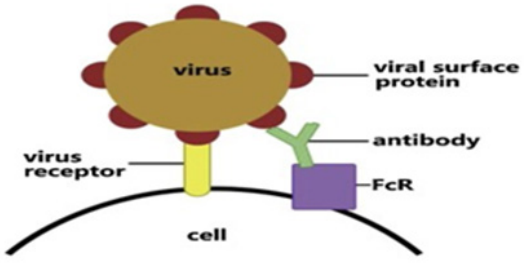

Figure 3: COVID-19 depends on IgG antibodies *via* ADE process for cell invading and entry.

Regarding MERS-CoV, this induces viral entry under the induction of binding between FcR and antibodies, which is similar to the traditional route of viral entry [103]. In 2007, Yiu Wing Kam et al. explored whether antibody against viral spike protein of SARS-CoV can induce viral invading and entry into FcR-bearing cells and inducing and evoke Antibody-dependent enhancement (ADE) [104]. The results demonstrated and showed that by increasing the affinity of SARS-CoV towards FcγRII-bearing cells, these antibodies cause infection. This increase is mediated by the Fc portion of FcRII on cells and anti-spike antibody, while Angiotensin-converting enzyme 2 (ACE2) is not needed or required in the process. Later research by Jaume et al. in 2014 showed that antibody against SARS-CoV viral spike protein strengthened the infection towards lymphocytes and monocytes, both of which do not express SARS-CoV receptors [105]. This was matching with the results obtained by Yiu Wing Kam's team. In the same year, researches conducted by Huang's team and Chen showed that Antibody-dependent enhancement (ADE) is mainly induced by diluted antibodies (Abs) against viral spike proteins rather than viral nucleocapsid protein [106]. These researches further showed that anti-spike antibodies induce Antibody-dependent enhancement (ADE) during infection of SARS-CoV, and this effect mainly works in cells of the immune system. The rhesus monkey was used as an animal model in 2018 to study the relationship between the vaccine-induced antibody titer and to induce antibody-dependent enhancement (ADE). The results showed that those vaccines that elicit low antibodytiters cannot induce antibody-dependent enhancement (ADE) after infection with SARS-CoV [107]. The highly diluted serum can in turn induce SARS-CoV infectivity. In addition, antibody responses to SARS-CoV viral spike (S) glycoprotein have been reported to have evolved significantly faster anti-Spike neutralizing antibody (NAbs) responses in deceased patients compared to recovered patients after the onset of clinical symptoms [108]. As World Health Organization (WHO) declared that COVID-19 and influenza viruses have a similar disease presentation [109]. Consistently, preexisting serum antibodies against influenza antigens were found to associate with poor outcomes and worse clinical severity in patients during the 2009 influenza pandemic [110,111]. In this case, we hypothesize that therapeutics such as metabolites of vitamin A (retinoic acid) that activate and induce IgA antibodies which is the first line of defense against viruses is recommended to be potential treatment and vaccine adjuvant.

IgA antibodies have no Fc receptor binding sites and may be less susceptible to ADE phenomena: Immunoglobulin A(IgA) is the first line of defense in the resistance against infection, *via* inhibiting bacterial and viral adhesion to epithelial cells and by neutralisation of bacterial toxins and virus, both extra- and intracellularly. In addition, Antibody-dependent enhancement (ADE) don't occur with antibodies of IgA because they are found in the lining layer of lungs and intestine. Unlike IgG serum antibodies, secretory IgA may form polymers and has a unique structure which may not have the Fc receptor binding sites in some forms as in Figures 4 and 5. Secreted IgA antibodies plays a crucial and potential role in the immune defense of mucosal surfaces, the first point of entry of SARS-CoV-2. IgA antibodies-based serology tests targeting COVID-19 specific Spike protein and nucleocapsid protein (NP) may thus represent an important therapeutic and diagnostic approach [112-114].

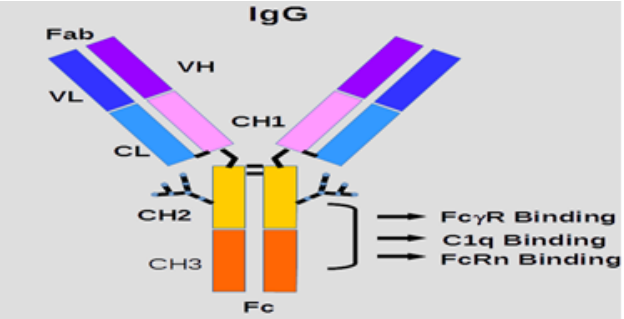

Figure 4: IgG antibodies and their Fc receptor binding site.

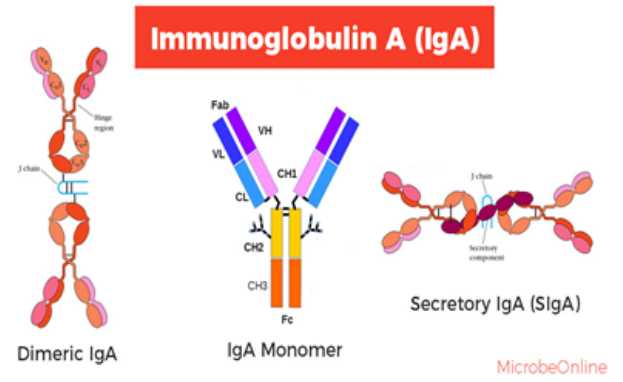

Figure 5: IgA antibodies and their uniqe dimeric form lacking Fc receptor binding site.

Retinoic acid is a potent IgA antibodies inducer and isotype switching

Vitamin A, given the vital role of its metabolites such as retinoic acid in imprinting a gut-homing capacity on B and T cells [115] as well as its effective and potential role in inducing the differentiation of IgA$^+$ ASCs [116,117]. So, it is not surprising that vit A deficiency is associated with decreased and impaired intestinal immune responses [118-120]. Consistent with a role for vit A in gut IgA antibodies production, mice or rats depleted of vit A have decreased mucosal antigen-specific IgA antibodies responses and levels of total IgA antibodies in intestinal lavages, which correlates with decreased protection against oral bacterial toxins and infections [121,122]. Furthermore, vitamin A supplementation prevents the decline in IgA antibodies levels showed in malnourished mice [123]. Moreover a direct effect on ASCs, it should be considered that deficiency in vitamin A could also decrease IgA antibodies production in the gut by decreasing the expression of the polymeric receptors of immunoglobulin, leading to a decrease in the production of dimeric IgA antibodies to the site of the gut lumen. Vitamin A deficient Mice-has decreased numbers of IgA+ ASCs in site of the small bowel lamina propria Consequently, mice deficient in the retinoic acid (RA) precursor vitamin A lacked IgA antibodies-secreting cells in the small intestine [124]. In addition, RA is considered to possess an activity of IgA isotype switching [125]. Furthermore, retinoic acid, performing as a highly specific IgA isotype switch factor, cooperates with transforming growth factor beta 1 (TGF-β1) to enhance the

overall IgA response [126]. In addition, retinoic acid enhances lactoferrin-induced IgA responses by increasing expression of betaglycan [127]. Conversely, supplementation of vitamin A correlates with a significant decrease in mortality and diarrhoea in HIV-infected patients or malnourished children [128,129]. Also, is not surprising that isotretinoin (13 cis retinoic acid) repair the function of IgA antibodies in subcorneal pustular dermatosis patients [130]. Concerning that the oral isotretinoin treatment can reduce mucosal thickness and lead to nasal and mucus dryness and crusting, which may increase the likelihood of contracting the disease spread by aerosol particles.

Hypersecretion of mucus in COVID-19: In COVID-19 patients, the forming of mucus plugs was found, causing airway obstruction and respiratory failure in a large proportion of these patients. 33 percent of COVID-19 autopsies have detected serious mucoid tracheitis [131].

Mucus secretion association with immune response: Although the respiratory mucosa functions as a defensive layer against pathogens owing to its ability to trap an invading pathogen through sticky secretions and then move it out *via* ciliary action [132]. But sungnank et al. confirmed that the nasal epithelial is the source of SARS-CoV-2 infection, from there it spreads to the lower respiratory tract. Arumugham et al. demonstrated that COVID-19 overstimulates the mucosa in a pathophysiology similar to dengue virus. This contributes to an inflammatory cascade being triggered and various inflammatory cytokines and chemokines being produced [133]. This is consistent with other studies showing that SARS-CoV-2 stimulates the inflammatory response and causes increased respiratory mucosal secretion [134].

The role of IL-4 and IL-5 in mucus development and cell recruitment mediated by TH2 cells is well defined in an experiment by Cohn et al. IL-4 activation of CD4 T cells allows th0 cells to be separated from th2 cells, which in turn activates IL-4 secretion and maintains a positive feedback loop [135]. By activation of the JAK3/STAT 6 pathway, interleukin 4 induces the transcription of MUC5AC. STAT 6 is involved in CLCA1 (calcium activated chloride channel 1) activation, which stimulates MAPK signalling, eventually contributing to the development of mucin. Th2 cells aid in attracting lymphocytes and eosinophils into the lungs, allowing MUC5AC to be over-secreted in the airway, resulting in goblet cell hygiene [136]. On eosinophils and T lymphocytes, Very-Late-Activation-Antigen-4 (VLA-4) is present, which has the ability to bind with Vascular Cell Adhesion Molecule 1 (VCAM-1) and allows selective entry of eosinophils into injured tissues [136].

Function of inflammation in hyper secretion of airway mucus: The symptoms in COVID-19 and elevated levels of inflammatory markers in patients indicate that a severe cytokine storm develops in this disease. Recent studies support that mucus hypersecretion is caused by inflammation. Studies have shown that most SARS-CoV-2 contaminated patients have normal WBC counts or lymphocytopenia in some cases. There are substantial rises in neutrophil levels in patients that show serious conditions. Their blood urea and D-dimer levels are both considerably high, while their lymphocyte count is decreased [137]. Multiple pro-inflammatory cytokines such as IL6, IL10, and TNF-alpha have elevated levels. In addition, rises in IL-2, IL-7, and IL-10 were reported in the blood tests of patients admitted to intensive care units (ICU) [138,139]. The inflammatory response can cause hypersecretion of mucus that can block the respiratory tract, reduce airflow and thus aggravate the already decreasing function of the lung [140].

In addition, the pro-inflammatory cascades modify mucus composition and compromise its clearance by cilia [141]. This leads to repeated airway tract infections, causing further respiratory tract obstruction, producing a vicious cycle. Patients with COVID-19 have higher levels of many pro-inflammatory markers, including IL-1, IL-6, IL-2, IL-13 and TNF alpha, along with their crosstalk markers [142]. Several other inflammatory cytokines are upregulated by the crosstalk of these cytokines and their downstream signalling. IL-2, IL-4, and IL-6 control IL-4, IL-5, IL-6, and IL-13 levels through STAT5, STAT6, and NFAT, respectively. IL-5 also upregulates levels of IL-6, IL-1, and TNF *via* STAT1. TNF alpha contributes to upregulation of IL-1beta and IL-8 by activation of NF-eterB. Histamine released from mast cell degranulation during inflammatory response results in EGF and adenosine synthesis *via* ERk1/2 upregulation in addition to cytokines. The inflammation caused by these cytokines may lead to hypersecretion of mucus that corresponds to the complication that occurs in patients with COVID-19.

Although in our clinical study (ClinicalTrials.gov; NCT04353180) we will use isotretinoin treatment for a period of no more than 14 days, which is considered a very short period and is not sufficient to cause any side effects. But based on previous information we can take the advantage of 13 cis retinoic acid in modulating both nasal clearance and mucus secretion for inhibiting of COVID-19 inflammatory complication associated with mucus hyper secretion in addition decreasing the chance of infection transmission among peoples *via* hyper secretions of infected patients as a study investigated the effect of oral isotretinoin on nasal mucociliary clearance (A process responsible for mucus secretion into the upper airways) and lung function in patients with acne vulgaris through three months of treatment found that there was no difference before and during the third month of treatment in Forced Vital Capacity (FVC), forced expiratory volume in 1s (FEV1), FEV1/FVC ratio, forced expiratory flow rate between 25% and 75% of FVC (FEF(25-75), and their predicted percentage ratios. But found that nasal clearance was significantly prolonged with treatment, and there was significant correlation between drug dose and mucociliary clearance time [143].

Isotretinoin caused signs and symptoms of dry nose and disturbed mucociliary clearance without affecting pulmonary function tests PFTs [143]. When we compared the results of this study with the results of a cross-sectional study in which the mean mucociliary clearance times of COVID-19 infected patients (15.53 ± 5.57 min) was high in comparison with control (9.50 ± 3.70 min) groups, were significantly different ($Z=4.675$, $p<0.001$) [144-147]. We hypothesize that nasal clearance was significantly prolonged with treatment because isotretinoin treatment leads to mucus drying and hypo-secretion in contrast to COVID-19 infection which lead to hyper nasal clearance prolongation because of the inflammatory response can cause hyper-secretion of mucus that can block the respiratory tract, reduce airflow and thus aggravate the already decreasing function of the lung [148-161].

CONCLUSION

Generally the mechanism of isotretinoin in reducing fluid secretion like sebum secretion and mucus secretion is owing to its ability to inhibit androgen attachment to its receptors as astudy found that ARfloxLysMCre males (males lacking androgen receptor (AR) showed significantly less mucus production in ARfloxLysMCre males after OVA exposure. Isotretinoin alone can decrease androgen levels *via* attenuating its effect and attachment to

androgenic receptors.

Therefore Isotretinoin doesn't damage mucocillia but it caused signs and symptoms of dry nose and disturbed mucociliary clearance without affecting pulmonary function tests PFTs.

ETHICAL APPROVAL

These Clinical studies are ethically approved by Research Ethics Committee-Faculty of Medicine-Kafrelsheikh University; Approval number: 9127907 and 9127918

COMPETING INTEREST

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

FUNDING

This research was funded by the faculty of Medicine Kafr-elshiekh university (Egypt). Kafr-elshiekh university takes the responsibility to initiate, manage and finance these clinical trials in addition to implementing and maintaining quality assurance and quality control systems with written SOPs to ensure that this trial is conducted and data are generated, documented, and reported in compliance with the protocol, GCP, and the applicable regulatory requirement(s).

AUTHOR'S CONTRIBUTION

ME, YA and TH wrote the first draft and contributed equally to the manuscript. All authors contributed substantially to the conception and acquisition for the work and approved the final approval of the version to be published. All authors have read and approved the final manuscript.

REFERENCES

1. Zhang H, Penninger JM, Li Y, Zhong N, Slutsky AS. Angiotensin-converting enzyme 2 (ACE2) as a SARS-CoV-2 receptor: Molecular mechanisms and potential therapeutic target. Intensive Care Med. 2020;46(4):586-590.
2. Townsend A. Autoimmunity to ACE2 as a possible cause of tissue inflammation in COVID-19. Med Hypotheses. 2020;144:110043.
3. Wan Y, Shang J, Graham R, Baric RS, Li F. Receptor recognition by the novel coronavirus from Wuhan: An analysis based on decade-long structural studies of SARS coronavirus. J virol. 2020;94(7):e00127-00220.
4. Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020;579(7798):270-273.
5. Zhao Y, Zhao Z, Wang Y, Zhou Y, Ma Y, Zuo W. Single-cell RNA expression profiling of ACE2, the receptor of SARS-CoV-2. Am J Respir Crit Care Med. 2020;202(5):756-759.
6. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus–infected pneumonia in Wuhan, China. JAMA. 2020;323(11):1061-1069.
7. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020;395(10223):497-506.
8. Wan Y, Shang J, Graham R, Baric RS, Li F. Receptor recognition by the novel coronavirus from Wuhan: An analysis based on decade-long structural studies of SARS coronavirus. J Virol. 2020;17;94(7):e0012720..
9. Davidson AM, Wysocki J, Batlle D. Interaction of SARS-CoV-2 and other coronavirus with ACE (angiotensin-converting enzyme)-2 as their main receptor: Therapeutic implications. Hypertension. 2020;76(5):1339-49.
10. Yang XH, Deng W, Tong Z, Liu YX, Zhang LF, Zhu H, et al. Mice transgenic for human angiotensin-converting enzyme 2 provide a model for SARS coronavirus infection. Comparative medicine. 2007 1;57(5):450-9.
11. Imai Y, Kuba K, Rao S, Huan Y, Guo F, Guan B, et al. Angiotensin-converting enzyme 2 protects from severe acute lung failure. Nature. 2005;436(7047):112-6.
12. Madjid M, Safavi-Naeini P, Solomon SD, Vardeny O. Potential effects of coronaviruses on the cardiovascular system: A review. JAMA Cardiol. 2020;5(7):831-840.
13. Alwan NA, Burgess RA, Ashworth S, Beale R, Bhadelia N, Bogaert D, et al. Scientific consensus on the COVID-19 pandemic: We need to act now. Lancet. 2020;396(10260):e71-72.
14. Wu Z, McGoogan JM. Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China: Summary of a report of 72 314 cases from the Chinese Center for Disease Control and Prevention. JAMA. 2020;323(13):1239-1242.
15. Wadman M, Couzin-Frankel J, Kaiser J, Matacic C. A rampage through the body. Science.
16. Lan J, Ge J, Yu J, Shan S, Zhou H, Fan S, et al. Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. Nature. 2020;581(7807):215-220.
17. Zheng YY, Ma YT, Zhang JY, Xie X. COVID-19 and the cardiovascular system. Nat rev cardiol. 2020;17(5):259-260.
18. Tseng CT, Sbrana E, Iwata-Yoshikawa N, Newman PC, Garron T, Atmar RL, et al. Immunization with SARS coronavirus vaccines leads to pulmonary immunopathology on challenge with the SARS virus. PloS One. 2012;7(4):e35421.
19. Clay C, Donart N, Fomukong N, Knight JB, Lei W, Price L, et al. Primary severe acute respiratory syndrome coronavirus infection limits replication but not lung inflammation upon homologous rechallenge. J virol. 2012;86(8):4234-4244.
20. Liu L, Wei Q, Lin Q, Fang J, Wang H, Kwok H, et al. Anti–spike IgG causes severe acute lung injury by skewing macrophage responses during acute SARS-CoV infection. JCI insight. 2019;4(4).
21. Zhang L, Zhang F, Yu W, He T, Yu J, Yi CE, et al. Antibody responses against SARS coronavirus are correlated with disease outcome of infected individuals. J med virol. 2006;78(1):1-8.
22. Zhao J, Yuan Q, Wang H, Liu W, Liao X, Su Y, et al. Antibody responses to SARS-CoV-2 in patients with novel coronavirus disease 2019. Clin Infect Dis. 2020;71(16):2027-2034.
23. Jia H. Pulmonary angiotensin-converting enzyme 2 (ACE2) and inflammatory lung disease. Shock. 2016;46(3):239-248.
24. Takahashi Y, Haga S, Ishizaka Y, Mimori A. Autoantibodies to angiotensin-converting enzyme 2 in patients with connective tissue diseases. Arthritis Res Ther. 2010;12(3):1-8.
25. Laforge M, Elbim C, Frère C, Hémadi M, Massaad C, Nuss P, et al. Tissue damage from neutrophil-induced oxidative stress in COVID-19. Nat Rev Immunol. 2020;20(9):515-516.
26. Chappell MC. Angiotensin-converting enzyme 2 autoantibodies: Further evidence for a role of the renin-angiotensin system in inflammation. Arthritis res ther. 2010;12(3):1-2.
27. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. lancet. 2020;395(10223):497-506.
28. Di Pisa G, Patricolo E, Reas P. Extracellular potassium requirement for cell cycle activation in fertilized Ascidia malaca eggs. Acta Embryol Morphol Exp (Italy). 1980.
29. Kiernan DC. Teachers and research in special education. Spec Educ Forward Trends. 1982;9(3):25-26.
30. Tang N, Li D, Wang X, Sun Z. Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. J Thromb Haemost. 2020;18(4):844-847.
31. Wu C, Chen X, Cai Y, Zhou X, Xu S, Huang H, et al. Risk factors associated with acute respiratory distress syndrome and death in patients with coronavirus disease 2019 pneumonia in Wuhan, China. JAMA Intern Med. 2020;180(7):934-943.

32. Guan WJ, Ni ZY, Hu Y, Liang WH, Ou CQ, He JX, et al. Clinical characteristics of coronavirus disease 2019 in China. N Engl j Med. 2020;382(18):1708-1720.
33. Klok FA, Kruip MJ, Van der Meer NJ, Arbous MS, Gommers DA, Kant KM, et al. Incidence of thrombotic complications in critically ill ICU patients with COVID-19. Thromb Res. 2020;191:145-147.
34. Zhang S, Liu Y, Wang X, Yang L, Li H, Wang Y, et al. SARS-CoV-2 binds platelet ACE2 to enhance thrombosis in COVID-19. J Hematol Oncol. 2020;13(1):1-22.
35. Sahai A, Bhandari R, Koupenova M, Freedman JE, Godwin M, McIntyre T, et al. SARS-CoV-2 receptors are expressed on human platelets and the effect of aspirin on clinical outcomes in COVID-19 patients. Res Sq. 2020.
36. Wu X, Luo D, Liu Y, Zeng Y, Gong Y. Continuous thrombocytopenia after SARS-CoV-2 nucleic acid negative in a non-severe COVID-19 patient for several months. BMC Infect Dis. 2020;20(1):1-5.
37. Levi M, Thachil J, Iba T, Levy JH. Coagulation abnormalities and thrombosis in patients with COVID-19. Lancet Haematol. 2020;7(6):e438-e440.
38. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: A retrospective cohort study. lancet. 2020;395(10229):1054-1062.
39. Borissoff JI, Spronk HM, Heeneman S, ten Cate H. Is thrombin a key player in the 'coagulation-atherogenesis' maze? Cardiovasc Res. 2009;82(3):392-403.
40. Spronk HM, Borissoff JI, ten Cate H. New insights into modulation of thrombin formation. Curr Atheroscler Rep. 2013;15(11):1-9.
41. Krishna TH, Kamalraj S, Anikisetty M, Naidu KA, Surin WR, Jayabaskaran C. Inhibition of thrombin, an unexplored function of retinoic acid. Biochemistry and Biophysics Reports. 2019;18:100636.
42. Camacho M, Rodríguez C, Salazar J, Martínez-González J, Ribalta J, Escudero JR, et al. Retinoic acid induces PGI synthase expression in human endothelial cells. J Lipid Res. 2008;49(8): 1707-1714.
43. Majed BH, Khalil RA. Molecular mechanisms regulating the vascular prostacyclin pathways and their adaptation during pregnancy and in the newborn. Pharmacol Rev. 2012;64(3): 540-582.
44. Sinha S, Cheng K, Schäffer AA, Aldape K, Schiff E, Ruppin E. In vitro and in vivo identification of clinically approved drugs that modify ACE 2 expression. Mol Fyst Biol. 2020;16(7):e9628.
45. Wu C, Chen X, Cai Y, Zhou X, Xu S, Huang H, et al. Risk factors associated with acute respiratory distress syndrome and death in patients with coronavirus disease 2019 pneumonia in Wuhan, China. JAMA Intern Med. 2020;180(7):934-43.
46. Tiwari N, Upadhyay J, Ansari MN, Joshi R. Novel β-Coronavirus (SARS-CoV-2): Current and future aspects of pharmacological treatments. Saudi Pharm J. 2020;28(10):1243-52.
47. Bahmad HF, Abou-Kheir W. Crosstalk between COVID-19 and prostate cancer. Prostate Cancer Prostatic Dis. 2020;23(4):561-3.
48. Chambon P. A decade of molecular biology of retinoic acid receptors. FASEB J. 1996;10(9):940-954.
49. Harms M, Péchère M, Krischer J, Studer E, Saurat JH. Oral isotretinoin in HIV-positive women with acne: Report of three cases. Dermatology. 1998;196(1):163.
50. Scully CH. Immune Responses and COVID-19 Outcomes. Nat Rev Immunol. (20).
51. Meng Y, Wu P, Lu W, Liu K, Ma K, Huang L, et al. Sex-specific clinical characteristics and prognosis of coronavirus disease-19 infection in Wuhan, China: A retrospective study of 168 severe patients. PLoS pathog. 2020;16(4):e1008520.
52. Recchia F, Saggio G, Cesta A, Candeloro G, Nuzzo A, Lombardo M. Interleukin-2 and 13-cis retinoic acid as maintenance therapy in advanced ovarian cancer. Int j oncol. 2005;27(4):1039-1046.
53. Recchia F, Saggio G, Cesta A, Candeloro G, Di Blasio A, Amiconi G, et al. Phase II study of interleukin-2 and 13-cis-retinoic acid as maintenance therapy in metastatic colorectal cancer. Cancer Immunol Immunother. 2007;56(5):699-708.
54. Pomponi F, Cariati R, Zancai P, De Paoli P, Rizzo S, Tedeschi RM. Retinoids irreversibly inhibit in vitro growth of Epstein-Barr virus-immortalized B lymphocytes.
55. Hammerschmidt SI, Friedrichsen M, Boelter J, Lyszkiewicz M, Kremmer E, Pabst O, et al. Retinoic acid induces homing of protective T and B cells to the gut after subcutaneous immunization in mice. J clin invest. 2011;121(8):3051-3061.
56. Veras FP, Pontelli MC, Silva CM, Toller-Kawahisa JE, de Lima M, Nascimento DC, et al. SARS-CoV-2–triggered neutrophil extracellular traps mediate COVID-19 pathology. J Exp Med. 2020;217(12).
57. Norris DA, Osborn R, Robinson W, Tonnesen MG. Isotretinoin produces significant inhibition of monocyte and neutrophil chemotaxis in vivo in patients with cystic acne. J invest dermatol. 1987;89(1):38-43.
58. Shen WJ, Azhar S, Kraemer FB. SR-B1: A unique multifunctional receptor for cholesterol influx and efflux. Annu Rev Physiol. 2018;10;80:95-116.
59. Julovi SM, Xue A, Thanh LE TN, Gill AJ, Bulanadi JC, Patel M, et al. Apolipoprotein A-II plus lipid emulsion enhance cell growth via SR-B1 and target pancreatic cancer in vitro and in vivo. PLoS One. 2016;11(3):e0151475.
60. Johnson AC, Yabu JM, Hanson S, Shah VO, Zager RA. Experimental glomerulopathy alters renal cortical cholesterol, SR-B1, ABCA1, and HMG CoA reductase expression. Am J Pathol. 2003;162(1):283-91..
61. Hardenberg JH, Luft FC. Covid-19, ACE2 and the kidney. Acta Physiol (Oxf).2020;230(1):e13539.
62. Zhang H, Li HB, Lyu JR, Lei XM, Li W, Wu G, et al. Specific ACE2 expression in small intestinal enterocytes may cause gastrointestinal symptoms and injury after 2019-nCoV infection. Int J Infect Dis. 2020;96:19-24.
63. Liu F, Long X, Zhang B, Zhang W, Chen X, Zhang Z. ACE2 expression in pancreas may cause pancreatic damage after SARS-CoV-2 infection. Clin Gastroenterol Hepatol.2020;18(9):2128-30.
64. Seah I, Agrawal R. Can the coronavirus disease 2019 (COVID-19) affect the eyes? A review of coronaviruses and ocular implications in humans and animals. Ocul Immunol Inflamm.2020;28(3):391-5.
65. Hollstein T, Schulte DM, Schulz J, Glück A, Ziegler AG, Bonifacio E, et al . Autoantibody-negative insulin-dependent diabetes mellitus after SARS-CoV-2 infection: a case report. Nat Metab. 2020;2(10):1021-4.
66. Cheng Y, Luo R, Wang K, Zhang M, Wang Z, Dong L, et al. Kidney disease is associated with in-hospital death of patients with COVID-19. Kidney Int. 2020;97(5):829-38.
67. Zhang C, Shi L, Wang FS. Liver injury in COVID-19: management and challenges. Lancet Gastroenterol Hepatol.2020;5(5):428-30.
68. Zhang H, Kang Z, Gong H, Xu D, Wang J, Li Z, et al. Digestive system is a potential route of COVID-19: an analysis of single-cell coexpression pattern of key proteins in viral entry process. Gut. 2020;69(6):1010-8.
69. Lobo GP, Hessel S, Eichinger A, Noy N, Moise AR, Wyss A, et al. ISX is a retinoic acid-sensitive gatekeeper that controls intestinal β, β-carotene absorption and vitamin A production. FASEB J. 2010;24(6):1656-66.
70. Wu C, Liu Y, Yang Y, Zhang P, Zhong W, Wang Y, et al. Analysis of therapeutic targets for SARS-CoV-2 and discovery of potential drugs by computational methods. Acta Pharm Sin B. 2020;10(5):766-88.
71. Lindner HA, Fotouhi-Ardakani N, Lytvyn V, Lachance P, Sulea T, Ménard R. The papain-like protease from the severe acute respiratory syndrome coronavirus is a deubiquitinating enzyme. J Virol. 2005;79(24):15199-208.
72. Zhang ZL, Hou YL, Li DT, Li FZ. Laboratory findings of COVID-19: a systematic review and meta-analysis. Scand J Clin Lab Invest. 2020;80(6):441-7.
73. Grifoni E, Valoriani A, Cei F, Lamanna R, Gelli AM, Ciambotti B, et al. Interleukin-6 as prognosticator in patients with COVID-19. J Infect. 2020;81(3):452-82.
74. Huang L, Zhao X, Qi Y, Li H, Ye G, Liu Y, et al. Sepsis-associated severe interleukin-6 storm in critical coronavirus disease 2019. Cell Mol Immunol. 2020;17(10):1092-4.
75. Xu X, Han M, Li T, Sun W, Wang D, Fu B, et al. Effective treatment of severe COVID-19 patients with tocilizumab. Proc Natl Acad Sci U

S A.2020;117(20):10970-5.

76. Herold T, Jurinovic V, Arnreich C, Lipworth BJ, Hellmuth JC, von Bergwelt-Baildon M, et al. Elevated levels of IL-6 and CRP predict the need for mechanical ventilation in COVID-19. J Allergy Clin Immunol.2020;146(1):128-36.
77. Aziz M, Fatima R, Assaly R. Elevated interleukin-6 and severe COVID-19: A meta-analysis. J Med Virol. 2020;92(11):2283-2285.
78. Zitnik RJ, Kotloff RM, Latifpour J, Zheng T, Whiting NL, Schwalb J, et al. Retinoic acid inhibition of IL-1-induced IL-6 production by human lung fibroblasts. J Immunol.1994;152(3):1419-27.
79. Zhou TB, Drummen GP, Qin YH. The controversial role of retinoic acid in fibrotic diseases: analysis of involved signaling pathways. Int J Mol Sci.2012;14(1):226-43.
80. Guruvayoorappan C, Kuttan G. 13 cis-retinoic acid regulates cytokine production and inhibits angiogenesis by disrupting endothelial cell migration and tube formation. J Exp Ther Oncol. 2008;7(3):173-82.
81. Dispenza MC, Wolpert EB, Gilliland KL, Dai JP, Cong Z, Nelson AM, et al. Systemic isotretinoin therapy normalizes exaggerated TLR-2-mediated innate immune responses in acne patients. J Invest Dermatol.2012;132(9):2198-205.
82. Saxon A, Keld B, Braun J, Dotson A, Sidell N. Long-term administration of 13-cis retinoic acid in common variable immunodeficiency: circulating interleukin-6 levels, B-cell surface molecule display, and in vitro and in vivo B-cell antibody production. Immunology. 1993;80(3):477-87.
83. Sarohan AR. COVID-19: Endogenous retinoic acid theory and retinoic acid depletion syndrome. Med Hypotheses. 2020;144:110250.
84. Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, et al. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell.2020;181(2):271-80.
85. Bertram S, Heurich A, Lavender H, Gierer S, Danisch S, Perin P, et al. Influenza and SARS-coronavirus activating proteases TMPRSS2 and HAT are expressed at multiple sites in human respiratory and gastrointestinal tracts. PloS One. 2012;7(4):e35876.
86. Hatesuer B, Bertram S, Mehnert N, Bahgat MM, Nelson PS, Pöhlman S, et al. Tmprss2 is essential for influenza H1N1 virus pathogenesis in mice. PLoS Pathog.2013;9(12):e1003774.
87. Glowacka I, Bertram S, Müller MA, Allen P, Soilleux E, Pfefferle S, et al. Evidence that TMPRSS2 activates the severe acute respiratory syndrome coronavirus spike protein for membrane fusion and reduces viral control by the humoral immune response. J Virol. 2011;85(9):4122-34.
88. Matsuyama S, Nagata N, Shirato K, Kawase M, Takeda M, Taguchi F. Efficient activation of the severe acute respiratory syndrome coronavirus spike protein by the transmembrane protease TMPRSS2. J Virol. 2010;84(24):12658-64.
89. A. Shulla, T. Heald-Sargent, G. Subramanya, J. Zhao, S. Perlman, T. Gallagher A transmembrane serine protease is linked to the severe acute respiratory syndrome coronavirus receptor and activates virus entry. J. Virol. 2011; 85(2):873-882.
90. Montopoli M, Zumerle S, Vettor R, Rugge M, Zorzi M, Catapano CV, et al. Androgen-deprivation therapies for prostate cancer and risk of infection by SARS-CoV-2: A population-based study (N=4532). Ann Oncol. 2020;31(8):1040-1045.
91. Stopsack KH, Mucci LA, Antonarakis ES, Nelson PS, Kantoff PW. TMPRSS2 and COVID-19: Serendipity or opportunity for intervention? Cancer Discov. 10:779-782.
92. Bahmad HF, Abou-Kheir W. Crosstalk between COVID-19 and prostate cancer. Prostate Cancer and Prostatic Dis. 2020;23(4):561-563.
93. Wambier CG, Goren A, Vaño-Galván S, Ramos PM, Ossimetha A, Nau G, et al. Androgen sensitivity gateway to COVID-19 disease severity. Drug Dev Res. 2020;81(7):771-776.
94. Lin B, Ferguson C, White JT, Wang S, Vessella R, True LD, et al. Prostate-localized and androgen-regulated expression of the membrane-bound serine protease TMPRSS2. Cancer res. 1999;59(17):4180-4184.
95. Pettersson A, Graff RE, Bauer SR, Pitt MJ, Lis RT, Stack EC, et al. The TMPRSS2: ERG rearrangement, ERG expression, and prostate cancer outcomes: A cohort study and meta-analysis TMPRSS2: ERG and Prostate Cancer Outcomes. Cancer epidemiol biomarkers prev. 2012;21(9):1497-509.
96. Lucas JM, Heinlein C, Kim T, Hernandez SA, Malik MS, True LD, et al. The Androgen-Regulated Protease TMPRSS2 activates a proteolytic cascade involving components of the tumor microenvironment and promotes prostate cancer metastasisTMPRSS2 influences prostate cancer metastasis. Cancer Discov. 2014;4(11):1310-1325.
97. Wu ZS, Zhang ZQ, Wu S. Focus on the crosstalk between COVID-19 and urogenital systems. J urol. 2020;204(1):7-8.
98. Cadegiani FA. Repurposing existing drugs for COVID-19: an endocrinology perspective. BMC Endocr Disord. 2020;20(1):1-9.
99. Andrade KR, Carvalho VK, Farinasso CM, Lima AA, Silva RB, Wachira VK, et al. Terapia medicamentosa para infecções por coronavírus em humanos: Revisão sistemática rápida. Ciência & Saúde Coletiva. 2020;25:3517-3554.
100. Wan Y, Shang J, Sun S, Tai W, Chen J, Geng Q, et al. Molecular mechanism for antibody-dependent enhancement of coronavirus entry. J virol. 2020;94(5):e02015-e02019.
101. Kam YW, Kien F, Roberts A, Cheung YC, Lamirande EW, Vogel L, et al. Antibodies against trimeric S glycoprotein protect hamsters against SARS-CoV challenge despite their capacity to mediate FcRII-dependent entry into B cells in vitro. Vaccine. 2007;25(4):729-740.
102. Yip MS, Leung NH, Cheung CY, Li PH, Lee HH, Daëron M, et al. Antibody-dependent infection of human macrophages by severe acute respiratory syndrome coronavirus. Virol j. 2014;11(1):1-1.
103. Wang SF, Tseng SP, Yen CH, Yang JY, Tsao CH, Shen CW, et al. Antibody-dependent SARS coronavirus infection is mediated by antibodies against spike proteins. Biochem biophys res commun. 2014;451(2):208-214.
104. Luo F, Liao FL, Wang H, Tang HB, Yang ZQ, Hou W. Evaluation of antibody-dependent enhancement of SARS-CoV infection in rhesus macaques immunized with an inactivated SARS-CoV vaccine. Virol Sin. 2018;33(2):201-204.
105. Zhang L, Zhang F, Yu W, He T, Yu J, Yi CE, et al. Antibody responses against SARS coronavirus are correlated with disease outcome of infected individuals. J med virol. 2006;78(1):1-8.
106. To KK, Zhang AJ, Hung IF, Xu T, Ip WC, Wong RT, et al. High titer and avidity of nonneutralizing antibodies against influenza vaccine antigen are associated with severe influenza. Clin Vaccine Immunol. 2012;19(7):1012-1018.
107. Monsalvo AC, Batalle JP, Lopez MF, Krause JC, Klemenc J, Hernandez JZ, et al. Severe pandemic 2009 H1N1 influenza disease due to pathogenic immune complexes. Nat med. 2011;17(2):195-199.
108. Chao YX, Rötzschke O, Tan EK. The role of IgA in COVID-19. Brain Behav Immun. 2020;87:182.
109. Petherick A. Developing antibody tests for SARS-CoV-2. Lancet. 2020;395(10230):1101-1102.
110. Kumar N, Arthur CP, Ciferri C, Matsumoto ML. Structure of the secretory immunoglobulin A core. Science. 2020;367(6481):1008-1014.
111. Iwata M, Hirakiyama A, Eshima Y, Kagechika H, Kato C, Song SY. Retinoic acid imprints gut-homing specificity on T cells. Immunity. 2004;21(4):527-538.
112. Mora JR, Iwata M, Eksteen B, Song SY, Junt T, Senman B, et al. Generation of gut-homing IgA-secreting B cells by intestinal dendritic cells. Science. 2006;314(5802):1157-1160.
113. Tokuyama H, Tokuyama Y. The regulatory effects of all-trans-retinoic acid on isotype switching: retinoic acid induces IgA switch rearrangement in cooperation with IL-5 and inhibits IgG1 switching. Cell immunol. 1999;192(1):41-47.
114. Wiedermann U, Hanson LA, Kahu H, Dahlgren UI. Aberrant T-cell function in vitro and impaired T-cell dependent antibody response in vivo in vitamin A-deficient rats. Immunology. 1993;80(4):581.
115. Sirisinha S, Darip MD, Moongkarndi P, Ongsakul M, Lamb AJ. Impaired local immune response in vitamin A-deficient rats. Clin exp immunol. 1980;40(1):127.

116. Mora JR, Von Andrian UH. Differentiation and homing of IgA-secreting cells. Mucosal immunol. 2008;1(2):96-109.
117. Stephan U, König W. Effect of Antigens from Nippostrongylus brasiliensis and Cytokines on the Ongoing IgE Synthesis in vitro. Int Arch Allergy Immunol. 1992;98(4):299-307.
118. Dauwerse JG, Jumelet EA, Wessels JW, Saris JJ, Hagemeijer A, Beverstock GC, et al. Extensive cross-homology between the long and the short arm of chromosome 16 may explain leukemic inversions and translocations. Blood.
119. Nikawa T, Odahara K, Koizumi H, Kido Y, Teshima S, Rokutan K, et al. Vitamin A prevents the decline in immunoglobulin A and Th2 cytokine levels in small intestinal mucosa of protein-malnourished mice. J nutr. 1999;129(5):934-941.
120. Sato A, Hashiguchi M, Toda E, Iwasaki A, Hachimura S, Kaminogawa S. CD11b+ Peyer's patch dendritic cells secrete IL-6 and induce IgA secretion from naive B cells. J Immunol. 2003;171(7):3684-3690.
121. Zitnik RJ, Kotloff RM, Latifpour J, Zheng T, Whiting NL, Schwalb J, Elias JA. Retinoic acid inhibition of IL-1-induced IL-6 production by human lung fibroblasts. J Immunol. 1994;152(3):1419-1427.
122. Seo GY, Jang YS, Kim HA, Lee MR, Park MH, Park SR, et al. Retinoic acid, acting as a highly specific IgA isotype switch factor, cooperates with TGF-β1 to enhance the overall IgA response. J leukoc biol. 2013;94(2):325-335.
123. Lee JM, Jang YS, Jin BR, Kim SJ, Kim HJ, Kwon BE, et al. Retinoic acid enhances lactoferrin-induced IgA responses by increasing betaglycan expression. Cell mol immunol. 2016;13(6):862-870.
124. Villamor E, Fawzi WW. Effects of vitamin A supplementation on immune responses and correlation with clinical outcomes. Clin microbiol rev. 2005;18(3):446-464.
125. Semba RD, Ndugwa C, Perry RT, Clark TD, Jackson JB, Melikian G, et al. Effect of periodic vitamin A supplementation on mortality and morbidity of human immunodeficiency virus–infected children in Uganda: a controlled clinical trial. Nutrition. 2005;21(1):25-31.
126. Gruss C, Zillikens D, Hashimoto T, Amagai M, Kroiss M, Vogt T, et al. Rapid response of IgA pemphigus of subcorneal pustular dermatosis type to treatment with isotretinoin. J Am Acad Dermatol. 2000;43(5):923-926.
127. Farooqi FI, Morgan RC, Dhawan N, Dinh J, Yatzkan G, Michel G. Airway hygiene in COVID-19 pneumonia: treatment responses of 3 critically Ill cruise ship employees. Am J Case Rep. 2020;21: e926596.
128. Bustamante-Marin XM, Ostrowski LE. Cilia and mucociliary clearance. Cold Spring Harb Perspect Biol. 2017;9(4): a028241.
129. Arumugham V. Immunological mechanisms explaining the role of IgE, mast cells, histamine, elevating ferritin, IL-6, D-dimer, VEGF levels in COVID-19 and dengue, potential treatments such as mast cell stabilizers, antihistamines, Vitamin C, hydroxychloroquine, ivermectin and azithromycin. SSRN. 2020.
130. Prompetchara E, Ketloy C, Palaga T. Immune responses in COVID-19 and potential vaccines: lessons learned from SARS and MERS epidemic. Asian Pac J Allergy Immunol. 2020;38(1): 1-9.
131. Cohn L, Homer RJ, Marinov A, Rankin J, Bottomly K. Induction of airway mucus production By T helper 2 (Th2) cells: a critical role for interleukin 4 in cell recruitment but not mucus production. J Exp Med. 1997;186(10):1737–1747.
132. Thai P, Chen Y, Dolganov G, Wu R. Differential regulation of MUC5AC/Muc5ac and hCLCA-1/mGob-5 expression in airway epithelium. Am J Respir Cell Mol Biol. 2005;33(6): 523–530.
133. Griffin DO, Jensen A, Khan M, Chin J, Chin K, Saad J, et al. Pulmonary embolism and increased levels of d-dimer in patients with coronavirus disease. Emerg Infect Dis. 2020;26(8): 1941–1943.
134. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020;395(10223): 497–506.
135. Wang W, Liu X, Wu S, Chen S, Li Y, Nong L et al. The definition and risks of cytokine release syndrome in 11 COVID-19-affected critically ill patients with pneumonia: Analysis of disease characteristics. J Infect Dis. 2020;222(9): 1444-1451.
136. Allinson JP, Hardy R, Donaldson GC, Shaheen SO, Kuh D, Wedzicha JA. The presence of chronic mucus hypersecretion across adult life in relation to chronic obstructive pulmonary disease development. Am J Respir Crit Care Med. 2016;193(6): 662–667.
137. Busse PJ, Zhang TF, Kamal S, Lin BP, Schofield B, Sealfon SC, et al. Chronic exposure to TNF-alpha increases airway mucus gene expression in vivo. J Allergy Clin Immunol. 2005; 116(6):1256–1263.
138. Girija ASS, Shankar EM, Larsson M. Could SARS-CoV-2-induced hyperinflammation magnify the severity of Coronavirus Disease (CoViD-19) leading to acute respiratory distress syndrome? Front Immunol. 2020;11:1206.
139. Gorpelioglu C, Ozol D, Sarifakioglu E. Influence of isotretinoin on nasal mucociliary clearance and lung function in patients with Acne vulgaris. Int J Dermatol. 2010;49(1): 87-90.
140. Feily A, Taheri T, Meier-Schiesser B, Rhinehart DP, Sobhanian S, Colon-Diaz M, et al. The effect of low-dose isotretinoin therapy on serum androgen levels in women with Acne vulgaris. Int J Womens Dermatol. 2020;6(2):102-104.
141. Feily A, Namazi MR. Decrease of insulin growth factor-1 as a novel mechanism for anti-androgen effect of isotretinoin and its reported association with depression in some cases. J Drugs Dermatol: JDD. 2011;10(7):793-794.
142. Karadag AS, Takci Z, Ertugrul DT, Bilgili SG, Balahoroglu R, Takir M. The effect of different doses of isotretinoin on pituitary hormones. Dermatology. 2015;230(4):354-9.
143. McMillan P, Uhal BD. COVID-19-A theory of autoimmunity to ACE-2. MOJ immunology. 2020;7(1):17.
144. Guan WJ, Ni ZY, Hu Y, Liang WH, Ou CQ, He JX, et al. Clinical characteristics of 2019 novel coronavirus infection in China. N Engl J Med. 2020;382(18):1708-1720.
145. Wei C, Wan L, Yan Q, Wang X, Zhang J, Yang X, et al. HDL-scavenger receptor B type 1 facilitates SARS-CoV-2 entry. Nat Metab.2020;2(12):1391-400.
146. Scully CH. Immune Responses and COVID-19 Outcomes. Nat Rev Immunol. (20).
147. Liu L, Wei Q, Lin Q, Fang J, Wang H, Kwok H, et al. Anti-spike IgG causes severe acute lung injury by skewing macrophage responses during acute SARS-CoV infection. JCI insight. 2019;4(4).
148. Mora JR, Iwata M, Eksteen B, Song SY, Junt T, Senman B, et al. Generation of gut-homing IgA-secreting B cells by intestinal dendritic cells. Science. 2006;314(5802):1157-1160.
149. Zitnik RJ, Kotloff RM, Latifpour J, Zheng T, Whiting NL, Schwalb J, Elias JA. Retinoic acid inhibition of IL-1-induced IL-6 production by human lung fibroblasts. J Immunol. 1994;152(3):1419-1427.
150. Kopecky-Bromberg SA, Martínez-Sobrido L, Frieman M, Baric RA, Palese P. Severe acute respiratory syndrome coronavirus open reading frame (ORF) 3b, ORF 6, and nucleocapsid proteins function as interferon antagonists. J Virol. 2007;81(2):548-557.
151. Siu KL, Kok KH, Ng MH, Poon VK, Yuen KY, Zheng BJ, et al. Severe acute respiratory syndrome coronavirus M protein inhibits type I interferon production by impeding the formation of TRAF3· TANK TBK1/IKKε complex. J Biol Chem. 2009;284(24):16202-16209.
152. Hacker H, Redecke V, Blagoev B, Kratchmarova I, Hsu LC, Wang GG, et al. Specificity in Toll-like receptor signalling through distinct effector functions of TRAF3 and TRAF6. Nature. 2006;439(7073):204-.
153. Sun L, Xing Y, Chen X, Zheng Y, Yang Y, Nichols DB, et al. Coronavirus papain-like proteases negatively regulate antiviral innate immune response through disruption of STING-mediated signaling. PloS One. 2012;7(2):e30802.
154. Narayanan K, Huang C, Lokugamage K, Kamitani W, Ikegami T, Tseng CT, et al. Severe acute respiratory syndrome coronavirus nsp1 suppresses host gene expression, including that of type I interferon, in infected cells. J virol. 2008;82(9):4471-4479.
155. Siu KL, Yeung ML, Kok KH, Yuen KS, Kew C, Lui PY, et al. Middle east respiratory syndrome coronavirus 4a protein is a double-stranded RNA-binding protein that suppresses PACT-induced activation of RIG-I and MDA5 in the innate antiviral response. J virol. 2014;88(9):4866-4876.

156. Niemeyer D, Zillinger T, Muth D, Zielecki F, Horvath G, Suliman T, et al. Middle East respiratory syndrome coronavirus accessory protein 4a is a type I interferon antagonist. J virol. 2013;87(22):12489-12495.
157. Totura AL, Whitmore A, Agnihothram S, Schäfer A, Katze MG, Heise MT, et al. Toll-like receptor 3 signaling via TRIF contributes to a protective innate immune response to severe acute respiratory syndrome coronavirus infection. mBio. 2015;6(3):e00638-00615.
158. Sprokholt J, Helgers LC, Geijtenbeek TB. Innate immune receptors drive dengue virus immune activation and disease. Future Virol. 2018;13(4):287-305.
159. Chuang HC, Lin HY, Liao PL, Huang CC, Lin LL, Hsu WM, et al. Immunomodulator polyinosinic-polycytidylic acid enhances the inhibitory effect of 13-cis-retinoic acid on neuroblastoma through a TLR3-related immunogenic-apoptotic response. Lab Invest. 2020;100(4):606-618.
160. Clerkin KJ, Fried JA, Raikhelkar J, Sayer G, Griffin JM, Masoumi A. COVID-19 and cardiovascular disease. Circulation. 2020;19(141):20.
161. Puntmann VO, Carerj ML, Wieters I, Fahim M, Arendt C, Hoffmann J, et al. Outcomes of cardiovascular magnetic resonance imaging in patients recently recovered from coronavirus disease 2019 (COVID-19). JAMA Cardiol. 2020;5(11): 1265–1273.