Molecular pathways triggered by COVID-19 in different organs: ACE2 receptor-expressing cells under attack? A review

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Abstract. – **OBJECTIVE:** In human pathology, SARS-CoV-2 utilizes multiple molecular pathways to determine structural and biochemical changes within the different organs and cell types. The clinical picture of patients with COVID-19 is characterized by a very large spectrum. The reason for this variability has not been clarified yet, causing the inability to make a prognosis on the evolution of the disease.

MATERIALS AND METHODS: PubMed search was performed focusing on the role of ACE 2 receptors in allowing the viral entry into cells, the role of ACE 2 downregulation in triggering the tissue pathology or in accelerating previous disease states, the role of increased levels of Angiotensin II in determining endothelial dysfunction and the enhanced vascular permeability, the role of the dysregulation of the renin angiotensin system in COVID-19 and the role of cytokine storm.

RESULTS: The pathological changes induced by SARS-CoV-2 infection in the different organs, the correlations between the single cell types targeted by the virus in the different human organs and the clinical consequences, COVID-19 chronic pathologies in liver fibrosis, cardiac fibrosis and atrial arrhythmias, glomerulosclerosis and pulmonary fibrosis, due to the systemic fibroblast activation induced by angiotensin II are discussed.

CONCLUSIONS: The main pathways involved showed different pathological changes in multiple tissues and the different clinical presentations. Even if ACE2 is the main receptor of SARS-CoV-2 and the main entry point into cells for the virus, ACE2 expression does not always explain the observed marked inter-individual variability in clinical presentation and outcome, evidencing the complexity of this disorder. The proper interpretation of the growing data available might allow to better classifying COVID-19 in human pathology.

Key Words:
  COVID-19, ACE2, SARS-COV-2, Molecular pathways.

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Introduction

The global pandemic emergency caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)[1] represents the most important world health problem currently ongoing with 32,886,465 confirmed cases worldwide since the beginning of the epidemic. Of these, 994,940 (3.02% lethality) have been fatal (data provided by the WHO Health Emergency Dashboard on September 27th, 10.00 am CET)[2].

The key clinical features of the patients with SARS-CoV-2, the causative agent of COVID-19, are characterized by a striking inter-individual variability, ranging from asymptomatic subjects to patients presenting with lower respiratory tract illness with fever, dry cough, and dyspnea. The clinical picture, in some patients, may worsen in a short time, with a hyper inflammation response, acute respiratory distress syndrome, ending with multiple organ failure. The disease is not restricted to the respiratory tract and to lungs. A subset of affected patients undergo disseminated intravascular coagulation (DIC), cerebral ischemic stroke, pulmonary thrombosis and myocardial infarction[3-6]. The reasons for this marked variability among subjects infected have not been clarified yet, given that the pathophysiology of the SARS-CoV-2 is not yet fully understood.

This aim of this paper is to show, in a simple and unifying drawing-based approach, the multiple molecular pathways through which SARS-CoV-2 targets the different cell types in the human organs, giving rise to the multiple different clinical presentations of the disease. We will try to focus on some simple molecular rules that can explain the different target lesions. The key points of this viral infection will be summarized, trying to correlate the known molecular events occurring in the different organs with the pathological changes observed at microscopy and with the clinical presentation.

We will also try to explain the reason why SARS-CoV-2 does not cause acute hepatitis and why survivors should be monitored, in order to exclude the insurgence of liver fibrosis and chronic liver disease; why patients with atherosclerosis should undergo strict follow-up, in order to prevent severe complications due to plaque inflammation and rupture, both events favored by the SARS-CoV-2 induced cytokine storm; why young subjects, asymptomatic for lower respiratory tract illness, have developed unexpected occurrence of ischemic events consistent with large-vessel stroke; why the prevalence of atrial fibrillation is increased following COVID-19 infection; why diabetic patients might show an accelerated kidney injury, ending with glomerulosclerosis. Finally, the molecular pathways at the basis of the vicious circle of inflammation-blood coagulation activation-inflammation induced by SARS-CoV-2, leading to disseminated intravascular coagulation (DIC), pulmonary thrombosis[7] and complicated atherosclerosis[8] are here discussed.

Materials and Methods

The review will discuss the molecular pathways through which SARS-CoV-2 leads to different clinical consequences. In order to address this purpose, a PubMed search focusing on the following items was performed: (1) the role of ACE 2 receptors in allowing the viral entry into cells; (2) the ability of the following ACE 2 down-regulation or loss in tissues in triggering tissue pathology or in accelerating previous disease states; (3) the consequences of the increased levels of Angiotensin II, including the endothelial dysfunction and the enhanced vascular permeability in different organs; (4) the dysregulation of the renin angiotensin system (RAS) with decrease in the anti-thrombotic and anti-fibrotic angiotensin levels; (5) the rise in inflammatory cytokines, including IL-6, IL-7, IL-22, CXCL-10 and their role in the exaggerated inflammatory response, ending with the cytokine storm. Original papers dealing with SARS-CoV-2 and COVID-19 have been taken into consideration. Reviews, meta-analysis and case reports/series have been excluded. The key points regarding the pathological changes induced by SARS-CoV-2 infection in the different organs are systematically described. The use of simple drawings will schematize the main molecular pathways involved in order to correlate the single cell types targeted by the virus in the different human organs and the clinical consequences.

Results

High-Risk Category Groups in Which COVID-19 May Be Severe

Some category groups have been shown to be at higher risk of developing COVID-19 and to undergo severe or even fatal outcome (Figure 1).

Elderly subjects (>65 years), mainly when obese, diabetic, smokers, or hypertensive, patients with chronic kidney disease (CKD), cancer carriers. Even patients with pre-existing lung disease, including chronic obstructive pulmonary disease (COPD) and asthma, are at higher risk. The inclusion in the high-risk group of patients affected by alpha-1-antitrypsin deficiency is proposed in this work, but further data are needed to confirm this hypothesis.

Patients with pre-existing cardiovascular disorders show an elevated vulnerability to SARS-CoV-2, often leading to severe outcome and high mortality rate[4]. The reason for the susceptibility to undergo severe outcome of these categories is often lacking. The overlapping between high-risk category groups for COVID and high-risk categories for atherosclerosis is intriguing and deserves further studies. Recently, some doubt has been arisen regarding the inclusion of COPD patients among the risk groups for COVID[9]. Regarding the susceptibility of elderly people to SARS-Cov-2 infection and to severe complications, the trace element status might play a relevant role. In particular, changes in zinc and copper, two trace elements essential for a competent immune and anti-oxidative reaction, including both innate and acquired immune response, are frequent among elderly subjects. An increased copper/zinc ratio is associated with increased risk of infection by SARS-CoV-2, and probably with a severe disease

Figure 1. High risk category groups in which COVID 19 may be sever or fatal.

outcome[10]. These data suggest that the trace element status of elderly subjects should be checked, in order to prevent their susceptibility to undergo SARS-CoV-2 infection.

Severe Complications Due to SARS-CoV-2

Severe pneumonia is the most frequent severe complication of SARS-CoV-2 infection. Two types of lung infection have been identified: type I, characterized by hypoxemia, mainly due to pulmonary hypertension, ending with right-to-left venous admixture around 50%; type II, in which severe hypoxemia is associated with acute respiratory distress syndrome (ARDS). This differential diagnosis is mandatory, given that high positive end-expiratory pressure (PEEP) and prone positioning improve oxygenation through recruitment of collapsed areas exclusively in type II patients[11]. Severe cardiac injury has been reported in 12-26% of COVID patients[4]. Among the major cardiovascular complications, myocardial injury, altered myocardial demand-supply ratio, arrhythmias and plaque rupture followed by coronary thrombosis have been reported. Multiple organ dysfunction syndrome has been reported in COVID patients, particularly in subjects under hemodialysis[12]. Neurological manifestations are generally mild (anosmia and ageusia), but occasionally severe encephalopathy has been reported[13].

Viral Entry Into Cells: the Role of ACE2 Receptors

Human ACE2 (angiotensin converting enzyme 2) is a zinc carboxypeptidase (Swiss-Prot code Q9BYF1), 805 amino acid residue long, with seven N-glycosylation sites and three disulfide bridges, located on the surface of the cell. It is a single-pass type I integral membrane glycoprotein, orientated with the N-terminus and the catalytic site facing the extracellular space (an ectoenzyme), where it can metabolize circulating peptides[14]. ACE2 is able to remove the C-terminal residue of various peptides, but its specific action is on the renin-angiotensin-aldosterone system (RAAS), where it modulates the plasmatic levels of angiotensin I and II. Indeed, it converts angiotensin I in angiotensin 1-9, a peptide with anti-hypertrophic effects in cardiomyocytes, and angiotensin II to angiotensin 1-7, a vasodilator[14]. The activity of ACE 2 is chloride ions dependent, but it is also down-regulated by the proteolytic shedding of its extracellular domain by TNF-α converting enzyme (TACE/ADAM-17, also called sheddase). The resulting

soluble protein is released into blood stream and ultimately is excreted into urine[15].

Some years ago, different studies evidenced that ACE2 is the main entry point into cells for some coronaviruses, such as SARS-CoV (the virus that causes SARS) and HCoV-NL63[16]. Recently, ACE2 has been also recognized as the receptor of SARS-CoV-2[17]. The ACE2 binding domain recognized by SARS-CoV-2 is almost identical to that one SARS-CoV. The viral envelope-anchored trimeric spike protein mediates coronavirus entry into host cells by binding to its host domain and subsequently fusing host and viral membranes. The spike protein consists of three segments, an ectodomain, a single-pass membrane anchor, and a short intracellular tail. The ectodomain can be divided into two subunits, the receptor-binding S1 subunit, which is responsible for the ACE2 recognition and a membrane-fusion S2 subunit. Twenty amino acid residues of the ACE2 N-terminal domain are in contact with seventeen amino acid residues of the S1 subunit[18]. The binding of the spike S1 protein of SARS-CoV-2 to ACE2 on the surface of cells results in endocytosis and translocation of both the virus and the enzyme into endosomes located within cells[16] (Figure 2). The molecular mechanisms leading to the lytic and non-lytic replication of the virus inside the cell have been less investigated, but they should be similar to those reported for other RNA viruses and might be taken into account for the development of appropriate therapies.
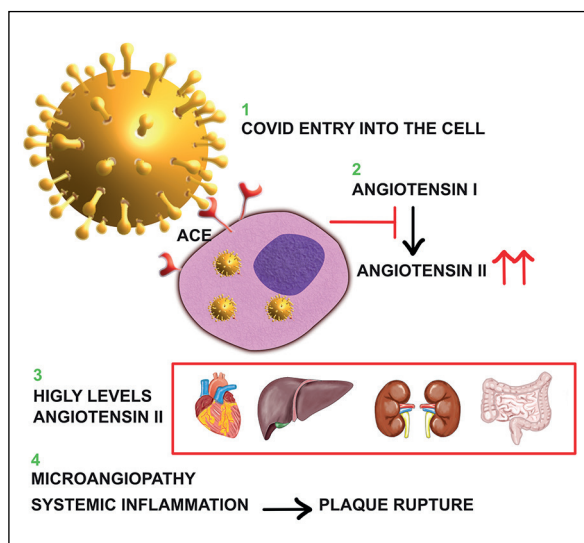

Figure 2. Schematic representation of SARS-COV-2 entry into cells.

Cells Highly Expressing ACE2 Receptors in Different Human Organs

The previous section highlighted as the interaction of SARS-CoV-2 with ACE2 is the key to understand the molecular mechanisms at the basis of the virus infection and of its deleterious effect on the health host. Therefore, several researches have been carried out in order to investigate the expression of ACE2 in different organs and tissue. Recently, Li et al[19] have compared the ACE2 expression levels in 31 normal tissues consulting the GTEx RNA-Seq gene expression profiling datasets available upon UCSC Xena project portal. A two-sided Student's *t*-test was utilized to establish statistically significant differences between males and females and between young (less than 49 y) and old (more than 49 y) people.

Expression levels of ACE2 were very high in the small intestine, testis, kidneys, heart, thyroid, and adipose tissue, while very low expression levels were found in the blood, spleen, bone marrow, brain, blood vessels, and muscles. Lungs, colon, liver, bladder, and adrenal glands have displayed medium expression levels (Figure 3). No significant different ACE2 expression levels were observed between males and females or between younger and older persons in any tissue. In the skin, digestive system, brain, and blood vessels, ACE2 expression levels were positively associated with immune signatures, consisting of CD8+ T cells, interferon response, B cells, and natural killer (NK) cells, in both males and females. In the thyroid and lungs, ACE2 expression levels were positively and negatively associated with immune signatures in males and females, respectively. In the lungs, they had a positive and a negative correlation in the older and younger groups, respectively.

Overall, results of this study indicated that on the basis of ACE2 expression, SARS-CoV-2 may infect different tissues, but probably with different virulence. The infection seems to not discriminate people with different sexes, ages, and races. However, the different host immune responses deriving from SARS-CoV-2 infection could in part explain why males and females, young and old people display markedly distinct disease severity.

Dysregulation of the Renin Angiotensin System (RAS)

The RAS system is a master regulator of human physiology, playing a key role in maintaining blood pressure homeostasis, regulating
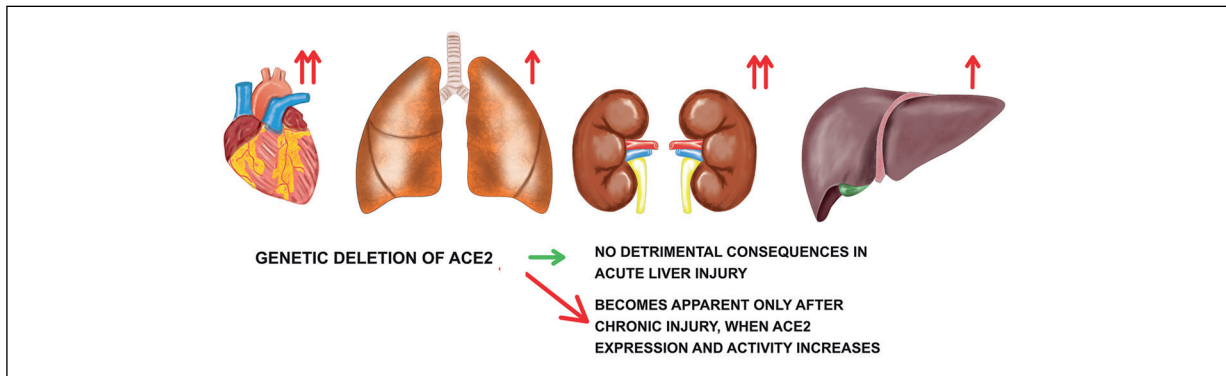
Figure 3. Baseline expression of ACE2.

fluid and salt balance by coordinating heart, kidneys, blood vessels and brain functions[20] (Figure 4). SARS-Cov-2 cell entry depends on ACE2, which is down-regulated following viral entry[21]. The loss of ACE2 activity causes a dysregulation of RAS, leading to the accumulation of undegraded Angiotensin II, ending with development of atherosclerosis and arterial neointima formation. Moreover, loss of ACE2 activity has detrimental effects on systemic fibroblast activation, triggering pulmonary, renal, cardiac and liver fibrosis[22]. Through the imbalance of renin and angiotensin II, COVID-19 might result in an overwhelming number of chronic and acute diseases[23].

The Role of the Increased Levels of Angiotensin II and of Decreased Levels of Angiotensin

The function of angiotensin and angiotensin II are opposite. Whereas the former has vasodilatative, anti-fibrotic, anti-thrombotic, anti-proliferative functions, the latter is characterized by
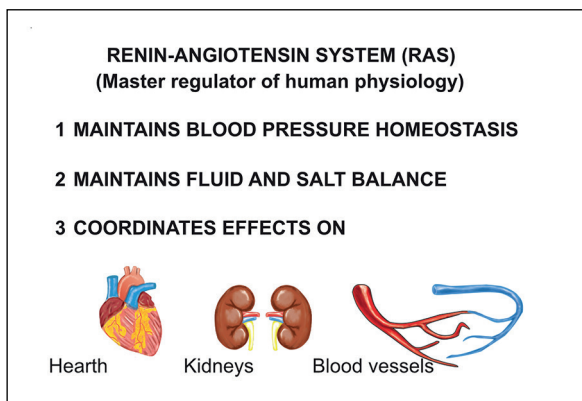
pro-inflammatory, pro-fibrotic, pro-thrombotic, proliferative action, and activates vasoconstrictive activities. In clinical practice, angiotensin confers endothelial protection and attenuates atherosclerotic lesions[24]. Increased levels of angiotensin II, due to SARS-Cov-2-mediated RAS dysregulation, might be responsible for endothelial dysfunction, enhanced vascular permeability, exaggerated neutrophil accumulation in lungs, as well in atherosclerotic plaques, with acute and chronic consequences on human health[25].

Given the dysregulation of RAS in patients with COVID-19, the withdrawn of drugs, such as inhibitors of the renin-angiotensin system has been suggested, in order to do not aggravate the ACE2-inhibitory effect due to SARS-CoV-2 on ACE2[26].

The Systemic Inflammatory Response, the Cytokine Storm

Patients with COVID-19 may develop a hyper-inflammatory syndrome defined as cytokine storm syndrome characterized by a marked increase of cytokines and macrophage activation. The cytokine storm is often associated with multi-organ failure, resulting in a rapid failure of respiratory function and very high mortality rate[27] (Figure 5). The clinical presentation is similar to that observed in viral-induced hemophagocytic lymphohistiocytosis and in the macrophage activation syndrome (MAS). The cytokine storm may induce an abnormal inflammatory response, including myocarditis, and hyper-coagulation status, worsening the endothelial dysfunction (Figure 6). In addition, thrombosis, blood hypertension and pulmonary embolism, observed in the COVID-19 infection, indicates that the endothelium represents a target organ of the infection. Indeed, anticoagulation has been proposed in the
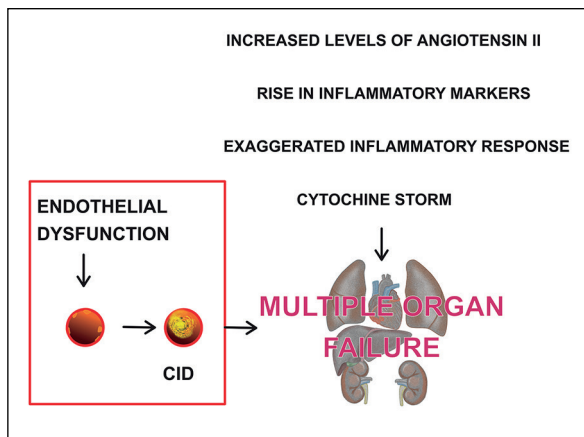

Figure 4. Main functions of the renin-angiotensin system.

Figure 5. Schematic representation of systemic inflammatory response ending with multiple organ failure.

treatment of COVID-19 complications and all of the drugs presenting a potential therapeutic strategy have been shown to improve endothelial function[28].

An increase in plasma levels of ferritin and several interleukins (IL), including IL-2, IL-6, IL-7, granulocyte colony stimulating factor (G-CSF), interferon (IFN) gamma, tumor necrosis factor (TNF) alpha, and monocyte chemoattractant protein-1 (MCP-1) has been observed in COVID-19 infection. Cytokine storm may induce T-cell apoptosis and necrosis, leading to a senescence status and count reduction. T-cells play a key role against viral infection, particularly through secretion of perforin, granzyme and IFN-γ. In COVID patients, a decrease of CD4+,
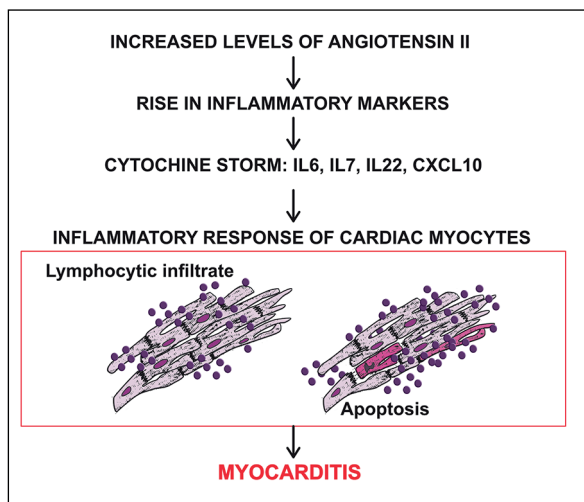

Figure 6. Inflammatory response of cardiac myocytes leading to myocarditis.

CD8+ and total T-cell numbers has been reported associated to lower survival[29].

Recently, high ferritin and IL-6 plasma values have been associated with lethality rate in patients with COVID-19 infection[30]. The lethality from COVID-19 infection varies from 2 to 9%, and is particularly high, up to 15%, in elderly patients over 65 years of age. The current therapeutic strategies to deal with infection is exclusively supportive and acute respiratory distress syndrome (ARDS) represents the major cause of mortality[30,31]. Tocilizumab, an IL-6 inhibitor, and Ruxolitinib that reduce the circulating values of pro-inflammatory interleukins such as IL-6 and TNF-alpha, have been proposed in the treatment of COVID-19 infection[32].

In children with COVID-19 infection, the respiratory involvement appears to have a more benign course. Nevertheless, increasing evidence suggests a correlation between COVID-19 and a severe Kawasaki-like disease, characterized by shock, multisystemic inflammation and systemic vasculitis that responds to a variety of immune-modulatory treatments, including intravenous immunoglobulins and corticosteroids. This entity has been provisionally called pediatric inflammatory multisystem syndrome temporally associated with SARS-CoV-2[33,34].

SARS COV-2 and Changes in Hemostasis and Thrombosis

Innate immune and blood coagulation are two ancestral defensive systems devoted to fight both viral and bacterial infections by trapping them into a fibrin network. However, an exaggerate response is dangerous because it can induce fatal vascular thrombosis. This mechanism may be common during COVID-19 infection because the virus enters cells which express the angiotensin converting enzyme 2 (ACE2) receptors (Figure 7). The attack of COVID-19 to the lungs may be devastating because it can induce a prothrombotic condition *via* a monocytes procoagulant activation and the release of several cytokines (IL-6, IL-1 and TNF alpha). The cytokine storm in turn causes a severe endothelial damage with both secondary pulmonary and alveolar micro-thrombosis resulting in a local consumption coagulopathy, as we recently suggested to occur in the COVID-19 infection[7] but also in other conditions, such as bacterial pneumonia and Chronic Obstructive Disease (COPD)[35].

However, the expression of ACE2 in the endothelium may allow COVID-19 entering so lead-
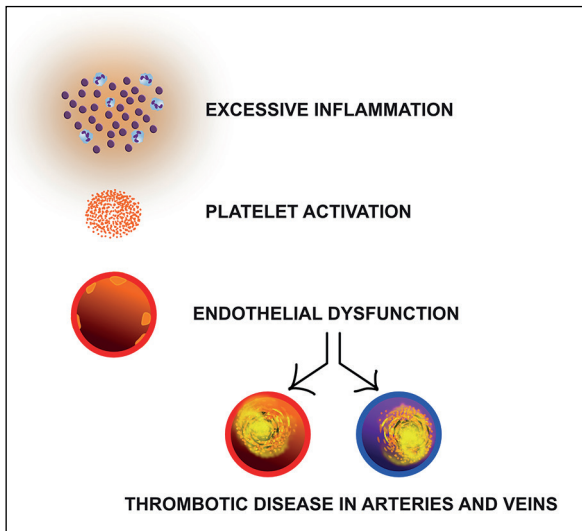
Figure 7. Schematic representation of SARS-COV-2 induced thrombotic disease.

ing to a severe endothelial damage with consequent venous and arterial thromboembolism. In the worsening of the disease, even a disseminated intravascular coagulation may occur. The most important test to follow during the disease is D-Dimer, a product of fibrinolysis activation, secondary to fibrin deposition. It reflects the amount of vascular thrombosis. High D-Dimer levels, more than 2 folds than the normal values, clearly indicate that a therapeutic anticoagulant approach is important by using either low molecular weight heparins or fondaparinux because of a prophylactic regimen failure.

The Systemic Fibroblast Activation

The vast majority of studies regarding SARS-CoV-2 have been focused on the acute consequences of the viral infection, and on its occasionally fatal outcome. Recently, the hypothesis is surfacing in the literature that COVID might have even important long-term implications[25]. The ability of angiotensin II to trigger fibrogenesis has been first described in hepatic stellate cells (HSCs), which are particularly susceptible to Angiotensin II, due to the high expression of ACE2 on their surface and to their ability into synthesize angiotensin II[36].

Up-regulation of angiotensin II favors transformation of HSCs in myofibroblasts, triggering liver fibrosis. Further studies showed that the fibrogenic effects of angiotensin II are not restricted to the liver. Fibroblastic activation due to angiotensin II is systemic, with possible involvement of lungs

(pulmonary fibrosis), heart (myocardial fibrosis, cardiac hypertrophy, hypertrophic myocardiopathy, atrial fibrosis with arrhythmias)[37] and kidneys (gomerulosclerosis, accelerated diabetic nephropathy)[38] (Figure 8).

The Respiratory Tract and Lung Disease Pathology

The early phases of lung involvement in patients affected by COVID-19 are characterized by edema, proteinaceous exudate, focal reactive hyperplasia of type II pneumocytes with patchy inflammatory cellular infiltration, and multinucleated giant cells, in the absence of hyaline membranes[39].

The histopathological picture in severe fatal cases is characterized by the typical markers of diffuse alveolar damage (DAD), including denuded alveoli due to type I pneumocyte death. Hyperplasia of type II pneumocytes, intra-alveolar fibrinous exudate, patchy interstitial lymphocytic infiltrate and loose interstitial fibrosis are the subsequent pathological events. At immunohistochemistry, SARS-CoV-2 was detected in damaged alveolar cells[40]. Moreover, disseminated intravascular thrombosis may be observed in septal interalveolar capillaries, due to a local blood coagulation activation probably triggered by SARS-CoV-2 induced endothelial damage[7].

Most of the papers related to SARS-CoV-2 and lung disease deal with the acute clinical pictures causes by COVID-19, often causing a severe acute respiratory distress syndrome, ending with
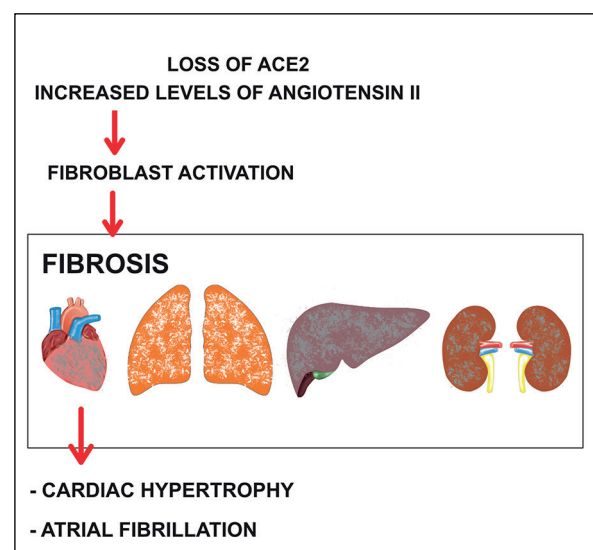

Figure 8. Systemic fibroblast activation by SARS-COV2.

fatal disease[41]. Previous data from other corona-virus infections, including severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS) suggest that survivors might undergo substantial fibrotic consequences[42] with significant long-term clinical implications. What is emerging from multiple studies, is that COVID-19 may present with multiple diverse courses, with a wide range of symptoms: at one extreme of the spectrum you can find asymptomatic subjects and at the other extreme, patients with respiratory failure[43]. Current clinical evidence indicate that pulmonary fibrosis might also complicate infection by SARS-CoV-2. The mechanisms underlying the association between COVID-19 and lung fibrosis are not fully understood. Advanced age, illness severity, smoking, chronic alcoholism and length of medical ventilation have been suggested as predictors of lung fibrosis in COVID-19 survivors[44]. Moreover, lung fibrosis was more likely to occur in patients with severe clinical conditions and with high inflammatory indicator[45].

SARS-CoV-2 has been shown to activate fibrosis-related genes, inducing transcriptional signatures in lung epithelial cells, ending with increased production of ACE2, TGFB1, CTGF and FN1 that are the drivers of lung fibrosis[46]. On these bases, Spagnolo et al[47] lays stress on pulmonary fibrosis secondary to COVID-19, suggesting a "call to arms" as the better answer to SARS-CoV-2 infection.

At histological level, the pathological report of three COVID-19 patients with fatal disease, who underwent minimal invasive autopsy, evidenced the presence of foci of organization of the exudate in some alveolar cavities, associated with interstitial fibrosis. The abnormal stimulation of myofibroblasts, evidenced by the foci of organization of the exudate, might represent the linkage between the acute damage caused by SARS-CoV-2 on alveolar cells and the development of interstitial fibrosis[48].

Cardiac Pathology: Thrombotic Events, Myocarditis, Arrhythmias, Myocardial Infarction, Hypertrophic Cardiomyopathy (Figure 9)

It is well known that different types of viruses, such as adenovirus, enterovirus and herpesvirus, but also coronavirus, may cause heart injury. Moreover, it is reported that the MERS-CoV can cause acute myocarditis and heart failure[49]. Some patients with SARS may present with
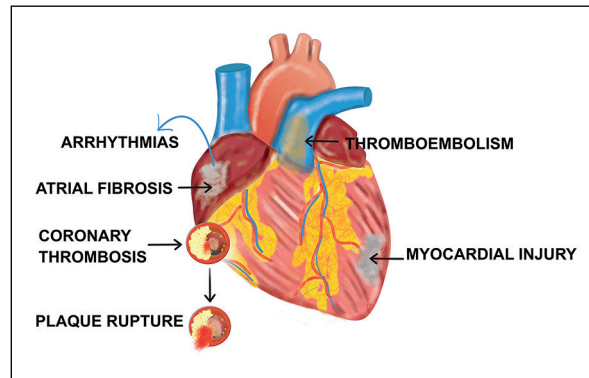

Figure 9. SARS-COV2: related cardiovascular complication.

a transient increase in myocardial enzymes. A challenge in coronavirus disease is represented by the occurrence of comorbidities, which may complicate patients' outcome. According to Wang et al[50], hypertension, diabetes mellitus, and cardiovascular diseases were the most common coexisting conditions in patients with COVID-19. Furthermore, compared with patients who did not received intensive care unit (ICU), patients who required it were more likely to have underlying comorbidities, such as those above described as well as cerebrovascular accidents.

COVID and Atherosclerosis: the Increased Risk of Developing Vulnerable Plaques

It is possible that the SARS-CoV-2 could play a significant role in the atherosclerotic pathway by triggering those molecular mechanisms related to the plaque's (both carotid and coronary arteries) vulnerability. This would also explain the increased prevalence of ischemic events in young subjects, carotid plaque-related, asymptomatic for lower respiratory tract illness, who have developed unexpected occurrence of ischemic events consistent with large-vessel stroke[51]. The consequences of the dysregulation of the renin-angiotensin system caused by the SARS-CoV-2 inside atherosclerotic plaques would determine endothelial dysfunction, leading to thrombosis; enhanced permeability of the endothelial barrier, favoring the invasion of the plaque by inflammatory cells and the insurgence of intra-plaque hemorrhage (IPH); accumulation of inflammatory cells, including neutrophils, activated monocytes, lymphocytes and plasma cells in the plaque.

The pro-inflammatory and pro-thrombotic activity of angiotensin II might transform a vul-

nerable plaque into a ruptured and complicated plaque. Based on these pathophysiological mechanisms, it appears possible that subjects infected with SARS-CoV-2 suffer an increased risk of conversion from asymptomatic, subclinical, atherosclerotic disease into an unstable state with vulnerable plaques in the carotid and/or coronary arteries due to the immunopathology associated with the viral infection.

Brain Disease, Cerebral Ischemic Stroke

The occurrence of acute neurological complications and of severe neurologic symptoms manifested as acute cerebrovascular disease and impaired consciousness was reported in patients with COVID-19. The few early imaging investigative efforts showed variable neuroimaging features of COVID-19 but dominated by acute ischemic and hemorrhagic disease, ranging from few foci of abnormal signal in the basal ganglia, thalami and subcortical regions to a large vascular territory infarct[52].

Similar to SARS and MERS, angiotensin-converting enzyme (ACE2) are functional receptors for SARS-CoV-2, also present in the central and peripheral nervous system and skeletal muscle[53]. Accumulating evidence suggests that a subgroup of patients with severe COVID-19 might have a cytokine storm syndrome which results in blood-brain-barrier breakdown, but without direct CNS viral invasion[27]. This may trigger for acute ischemic stroke, probably related to the prothrombotic effect of the inflammatory response. Although, it is still unclear whether some of the neurological manifestations might be owing to critical illness-related effects due to patient comorbidities, or from direct central nervous system CNS invasion of SARS-CoV-2.

Kidney Disease

Clinical and laboratory signs of kidney injury, including increased serum creatinine levels and new-onset hematuria or proteinuria, are frequently observed in up to 75% of patients affected by severe COVID[54]. The histopathological study of kidney samples in patients died following severe COVID evidenced diffuse proximal tubule injury with loss of the brush border, non-isometric vacuolar degeneration, and focal necrosis.

Moreover, red blood cells aggregates were observed in the lumen of peritubular capillaries[55]. In the same study, by transmission electron microscopy, viral particles were detected in proximal tubular cells and in podocytes, confirming the ability of SARS-Cov-2 to enter into these renal cells. A study[56] carried out on a large series of patients from Wuhan, China, evidenced that acute kidney injury is uncommon in COVID-19, and that SARS-CoV-2 infection does not aggravate pre-existing chronic kidney disease (CKD).

Gastrointestinal Tract Involvement

Some patients infected by SARS-CoV-2 present with gastrointestinal symptoms, including vomiting, diarrhea, and abdominal pain[57]. SARS-CoV-2 RNA has been detected in stool specimens of COVID-19 patients[58]. Moreover, the SARS-CoV-2 viral receptor angiotensin converting enzyme 2 (ACE2) is highly expressed in enterocytes, particularly in the ileum, suggesting the hypothesis that COVID-19 might cause gastrointestinal tract injury[59]. All these data taken together suggest that SARS-CoV-2 can actively infect and replicate in the gastrointestinal tract, being responsible of acute and, possibly, chronic consequences due to the systemic pro-fibrogenic effect of SARS-CoV-2.

Liver Acute and Chronic Pathology

The liver has been theorized to escape acute damage by SARS-Cov-2 infection, since abnormal liver function tests have been more frequently reported in patients admitted to intensive care unit than in patients with less severe disease. Liver injury was more evident during hospitalization especially related to certain medications including lopinavir/ritonavir that was discovered to may cause liver damage. Thus, the hypothesis of an iatrogenic drug-induced liver disease seems to be more likely than the virus wound[60].

In fact, no case of severe acute hepatitis due to SARS-Cov-2 has been described yet, besides hepatocytes have low/intermediate baseline expression of ACE-2, which represents the mandatory way allowing the virus entry into human cells. On the other hand, cholangiocytes[61] and hepatic stellate cells (HSCs) showed the expression of ACE-2 on their surface[36]. Thus, cholangiocytes and HSCs are the liver cells into which SARS-CoV-2 could actually enter. The link between ACE-2 and SARS-CoV-2 leads to the loss of ACE-2, leading to the decrease of the anti-inflammatory and anti-proliferative angiotensin. The increased levels of angiotensin II promote endothelial dysfunction, inflammation and fibrosis. The main consequence in the liver is the transformation of the quiescent stellate cells into

activated myofibroblasts, triggering liver fibrosis[36] whereas bile duct cell damage might induce the development of biliary fibrosis[61] (Figure 10).

Dysregulation of Iron Homeostasis: the Increase in Ferritin Serum Levels

Increased serum levels of D-dimer, Ferritin, interleukin-6 (IL-6), troponin, myoglobin and C-reactive protein (CRP) are considered important markers for the prognostic stratification of patients with COVID-19 admitted to hospital[62]. Ferritin is one of the most important actors in iron metabolism, being generally accepted as an ancestral defensive mechanism against infections[63]. During infections, viruses utilize iron stored in the host cells for their replication. Innate immunity, in order to halt infection, triggers production of IL-6, which stimulates hepatocytes to produce hepcidin that on one hand, halts iron absorption in the gut, and on the other hand hides iron stores in ferritin, blocked inside Kupffer cells[64]. Serum ferritin levels are very sensitive to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection but cannot be used for disease assessment[65].

Muscles Involvement

Myalgia is a common symptom at clinical presentation of COVID, being present in more than 30% of patients[66]. It has been claimed that skeletal muscle pain might be considered a negative prognostic factor, but a recent metanalysis indicates that the presence of myalgia should not be considered a prognostic factor for severe COVID-19 disease[67]. The pathological changes underlying myalgia in COVID patients is not always clarified. In rare cases, rhabdomyolysis has been reported as the cause of muscle pain[68].

Placenta Involvement

Placental infection with SARS-CoV-2, when occurring in pregnant women with severe acute respiratory syndrome (SARS), can induce placental insufficiency with severe maternal and fetal consequences, including miscarriage, preeclampsia, intra-uterine growth restriction (IUGR) and perinatal death (7-11%)[69]. The placenta has many defenses against hemorrhage that predispose it to thrombosis, most notably the high levels of tissue factor (TF) in placental trophoblasts and Plasminogen Activator Inibitor-2 (PAI-2) production. Histologic examination of COVID19-positive placentas showed features of fetal vascular malperfusion.

The main pathological changes were thrombosis in larger vessels in the fetal circulation, foci of avascular villi and villous stromal-vascular karyorrhexis, appearing as nuclear dust. Peri-villous and inter-villous fibrin deposition are also histological pathological changes always present in placentas infected by SARS-CoV-2[70]. The possibility of SARS-CoV-2 vertical transmission is still controversial[71,72], with only occasionally cases reported, accounting for less than 1% in some studies[73].

Conclusions

This review evidences the complexity of the interaction between SARS-CoV-2 and human cells. We tried to focus on the main known pathways involved in this viral infection, trying to link changes at molecular level with pathological changes occurring in multiple tissues and the different clinical presentations of the disease. Data here reported evidence that ACE2 is the main receptor of SARS-CoV-2 and the main entry point into cells for the virus. The expression of ACE2 in the majority of human cells does not explain the marked inter-individual variability in clinical presentation and outcome observed in subjects affected by COIVID-19[74]. Similar levels of ACE2 in the lungs and in the liver cannot explain the high frequency of lung involvement and the rarity of severe liver involvement. Moreover, no significant different ACE2 expression levels
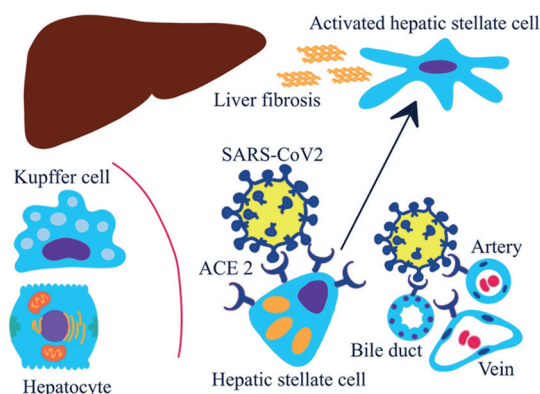

Figure 10. Schematic representation of liver changes due to SARS-COV2 infection.

were detected between younger and older people, nor between males and females, suggesting that other factors are involved in the pathogenesis of COVID-19[74].

The viral effects on the multiple cell types involved evidence the complexity of this disorder, and we are well aware that many questions remain without a certain answer.

Why some organs, including vessels, lungs, heart and brain are severely damaged and other apparently are less involved in the acute phases of COVID-19? What will happen, in survivors, in the multiple organs affected, following the acute phase of the disease? Will pulmonary fibrosis develop in affected lungs? Might organs like liver, rarely affected in the acute phase of the disease undergo chronic disease due to activation of stellate cells, though their transformation into myofibroblasts, ending with liver fibrosis?

A key point in the pathogenesis of COVID-19 is the interaction of the viral spike protein with ACE2 expressed on the cell surface of multiple cells types. However, the expression of ACE2 is not the single factor at the basis of the selective involvement in this disease. Currently the knowledge of the interactions between COVID-19 and human cells is partial and further studies are warranted to better clarify the relationship between molecular, pathological and clinical events following this viral infection. Since patients infected by SARS-CoV-2 frequently show increased cytokine production associated with deregulated T-cell homeostasis, typical features of older and diabetic subjects with hypomagnesemia[75], we may hypothesize that magnesium deficiency may predispose to a severe outcome of COVID-19. The interpretation of the growing imaging and tissue data available might receive an important support from artificial intelligence-based characterization techniques[76], which will allow a prognostic classification of acute and chronic COVID-19-related human pathology.

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