The enzymes in COVID-19: A review

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A B S T R A C T

COVID-19 brought a scientific revolution since its emergence in Wuhan, China, in December 2019. Initially, the SARS-CoV-2 virus came to attention through its effects on the respiratory system. However, its actions in many other organs also have been discovered almost daily. As enzymes are indispensable to uncountable biochemical reactions in the human body, it is not surprising that some enzymes are of relevance to COVID-19 pathophysiology. Past evidence from SARS-CoV and MERS-CoV outbreaks provided hints about the role of enzymes in SARS-CoV-2 infection. In this setting, ACE-2 is an enzyme of great importance since it is the cell entry receptor for SARS-CoV-2. Clinical data elucidate patterns of enzymatic alterations in COVID-19, which could be associated with organ damage, prognosis, and clinical complications. Further, viral mutations can create new disease behaviors, and these effects are related to the activity of enzymes. This review will discuss the main enzymes related to COVID-19, summarizing the findings on their role in viral entry mechanism, the consequences of their dysregulation, and the effects of SARS-CoV-2 mutations on them.

Contents

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1. SARS-CoV-2 and COVID-19

First reported in December 2019, it is possible to state that since its emergence in Wuhan, Hubei Province, China, the SARS-CoV-2 virus brought a scientific revolution in the year 2020, but not for the best reasons [1] COVID-19 has shown in recent months how devastating and deadly a virus can be. Initially indicated as a virus affecting the respiratory system, recent studies have discovered effects in several of the organs of the human body, and biological pathway [2].

Among the 10–20% of the individuals who contracted the disease require hospitalization or admission to intensive care. SARS-CoV-2 enters the human body cells through the angiotensin-converting enzyme 2 (ACE-2) on the cell surface, which is responsible for controlling blood pressure, therefore they can be found in almost every tissue of body and the number of receptors is closely related to how the virus affects each organ [3]. After invasion, the immune system reaction is responsible for the cytokine storm, which leads to inappropriate responses that cause tissue damage and hence multi-organ failure and death [4].

A recent report demonstrated the cleavage of human proteins by the SARS-CoV-2 papain-like protease (PLpro) affected proteins, reported were: MYH7 and MYH6 (cardiac myosins linked to several cardiomyopathies), FOXP3 (transcription factor), ErbB4 (HER4) and plasma protein S (PROS1), an anticoagulation protein. Cleaving these proteins reduces their activity and may be associated with several serious complications of COVID-19 [5,6].

COVID-19 has infected up to 300 million people and have already killed more than 5.5 million around the world (World Health Organization) and the biggest challenge has been finding a cure or approved treatment for the disease. Furthermore, the detection of new variants has created a concern among the scientific community since it is not yet clear as to whether newly developed vaccines are protective against all variants.

2. Enzyme dysregulation consequences

Enzymes are indispensable to uncountable biochemical reactions in the human body, with roles in health and disease [7–9]. Thus, it is not surprising that some enzymes are of relevance to COVID-19 pathophysiology.

The SARS-CoV-2 interaction with host enzymes is responsible for a variety of physiological changes (Table 1). At the biochemical level, severe COVID-19 patients have significant disorders such as high levels of C-reactive protein (CRP) [10], Alanine, Aspartate aminotransferase (ALT and AST) [12–14], lipase [15–17], high levels of changes in the level of thrombin [18] low levels of lymphocytes (lymphopenia) [11] and protein S [5].

It's known that SARS-CoV-2 uses ACE-2 as a viral receptor to enter the human host cell, so it is not surprising that the physiological changes and ACE-2 expression increase in some organs such as lung and stomach are present in many recent studies [19–27]. The Creatine Kinase Myocardial Band (CK-MB) elevation in some patients with COVID-19 was associated with heart failure, was suggest by Qin et al. [28] and Shafi et al., 2020 [29]. Enzymatic alterations also are evident in the brain with DPP4 and APN, but more studies are necessary, because it's not clear yet how these alterations occur [30].

Past knowledge about SARS-CoV and MERS-CoV outbreaks provided a start point to investigate SARS-CoV-2 infection mechanisms, especially regarding viral entry mediated by enzymes, such as ACE-2, TMPRSS2 and DPP4, both enzymes present on the membrane surface that is also be the coronavirus receptor for cell entry — TMPRSS2 For SARS-CoV and DPP4 for MERS-CoV [3,31,32]. A vast amount of clinical data has been revealing COVID-19 enzymatic alterations patterns, including cardiac, pancreatic, and hepatic ones associated with organ damage (Fig. 1) [12,16,28]. Beyond that, some of these alterations could have a role in prognosis [12] or cause a clinical complication directly [33].

2.1. Angiotensin-converting enzyme-2

Angiotensin-converting enzyme 2 (EC 3.4.17.23; ACE-2) is responsible for regulating the level of angiotensin II in the organism, in which it has a potent vasoconstrictor effect, promotes apoptosis, angiogenesis and cell proliferation in several cell types [34]. ACE-2 removes only the last amino acid from the protein substrate at the carboxyl end, transforming the octapeptide angiotensin II into angiotensin 1-7, which has the opposite action [35].

Several studies show that to enter human cells, the SARS-CoV-2 Spike protein interacts with human ACE-2 receptor present on the surface of alveolar epithelial cells, in venous and arterial endothelial cells, in macrophages, as well as in most organs, especially the lung, heart and kidneys [31,34,36–38].

It was observed in acute lung injury or in acute respiratory distress syndrome (ARDS) that angiotensin II participates in worsening lung damage by having a vasoconstrictor, pro-inflammatory, thrombosis and apoptotic action [38].

Table 1
SARS-CoV-2 related to host enzymes.

ORGAN	REMARKS	REFERENCES
BRAIN	Lack of ACE-2 expression in neuronal cells. DPP4 and APN as potential virus receptors.	Cheng et al., Saleki et al., 2020; Steardo et al., 2020
HEART	The MYH6/7 are dysregulated by PLpro. Elevated CK-MB were significantly increased cardiac injury and risk of COVID-19 death.	Reynolds et al., 2021; Perez-Bermejo et al., 2021; Qin et al., 2020; Shafi et al., 2020
EYES	ACE-2 is expressed in conjunctival samples at a low level, while BSG and TMPRSS2 are expressed at intermediate levels in both conjunctiva and cornea.	Leonardi et al., 2020
LIVER	Alterations in liver enzymes (ALT, AST) may correlate with worse clinical course. These changes can be due to either the use of antiviral and antibacterial agents or related to a hyper-inflammatory status and thrombotic microangiopathy.	Medetalibeyoglu et al., 2020; Bertolini et al., 2020; Boregowda et al., 2020 Agarwal et al., 2020; Puli et al., 2020
PANCREAS	Lipase elevation is seen in COVID-19 and associated with worse disease outcomes.	Wang et al., 2020; Barlass et al., 2020.
LUNGS	ACE-2 acts as a receptor to enter human cells. Its expression was mainly associated with innate and acquired immune responses, and regulation of B cell mediated immunity, as well as cytokines. Higher expression may prolong the virus life cycle. TMPRSS2 also facilitates entry to cells. Nonmuscle myosin MYH9 heavy chain IIA as an ACE2 coreceptor to promote infection.	Li et al., 2020; Thunders and Delahunt, 2020; Chen et al., 2021.
KIDNEYS	ACE-2 is found in abundance in the kidney then may be one of the targets of SARS-CoV-2 infection.	Hassanein et al., 2020; Martinez-Rojas et al., 2020
BLOOD	The viral enzyme PLpro cut Protein S, a cofactor to Protein C. Thrombin function is affected resulting in pro-inflammatory events, venous thromboembolism and stroke.	Reynolds et al., 2021; Li et al., 2020

Abbreviations: ACE-2- Angiotensin-converting enzyme 2; ALT- Alanine Aminotransferase; APN- Aminopeptidase N; AST- Aspartate aminotransferase; CK-MB- Creatine kinase; DPP4- Dipeptidyl peptidase-4; LDH- lactate dehydrogenase; MYH6/7 — Cardiac myosin; PLpro — Papain-like protease and TMPRSS2 -Transmembrane serine protease 2.
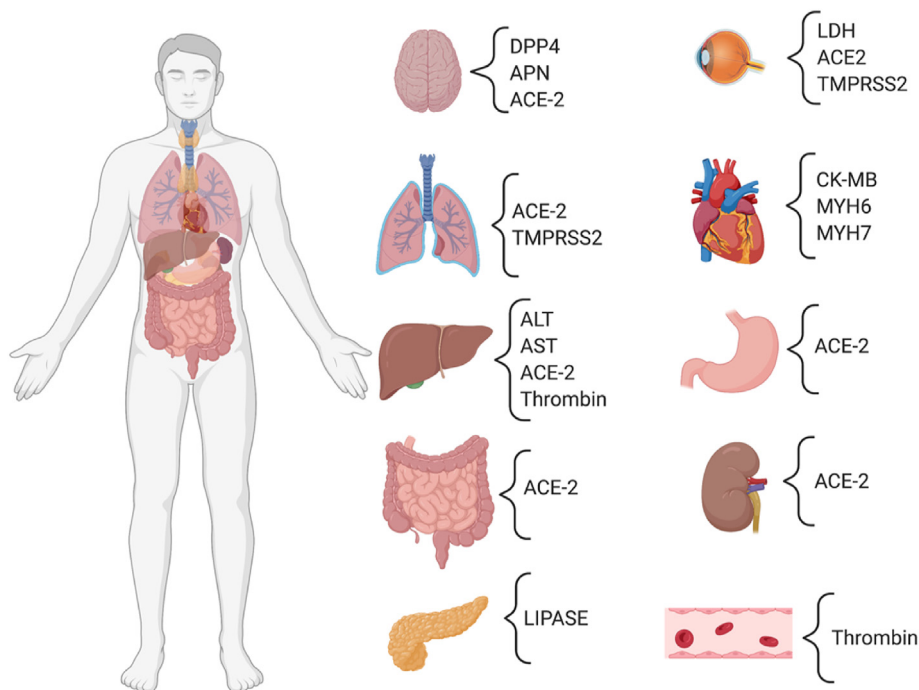
Fig. 1. Research evidence of enzymatic alterations. Made by biorender.com.

Cells infected with SARS-CoV-2 were analyzed suggesting that ACE-2 is not only the receptor, but also acts in the post-infection phase, with immune response, the amino-terminal head domain of MYH9 possesses ATPase activity and facilitates entry [6], cytokines and viral genome replication [1]. Then, the negative regulation of ACE-2 due to the action of SARS-CoV-2 results in the accumulation of angiotensin II in the body, aggravating the disease and causing lung injury [38].

Li et al. [31] showed in their work that the expression of ACE-2 increased after 24 h of SARS-CoV 2 infection and that even after 48 h, the expression of ACE-2 remained at a high level, indicating that ACE-2 acts in viral susceptibility. Still in their work, Li et al. [31] speculated that the high expression of ACE-2 resulted in the cytokine storm, further aggravating the symptoms of ARDS, as well as increasing the expression of genes involved in viral replication, which may increase the ability of the virus to enter host cells.

2.2. Transmembrane serine protease 2

Transmembrane serine protease 2 (EC 3.4.21; TMPRSS2) is a serine protease that acts on the cleavage of peptide bonds that have serine as the main amino acid at the active site. Expressed mainly on the cell surface of the respiratory and digestive endothelium, it can be found in heart, liver, and corneal cells [39–42].

TMPRSS2 is produced as a zymogen that undergoes catalytic activation. The exact biological function of TMPRSS2 is still unknown, however, several studies have been associated with physiological and pathological processes such as digestion, inflammatory responses, tumor cell invasion, apoptosis, and viral entry [39–45].

As in influenza, SARS-CoV-2 is dependent on TMPRSS2 for viral entry and spread in the host. For a better understanding, first, the viral spike protein binds to the angiotensin-converting enzyme 2 (ACE-2), expressed in respiratory epithelial cells. Subsequently, the spike is cleaved to activate the internalization of the virus. However, this step only occurs in the presence of proteases from the host cell, particularly TMPRSS2 [39,44,45].

In some studies, healthy wild rats infected with coronavirus developed acute pneumonia, lost weight, and had a strong expression of TMPRSS2. In comparison, knockout rats with negative TMPRSS2 expression were also infected and did not develop pneumonia, presenting a lower viral replication in the lungs and reduced inflammatory responses when compared with wild type mice, suggesting that TMPRSS2 is indispensable for viral replication [46].

Also, Hoffmann et al., 2020 [44] presented a study with evidence of the dependence of ACE-2 and TMPRSS2 for the host cell entry of SARS-CoV-2. The authors also found that inhibiting the serine protease using Camostat mesylate, a drug approved in Japan for pancreatitis treatment, can block the SARS-CoV-2 entry, suggesting an attractive option to COVID-19 treatment.

Several studies mentioned the ACE-2 and TMPRSS2 presence expressed highly in intestinal epithelial cells, which are the target cells for many enteric viruses [40]. Many patients with COVID-19 have symptoms such as abdominal pain and diarrhea, often before having respiratory symptoms.

Zang et al., 2020 [47] used CRISPR/Cas9 gene editing tool to decrease TMPRSS2 expression in intestinal epithelial cells, evidencing ACE-2 and TMPRSS2 higher levels are correlated with SARS-CoV-2 infection. Also in their study, they tested the inhibition of TMPRSS2 using Camostat mesylate, reducing the viral replication and consequently the SARS-CoV-2 infection.

Recent studies have been found both ACE-2 and TMPRSS2 expressed in human corneal epithelium (at low and intermediate levels respectively) suggest ocular surface cells could also be potential viral entry points [41,42].

Thus, TMPRSS2 actively participates in the mechanism of viral entry and replication and can also be contained with the use of protease inhibitors, being the target of many future studies.

2.3. Creatine Phosphokinase-MB

COVID-19 is viral infection with predominately respiratory manifestations, but, since patients with cardiovascular comorbidities have higher mortality, and the severity of disease correlates

with cardiovascular manifestations, it is important to understand the interaction of COVID-19 and cardiovascular disease complications [29,48]. The cardiac myosin heavy chain (MYH6 and MYH7) are mechanical enzymes involved in cardiac muscular contractility. Mutations in this myosins are associated with cardio myopathies. The new reports show the MYH6/7 are cleaved with the viral PLpro resulting in myofibril damage after SARS-CoV-2 infection [5,49]. The presence of secondary myocardial injury was associated with more than 50% mortality in COVID-19, increasing the importance of monitoring cardiac biomarkers [29].

Creatine kinase or Creatine phosphokinase (EC 2.7.3.2; CK) is responsible for the conversion of creatine and uses adenosine triphosphate (ATP) to create phosphocreatine and adenosine diphosphate (ADP). This enzyme is composed by the union of two subunits: type B and/or M, which correspond to the isoenzymes CK-BB (brain), CK-MB (heart) and CK-MM (muscle) [26]. The MB isoenzyme has high sensitivity and specificity for the diagnosis of cardiac injury and the raising it correlates with the volume of injured tissue and the prognosis. In clinical medicine, CK-MB is an essential biomarker used for the diagnosis of cardiovascular diseases and has been studied in forensic medicine for approximately 20 years [27].

Qin et al. (2020) [28] demonstrated an evaluation of cardiac biomarkers included CK-MB related to COVID-19 mortality. The authors related severe symptoms and disease progression associated with elevated levels of cardiac biomarkers (troponin I and NT-pro BNP, CK-MB, and MYO) and associated those patients with increased CK-MB and other biomarkers had a decreased survival rate and more occurrences of heart failure, sepsis, or multiorgan failure compared with those with normal levels.

Many studies connect CK-MB elevated in critical patients group compared to noncritical; in 31.68% patients at admission to ICU and in 55.45% in patients at 48 h to death [29,50–52]. Thus, the authors concluded these findings are consistent with that cardiac injury biomarkers are associated with an increased risk of COVID-19 mortality.

2.4. Thrombin

Many reports address an altered coagulation status and the occurrence of thrombotic events in patients with COVID-19, like venous thromboembolism and stroke [3,31,53–55]. Thrombin (EC 3.4.21.5) is a crucial enzyme in the blood coagulation cascade, and its excessive generation can cause thrombotic complications [56]. Several stimuli, mainly vascular injury, trigger thrombin generation from prothrombin by the action of factor Xa [57].

Thrombin is well-known for converting fibrinogen into fibrin, enabling fibrin clot formation, but it also acts on multiple substrates. Thrombin cleaves protease-activated receptors (PARs) 1 and 4, leading to platelet activation; further, it activates the factors V, VIII, and XI, which create a burst of its generation [58]. Moreover, thrombin activates the fibrin-stabilizing factor (factor XIII) and the thrombin-activatable fibrinolysis inhibitor (TAFI). All these actions contribute to clot formation and maintenance. This enzyme yet has an anticoagulant function − thrombin activates protein C down-regulating the coagulation cascade [59,60].

Severe COVID-19 patients often develop a hyperinflammatory systemic response and a cytokine storm, presenting a pronounced increase in cytokines, chemokines, and other inflammatory markers, like IL-6, IL-1, MCP-1, TNF-α, and G-CSF [18,28,61]. Although SARS-CoV-2 can directly affect the endothelium and hence the thrombin generation and the coagulation state, inflammation, as well, is likely to disrupt the pro-coagulant and anticoagulant balance in these patients [62].

Besides its coagulation action, thrombin is related to inflammation, through PARs especially. Endothelial cells, platelets, leukocytes,

fibroblasts, and vascular smooth muscle cells express these receptors, for instance. In conforming to the cell type, PARs activation drives the release of cytokines, chemokines, and adhesion molecules, such as IL-1, IL-6, IL-8, MCP-1, and P-selectin [63]. Thrombin also can activate PARs 1 and 4 from sources other than platelets, still activating PARs 3. This allows thrombin to modulate cell responses. Many variables, like thrombin concentration, determine whether these effects are pro-inflammatory or anti-inflammatory [64].

Likewise, inflammation stimulates the thrombin generation by expressing tissue factor, which activates coagulation. Inflammation and coagulation, therefore, are linked and affect each other [65]. More, under inflammation, coagulation participates in immuno-thrombosis, an element of the innate immune response. Immuno-thrombosis arises with the recognition of pathogens or damaged cells. Its pathways include an enhanced thrombin generation, resulting in microthrombi formation as a protective and containment mechanism against pathogens [66,67]. Such complex interactions corroborate to thrombin to play a role in some viral infections [68,69]. Immunothrombosis itself has been proposed as a mechanism in COVID-19 pathogenesis [57,70]. As the crosstalk between coagulation and inflammation is a critical element in COVID-19, thrombin exerts a significant influence in this process [33,71].

Several articles describe thrombin generation in patients with COVID-19. A prospective cohort study assessed thrombin generation (prothrombin fragment 1 + 2 (PF 1 + 2)) in COVID-19 patients with acute respiratory distress syndrome (ARDS) [72,73]. Elevated thrombin generation was observed, which was similar at baseline in survivors and non-survivors. At follow-up, it was noticed a decrease in thrombin generation in survivors. However, patients that eventually evolved to death (non-survivors) maintained initial levels or even higher ones. A retrospective cross-sectional study evaluated thrombin generation (PF 1 + 2, thrombin-antithrombin complex (TAT), thrombography) in critical and noncritical COVID-19 patients, which was not significantly different between these groups [74]. These two studies possibly highlight that one could not use thrombin generation markers to discriminate disease severity with one only measure. Furthermore, their behavior through time could perhaps indicate how the disease is evolving. It remains unknown whether thrombin generation routine assessment is advantageous to COVID-19 clinical practice and clinical outcomes, requiring further research.

Thrombin indirect inhibition by heparin has been used as thromboprophylaxis for COVID-19 patients since it was associated with lower mortality [75,76]. Considering also that thrombin plays multiple roles aside from coagulation, its inhibition could maybe present anti-inflammatory and anti-viral effects [62]. However, there is yet a lack of evidence about appropriate anticoagulation therapy [5,8,77]. An investigation assessed thrombin generation (thrombography) in COVID-19 patients who received heparin thromboprophylaxis [78]. The analyzed parameters were normal, even in anticoagulation. It suggests an increased thrombin generation capacity and a procoagulant state that was still uncontrolled [79]. This result appears to agree with reports that COVID-19 patients could present thromboembolic events, despite thromboprophylaxis [80,81].

Additionally, regarding coagulation disorders in COVID-19, protein S is noteworthy. Protein S is a vitamin k-dependent glycoprotein, mainly known as an anticoagulant cofactor for activated protein C and tissue factor pathway inhibitor [82]. Still acting as an anticoagulant, this protein inhibits prothrombinase and intrinsic tenase. Its influence on coagulation is palpable since protein S deficiencies are related to elevated thrombotic risk [83]. Clinical data revealed low activity of protein S in COVID-19 patients, suggesting this may contribute to coagulopathy in COVID-19 [84,85]. Also, backing this suggestion, SARS-CoV-2 papain-like protein cleaves a sequence within protein S, and this cut may result in impaired

protein S function or secretion in the course of the viral infection [5].

2.5. Dipeptidyl peptidase 4

Dipeptidyl peptidase 4 (EC 3.4.14.5) is a serine peptidase that exists both bound in the cell surface and as a soluble form in plasma and other body fluids [86]. DPP4 is a functional entry receptor in MERS-CoV, which raised questions about its role in COVID-19 [31,32]. Its catalytic activity consists of releasing N-terminal di-peptides whenever proline or alanine is the penultimate amino acid [87,88]. DPP4 acts on several substrates through its enzymatic function, like chemokines, cytokines, and growth factors [89]. Its actions on inactivating incretin hormones allow using DPP4 inhibitors, or gliptins, as anti-diabetic drugs in type 2 Diabetes Mellitus (T2DM) treatment [90].

Many cells express DPP4, such as epithelial, endothelial, and immune cells, in kidneys, lungs, liver, spleen, bone marrow, pancreas, and intestine [91]. Its expression on immune cells is broad; indeed, DPP4 is also known as T-cell antigen CD26 due to its co-stimulatory function in T-cell activation [92]. Likewise, DPP4 acts on apoptosis, chemotaxis modulation, and cell adhesion [93]. Since this enzyme participates in immune responses and inflammation, DPP4 may be involved in immune and inflammatory diseases [94]. Accordingly, DPP4 inhibitors have been related to some anti-inflammatory effects [95]. As inflammation in COVID-19 immunopathogenesis is a concern, DPP4 inhibitors could narrow disease progression to severe forms [96,97]. Further, considering diabetes is a risk factor for COVID-19 adverse outcomes, it is of interest to study the associations between clinical outcomes and gliptins use [98].

Computational approaches predicted that SARS-CoV-2 spike protein and human DPP4 could interact, hinting DPP4 may play a role in virus entry into host cells along with ACE-2 [31,99]. Such prediction paved the way for several hypotheses about DPP4 inhibitors antiviral effects in COVID-19, besides anti-inflammatory and immunoregulatory effects already proposed [96,100,101]. Another in silico study suggested DPP4 inhibitor could also exert an antiviral activity by inhibiting SARS-CoV-2 main protease [102]. However, functional studies demonstrated that the spike protein and DPP4 do not bind, therefore, suggesting this enzyme does not act as a cell entry receptor for SARS-CoV-2 [103,104]. As well, gliptins did not experimentally inhibit the main protease [105]. Other pathways to SARS-CoV-2 interact with DPP4 are still being hypothesized [106,107].

Many observational studies evaluated gliptins use and COVID-19 outcomes in T2DM patients, although the results are quite contrasting [108]. Several of them couldn't associate DPP4 inhibitors use with COVID-19 evolution, such as clinical severity or death [63,109–112]. Another study reported that treatment with sitagliptin throughout hospitalization was associated with decreased mortality and improved clinical outcomes [113]. Also, DPP4 inhibitors treatment before hospitalization was associated with lower mortality risk [114]. All these studies have limitations due to their observational and retrospective nature, but the ones that presented positive results [113,114] received critics regarding methodology, confounders, or shortcomings. In this setting, there is no established evidence of DPP4 inhibitors being beneficial to COVID-19 clinical outcomes. Randomized and prospective clinical trials are required to assess this properly [106,109].

2.6. Aminopeptidase-N

Aminopeptidase N (EC: 3.4.11.2; APN), also known as CD13, is a cell-surface and zinc-dependent metalloprotease [115]. It cleaves N-terminal amino acids from unsubstituted oligopeptides, as long as proline is not the penultimate amino acid [116]. Several cells and tissues express this ectopeptidase, like epithelial cells, endothelial cells, leukocytes, fibroblasts, mucosal cells of the small intestine, and synaptic membranes [40,115]. Also, APN exists in a soluble form in plasma [117].

Besides its enzymatic activity, APN mechanisms include endocytosis and signal transduction. These three mechanisms often overlap to achieve a function [118]. APN is extensively present in immune cells, regulating their development and activities. Further, APN catalytic action on hormones, chemokines, and cytokines modulates inflammation. Through this inflammation role, APN are related to some inflammatory conditions [119]. Of note, HCoV-229E is a coronavirus that causes common cold, and it uses human APN as a receptor to enter host cells [120,121].

Considering a possible role for APN as a SARS-CoV-2 receptor, a few studies assessed its expression in tissues, along with ACE-2. Ocular conjunctiva expressed APN at low levels, as well as ACE-2 [122]. In chronic colitis, neither ACE-2 nor APN expression in the gut was different compared to healthy controls [123]. However, evidence showed that aminopeptidase N is not a SARS-CoV-2 entry receptor [44,112]. Despite that, APN and ACE-2 coexpression in human tissues could suggest a function as an auxiliary protein [124]. In this regard, Devarakonda et al. [125] proposed that APN could affect SARS-CoV-2 pathogenesis by other pathways, such as immune response amplification and altering infectivity.

2.7. Alanine and aspartate aminotransferase

Aspartate aminotransferase (EC 2.6.1.57; AST) and alanine aminotransferase (EC 2.6.1.2; ALT) are intracellular enzymes, found specially in the liver. They catalyze reactions inside the cells, which means that their presence in the systemic circulation might indicate liver disfunction or damage, mainly those involved in liver cell membrane disruption [126].

It is already clear that the COVID-19 virus enters the cell from, among others, the ACE-2 (angiotensin-converting enzyme 2) receptor. ACE-2 helps the conversion of Angiotensin II (Ang II) in Angiotensin 1-7 and, therefore, helps with the vascular pressure regulation. The virus competes with Ang II and the blood pressure control stays in deficit. It is important to notice that people with some comorbidities, like hypertension, express more ACE-2 receptors, due to upregulation, which can augment the virus effect on the organs. This can also happen in the liver and the bile duct: because of ACE-2 high expression in the bile duct cells, it can lead to damage to these cells, which can promote liver injury [22]. It is important to point that it is still not clear while the damage to the liver is due to the cytokine storm or the direct cell damage promoted by the virus or both. Still, there is no denying the damage that the disease can do to the liver. It is shown that SARS-CoV-2 can produce liver damage, especially in people with other comorbidities, and therefore increase ALT and AST, which can show liver damage [12]. According to this study, the bigger the ratio between ALT and AST, the worse is the patient prognosis, which means that patients with ALT/AST >1 reported more hospital time and intensive care unit hospitalization, demonstrating the relation between liver damage and progression of the disease.

Other studies also correlated the elevation of serum ALT and AST to worse prognosis and higher mortality [13,14]. It is interesting to notice that liver abnormalities, proved by elevated ALT and AST were found in up to 54% of patients, when comparing studies. Even with the liver damage, there has been no reports on liver failure due to COVID-19 [23]. According to Boregowda et al., 2020 [14], when serum liver enzymes were analyzed among the non-survivors, they were elevated compared to survivors.

These results are important to help the treatment of COVID-19 in the Intensive Care Units, so that healthcare workers can avoid prescribing drugs that can promote liver damage and probably worsen the patient's prognosis.

2.8. Lipase

Lipase (3.1.1.3) is an enzyme produced by the pancreas and is responsible for hydrolyzing triacylglycerol into fatty acid and glycerol for absorption in the duodenum [127]. It can be normally found in the blood stream, but its elevation may indicate pancreatic injury. It is interesting to note that the pancreas has a great number of ACE-2 receptors, even more than the respiratory system [16], which would make this organ more vulnerable to SARS-CoV-2 infection, however lipase serum levels are only elevated in more severe initial presentations of COVID-19 [17]. Up to 17% of severe respiratory syndrome patients were reported with lipase serum levels higher than normal in COVID-19 [15], but severe pancreatitis has not been reported [128]. According to Wang et al., 2020 [15], the pancreas damage can be a result to a direct viral infection, a hypoxic injury due to respiratory failure or due to the cytokine storm promoted by the immune system. Patel et al., 2020 [128] also reported a pancreatic injury due to antipyretics. About the prognosis, McNabb-Baltar et al., 2020 [17] correlated an increase in lipase to severe presentations, but the outcomes were not always bad; while Barlass et al., 2020 [16] suggested worst outcomes due to lipase elevations.

It is interesting to notice that lipase elevations can happen because of nonpancreatic diseases, like gastritis and enteritis [17] due to the gastrointestinal system infection by SARS-CoV-2. This shows why pancreatic damage is not always a COVID-19 symptom, but elevated levels of serum lipase can help the diagnosis.

2.9. Lactate dehydrogenase

During glycolysis pyruvate can be converted into lactate in cases of lack of oxygen, such as hypoxia and anaerobic conditions. This reaction provides a fast source of energy and is catalyzed by lactate dehydrogenase (EC 1.1.1.27; LDH). After that, the produced lactate is taken to the liver, where is reconverted to glucose and can be reused to produce more energy [126].

This enzyme was previously used to evaluate damage in cardiac or skeletal muscles, because its serum levels increase substantially when there is not enough oxygen for tissue. LDH levels also get higher in severe infections [129], due to tissue damage. During COVID-19 pandemic, some systemic reactions were noticed, such as the cytokines storm, elevated lymphocyte levels and lung lesions, among others [130].

Some authors also noticed an elevation of lactate dehydrogenase levels in severe cases of COVID-19 and that this enzyme is related to worse outcomes [31,129,130]. This enzyme is released in tissue damage, the cytokines storm can explain the enzyme elevation, because the multisystemic inflammation promotes mostly lung damage, which can lead to pneumonia, but also cardiac, liver, and renal damage. LDH is also highly expressed in the lung tissue, according to Henry et al., 2020 [130], so patients with severe disease, mostly the ones affected by acute respiratory syndrome, have presented elevated LDH levels. These authors have also noticed that this enzyme elevation occurred in Middle East Respiratory Syndrome (MERS), confirming the presence of the enzyme in lung tissue.

Another author pointed LDH as a more effective marker for severe disease when compared to lymphocyte counts and D-dimer [31], with more sensitivity and specificity. Another author studied the LDH/lymphocyte ratio to evaluate worse COVID-19 outcomes
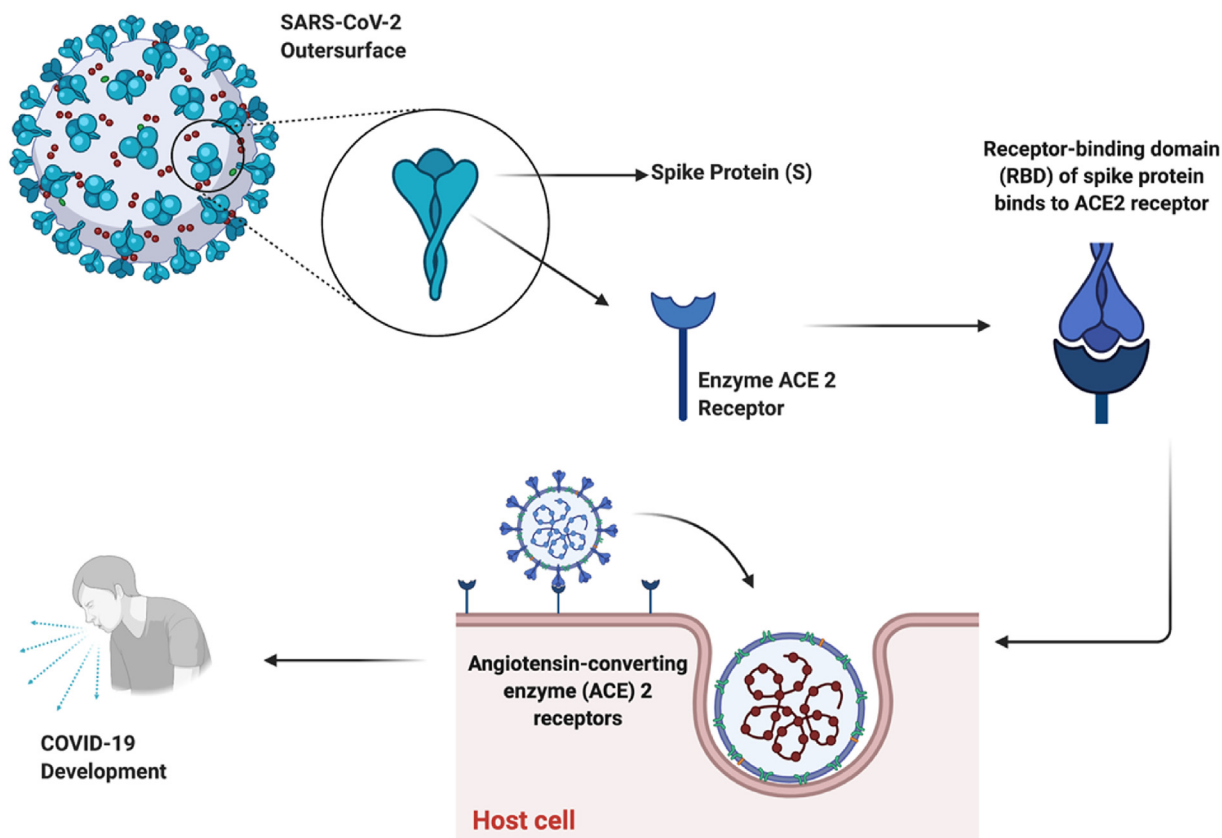

Fig. 2. Angiotensin-converting enzyme 2 (ACE-2), SARS-CoV-2 and the pathophysiology of coronavirus disease 2019 (COVID-19). Made by biorender.com.

[131]. According to this study, the ratio can be used to facilitate diagnosis, since PCR has shown a great number of false negatives. However, it may not be used as a differential diagnosis since this ratio is not known in other diseases. Lactate dehydrogenase has shown to be a powerful biomarker and predictive to worse outcomes in COVID-19 disease, which can lead to more attention to patients presented with higher levels of the enzyme.

3. Entrance and viral enzymes

As described above, SARS-CoV-2 is a type of coronavirus which is dependent of the successful host cell entry for their replication and release of new virions.

It's widely known that the SARS-CoV-2 have the crown appearance due the spike glycoprotein presents in their envelope surface (Fig. 2). This large glycoprotein (approx. 180 kDa), Spike

protein, or S protein has two domains (S1 and S2). Initially, the S protein binds a specific cellular receptor which leads to a series of proteolytic events resulting in the fusion of cell and viral membranes. Specifically, the receptor-binding domain (RBD) present on S1, which is responsible for binding the cellular receptor angiotensin-converting enzyme 2 (ACE-2) as mentioned above (Topic 2.1), and S2 domain is responsible for start the membranes fusion event (Fig. 3) [132,133].

For the successful viral replication occur, depending on SARS-CoV-2 strain and host cell type, several proteases as ACE-2, transmembrane serine proteases 2 (TMPRSS2) Furin and Cathepsin L is involved to active and cleavage of the S protein [134,135]. After the SARS-CoV-2 binding onto the ACE-2 receptor (Fig. 3, step 1), the S1 subunit proteolysis may facilitated by plasma membrane-bound serine protease TMPRSS2 and Cathepsin L [134]. As described previously (Topic 2.2), TMPRSS2 is a cell-surface protein which has
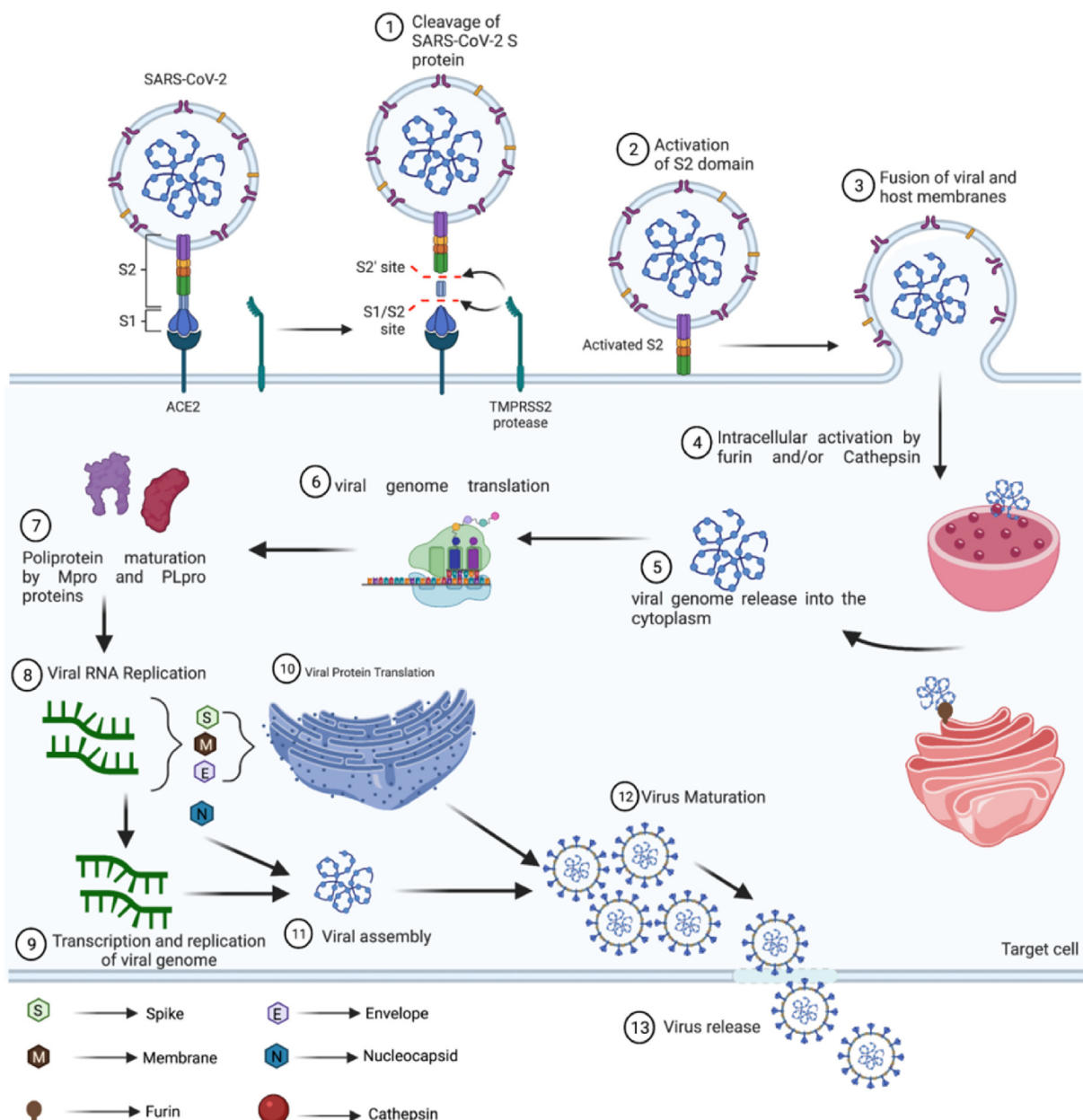

Fig. 3. Schematic representation of SARS-CoV-2 host cell entry. Adapted for Gioia et al., 2020. Made by biorender.com.

been involved in the proteolytic activation of S protein and consequently facilitating the entry of the virus into the human cell [132,133]. Cathepsin L (EC 3.4.23.25) also mediates the membrane fusion [134].

Evidence indicates that while TMPRSS2 acts locally at the host cell plasma membrane and possibly during the formation of endocytic vesicle on neutral pH, the Cathepsin L terminate the S1 degradation in the acidic endosome and lysosome [134—136]. Both process (TMPRSS2 and Cathepsin L) may continue during virus endocytosis [134].

Mediated by cathepsin in lysosomes, cleavage induces membrane fusion, forming a pore for viral passage into the cytoplasm [132,133].

Intracellular activation of protein S can also be performed by Endoprotease Furin (EC 3.4.21.75) in the Golgi complex (Fig. 3 step 4). Among the host proteases involved in viral infection, Furin is the most widely present in a variety of cell types and for its ability to activate other cellular proteins and in SARS-CoV-2.

Among host proteases involved in the viral infection, Furin is the one most widely present in a variety of cell types. It is an endoprotease which shows ability to activate other cellular proteins, and in SARS-CoV-2 activation is not clear.

But in theory, Furin activates surface glycoproteins by fusion, attacking the cleavage site facilitating the virus entry [132,133]. Moreover, acting in the virus entry, Furin also contributes during the transport of virions improving the virus diffusion [132].

The expression profile of Furin and ACE-2 in human cells could explain why SARS-CoV-2 is so efficient in spreading virus particles, since they are present throughout the body in endothelial cells with particularly increased levels in cells lying in alveoli and small intestine [132,133].

Once inside the host cell, the life cycle of SARS-CoV-2 started with the viral genome translation (Fig. 3, step 6), which 75% is translated in polyproteins accountable for the replication [132,133].

Two enzymes are involved in the cleaving viral polyproteins process: the papain-like protease (PLpro) and the main protease (Mpro) (Fig. 3, step 7). The two proteases have an important role in the SARS-CoV-2 life cycle. Mpro (EC 3.4.22.69) in their active form is a homodimer protein and their structure resembles the cysteine protease and 3 chymotrypsin protease. PLpro (EC 3.4.22.B14) is responsible for cleavages located at the N-terminus of the replicase polyprotein [132,133]. Some enzymes like the PLpro recognize host proteins such as Ubiquitin and acts preventing mutations in the PLpro for instance.

The other 25% of viral genome is translated to accessory and structural proteins: Spike (S), Envelope (E), Membrane (M) and Nucleocapsid (N) (Fig. 3, step 8) [132,133]. The transcription and replication of viral genome starts and amplifies the number of virus genome copies. The viral protein translation starts at the endoplasmic reticulum (Fig. 3, step 10) and the virion assembly occurs. After virion maturation, the newly viral particles are release [132,133].

4. The effect virus mutation on enzymes

Mutations can happen to RNA viruses since their replicatory enzymes, like RNA polymerase, are not fail-safe like DNA viruses or DNA cells. Because of that, alterations in the RNA virus genome are much more common when compared to other viruses and cells [137,138]. SARS-CoV-2 is an RNA virus, so mutations can easily happen. Since December 2019, a new variant of the SARS-CoV-2 virus has emerged and created some questions, such as if the variant can alter in some way the disease in the human body.

The most common mutation found was in the spike protein D614G [139]. Spike proteins are the ones that make the crown shape of the virus, and they are usually associated with the interaction between the virus and the cell [140]. This mutation affects the replication speed of the virus, but not disease severity [141,142]. It is interesting to notice that higher levels of viral RNA are found in people infected with the new variant, but the new variant is not related to more severe cases [139]. Instead, the mutation only affects the replication speed of the virus, making it much more contagious [141]. This can be explained by an increase in the affinity with the ACE-2 receptors and a more effective entry in the cells from the respiratory tract [143].

Other variants were found that justify some of the more affected organs and systems, but the mutation in the spike protein D614G was prevalent. According to Hu et al. (2020) [137] these other variants can increase respiratory symptoms, heart disease, thromboembolic events, among others, because of the affinity with each cell. It is important to point that it is not known yet if the new variant affects the effectiveness of the vaccines, but we suspect does not, since the new protein is not bind immune cells [139].

Declaration of competing interest

The authors declare no conflict of interest.

References

[1] A.R. Bourgonje, A.E. Abdulle, W. Timens, J.L. Hillebrands, G.J. Navis, S.J. Gordijn, M.C. Bolling, G. Dijkstra, A.A. Voors, A.D. Osterhaus, P.H. van der Voort, D.J. Mulder, H. van Goor, Angiotensin-converting enzyme 2 (ACE2), SARS-CoV-2 and the pathophysiology of coronavirus disease 2019 (COVID-19), J. Pathol. 251 (3) (2020) 228—248, https://doi.org/10.1002/path.5471. Jul.
[2] C. Robba, D. Battaglini, P. Pelosi, P.R.M. Rocco, Multiple organ dysfunction in SARS-CoV-2: MODS-CoV-2, Expet Rev. Respir. Med. 14 (2020) 865—868, https://doi.org/10.1080/17476348.2020.1778470.
[3] Y. Li, M. Li, M. Wang, Y. Zhou, J. Chang, Y. Xian, D. Wang, L. Mao, H. Jin, B. Hu, Acute cerebrovascular disease following COVID-19: a single center, retrospective, observational study, Stroke Vascu. Neurol. 5 (3) (2020) 279—284, https://doi.org/10.1136/svn-2020-000431.
[4] M. Soy, G. Keser, P. Atagündüz, F. Tabak, I. Atagündüz, S. Kayhan, Cytokine storm in COVID-19: pathogenesis and overview of anti-inflammatory agents used in treatment, Clinic. Rheumatol. (2020), https://doi.org/10.1007/s10067-020-05190-5.
[5] N.D. Reynolds, M.N. Aceves, J.L. Liu, J.R. Compton, D.H. Leary, B.T. Freitas, S.D. Pegan, K.Z. Doctor, F.Y. Wu, X. Hu, P.M. Legler, The SARS-CoV-2 SSHHPS recongnized by the papain-like protease, ACS Infect. Dis. 7 (2021) 1483—1502, https://doi.org/10.1021/acsinfecdis.0c00866.
[6] J. Chen, J. Fan, Z. Chen, M. Zhang, H. Peng, J. Liu, L. Ding, M. Liu, C. Zhao, P. Zhao, S. Zhang, X. Zhang, J. Xu, Nonmuscle myosin heavy chain IIA facilitates SARS-CoV-2 infection in human pulmonary cells, Proc. Natl. Acad. Sci. Unit. States Am. (50) (2021) 118, https://doi.org/10.1073/pnas.2111011118.
[7] C.C. Leslie, Cytosolic phospholipase A$_2$: physiological function and role in disease, J. Lipid Res. 56 (8) (2015) 1386—1402, https://doi.org/10.1194/jlr.R057588. Epub 2015 Apr 2. Aug.
[8] P.K. Robinson, Enzymes: principles and biotechnological applications, Essays Biochem. 59 (2015) 1—41, https://doi.org/10.1042/bse0590001. Erratum in: Essays Biochem. 2015;59:75.
[9] D.C. Whitcomb, M.E. Lowe, Human pancreatic digestive enzymes, Jan, Dig. Dis. Sci. 52 (1) (2007) 1—17, https://doi.org/10.1007/s10620-006-9589-z. Epub 2007 Jan 5.
[10] Q. Ruan, K. Yang, W. Wang, L. Jiang, J. Song, Clinical predictors of mortality due to COVID-19 based on an analysis of data of 150 patients from Wuhan, China, Intensive Care Med. (2020), https://doi.org/10.1007/s00134-020-05991-x.
[11] J.L. Frater, G. Zini, G. d' Onofrio, H.J. Rogers, COVID-19 and the clinical hematology laboratory, Int. J. Lab. Hematol. (2020), https://doi.org/10.1111/ijlh.13229.

[12] A. Medetalibeyoglu, Y. Catma, N. Senkal, A. Ormeci, B. Cavus, M. Kose, O.F. Bayramlar, G. Yildiz, F. Akyuz, S. Kaymakoglu, T. Tukek, The effect of liver test abnormalities on the prognosis of COVID-19, Nov-Dec, Ann. Hepatol. 19 (6) (2020) 614—621, https://doi.org/10.1016/j.aohep.2020.08.068. Epub 2020 Sep. 10.

[13] A. Bertolini, I.P. van de Peppel, F.A.J.A. Bodewes, H. Moshage, A. Fantin, F. Farinati, R. Fiorotto, J.W. Jonker, M. Strazzabosco, H.J. Verkade, G. Peserico, Abnormal liver function tests in patients with COVID-19: relevance and potential pathogenesis, Nov, Hepatology 72 (5) (2020) 1864—1872, https://doi.org/10.1002/hep.31480. Epub 2020 Oct 20.

[14] U. Boregowda, M.M. Aloysius, A. Perisetti, M. Gajendran, P. Bansal, H. Goyal, Serum activity of liver enzymes is associated with higher mortality in COVID-19: a systematic review and meta-analysis, Jul 22, Front. Med. 7 (2020) 431, https://doi.org/10.3389/fmed.2020.00431.

[15] F. Wang, H. Wang, J. Fan, Y. Zhang, H. Wang, Q. Zhao, Pancreatic injury patterns in patients with coronavirus disease 19 pneumonia, Jul, Gastroenterology 159 (1) (2020) 367—370, https://doi.org/10.1053/j.gastro.2020.03.055. Epub 2020 Apr 1.

[16] U. Barlass, B. Wiliams, K. Dhana, D. Adnan, S.R. Khan, M. Mahdavinia, F. Bishehsari, Marked elevation of lipase in COVID-19 disease: a cohort study, Jul, Clin. Transl. Gastroenterol. 11 (7) (2020), e00215, https://doi.org/10.14309/ctg.0000000000000215.

[17] J. McNabb-Baltar, D.X. Jin, A.S. Grover, W.D. Redd, J.C. Zhou, K.E. Hathorn, T.R. McCarty, A.N. Bazarbashi, L. Shen, W.W. Chan, Lipase elevation in patients with COVID-19, Am. J. Gastroenterol. 115 (8) (2020) 1286—1288, https://doi.org/10.14309/ajg.0000000000000732. Aug.

[18] A. Ribes, F. Vardon-Bounes, V. Mémier, M. Poette, J. Au-Duong, C. Garcia, V. Minville, P. Sié, A. Bura-Rivière, S. Voisin, B. Payrastre, Thromboembolic events and covid-19, Adv. Biol. Regulat. 77 (2020) 100735, https://doi.org/10.1016/j.jbior.2020.100735.

[19] K. Saleki, M. Banazadeh, A. Saghazadeh, N. Rezaei, The involvement of the central nervous system in patients with COVID-19, Rev. Neurosci. 31 (4) (2020) 453—456, https://doi.org/10.1515/revneuro-2020-0026. May 26.

[20] L. Steardo, L. Steardo Jr., R. Zorec, A. Verkhratsky, Neuroinfection may contribute to pathophysiology and clinical manifestations of COVID-19, Jul, Acta Physiol. (Oxf) 229 (3) (2020), e13473, https://doi.org/10.1111/apha.13473. Epub 2020 Apr 11.

[21] A. Leonardi, U. Rosani, P. Brun, Ocular surface expression of SARS-CoV-2 receptors, Ocul. Immunol. Inflamm. 28 (5) (2020) 735—738, https://doi.org/10.1080/09273948.2020.1772314.

[22] A. Agarwal, A. Chen, N. Ravindran, C. To, P.J. Thuluvath, Gastrointestinal and liver manifestations of COVID-19, May-Jun, J. Clin. Exp. Hepatol. 10 (3) (2020) 263—265, https://doi.org/10.1016/j.jceh.2020.03.001. Epub 2020 Apr 1.

[23] S. Puli, M. Baig, S. Walayat, Gastrointestinal symptoms and elevation in liver enzymes in COVID-19 infection: a systematic review and meta-analysis, Aug 24, Cureus 12 (8) (2020) e9999, https://doi.org/10.7759/cureus.9999.

[24] M. Hassanein, Y. Radhakrishnan, J. Sedor, T. Vachharajani, V.T. Vachharajani, J. Augustine, S. Demirjian, G. Thomas, COVID-19 and the kidney, Oct 1, Cleve. Clin. J. Med. 87 (10) (2020) 619—631, https://doi.org/10.3949/ccjm.87a.20072.

[25] M.A. Martinez-Rojas, O. Vega-Vega, N.A. Bobadilla, Is the kidney a target of SARS-CoV-2?, Jun 1, Am. J. Physiol. Ren. Physiol. 318 (6) (2020) F1454—F1462, https://doi.org/10.1152/ajprenal.00160.2020. Epub 2020 May 15.

[26] R. Bhayana, A. Som, M.D. Li, D.E. Carey, M.A. Anderson, M.A. Blake, O. Catalano, M.S. Gee, P.F. Hahn, M. Harisinghani, A. Kilcoyne, S.I. Lee, A. Mojtahed, P.V. Pandharipande, T.T. Pierce, D.A. Rosman, S. Saini, A.E. Samir, J.F. Simeone, D.A. Gervais, G. Velmahos, J. Misdraji, A. Kambadakone, Abdominal imaging findings in COVID-19: preliminary observations, Radiology 297 (1) (2020) E207—E215, https://doi.org/10.1148/radiol.2020201908. Epub 2020 May 11. Oct.

[27] C. Xu, T. Zhang, B. Zhu, Z. Cao, Diagnostic role of postmortem CK-MB in cardiac death: a systematic review and meta analysis, Forensic Sci. Med. Pathol. (2020), https://doi.org/10.1007/s12024-020-00232-5.

[28] J.J. Qin, X. Cheng, F. Zhou, F. Lei, G. el al Akolkar, Redefining cardiac biomarkers in predicting mortality of inpatients with COVID-19, Hypertension (2020) 76, https://doi.org/10.1161/HYPERTENSIONAHA.120.15528.

[29] A.M.A. Shafi, S.A. Shaikh, M.M. Shirke, S. Iddawela, A. Harky, Cardiac manifestations in COVID-19 patients - a systematic review, J. Card. Surg. (2020) 35, https://doi.org/10.1111/jocs.14808.

[30] Q. Cheng, Y. Yang, J. Gao, Infectivity of human coronavirus in the brain, EBioMedicine 56 (2020), https://doi.org/10.1016/j.ebiom.2020.102799.

[31] W. Li, M.J. Moore, N. Vasilieva, J. Sui, S.K. Wong, M.A. Berne, M. Somasundaran, J.L. Sullivan, K. Luzuriaga, T.C. Greenough, H. Choe, M. Farzan, Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus, Nature 426 (6965) (2003) 450—454, https://doi.org/10.1038/nature02145. Nov 27.

[32] V.S. Raj, H. Mou, S.L. Smits, D.H.W. Dekkers, M.A. Müller, R. Dijkman, D. Muth, J.A.A. Demmers, A. Zaki, R.A.M. Fouchier, V. Thiel, C. Drosten, P.J.M. Rottier, A.D.M.E. Osterhaus, B.J. Bosch, B.L. Haagmans, Dipeptidyl peptidase 4 is a functional receptor for the emerging human coronavirus-EMC, Nature 495 (7440) (2013) 251—254, https://doi.org/10.1038/nature12005.

[33] J.D. McFadyen, H. Stevens, K. Peter, The emerging threat of (Micro)Thrombosis in COVID-19 and its therapeutic implications, Circ. Res. 127 (4) (2020) 571—587, https://doi.org/10.1161/CIRCRESAHA.120.317447.

[34] G. Mery, O. Epaulard, A.L. Borel, B. Toussaint, A. Gouellec, COVID-19: underlying adipokine storm and angiotensin 1-7 umbrella, Front. Immunol. 11 (2020), https://doi.org/10.3389/fimmu.2020.01714.

[35] B. Vecchiatto, The role of ACE-2/Ang 1-7/Mas axis on skeletal muscle on the prevention of metabolic diseases by aerobic exercise training. https://doi.org/10.11606/D.100.2019.tde-01102019-220112, 2019.

[36] J. Gumashta, R. Gumashta, Role of the backbenchers of the renin-angiotensin system ACE-2 and AT2 receptors in COVID-19: lessons from SARS, Cureus 12 (2020), https://doi.org/10.7759/cureus.8411.

[37] J. Gomez, G.M. Albaiceta, M. Garcia-Clemente, C. López-Larrea, L. Amado-Rodríguez, I.L. Alonso, T. Hermida, A.I. Enríquez, P. Herrero, S. Melon, M.E. Alvarez-Arguelles, et al., Angiotensin-convertingenzymes (ACE, ACE-2) gene variants and COVID-19 outcome, Gene 762 (2020), https://doi.org/10.1016/j.gene.2020.145102.

[38] F. Potus, V. Mai, M. Lebret, S. Malenfant, E. Breton-Gagnon, A.C. Lajoie, O. Boucherat, S. Bonnet, S. Provencher, Novel insights on the pulmonary vascular consequences of COVID-19, Am. J. Physiol. Lung Cell Mol. Physiol. 319 (2020) 277—288, https://doi.org/10.1152/ajplung.00195.2020: 10.1152/ajplung.00195.2020.

[39] M. Thunders, B. Delahunt, Gene of the month: TMPRSS2 (transmembrane serine protease 2), J. Clin. Pathol. (2020), https://doi.org/10.1136/jclinpath-2020-206987.

[40] X. Zhang, H. Fang, J. Zhang, Y. Yuan, W. Xu, Recent advance in aminopeptidase N (APN/CD13) inhibitor research, Curr. Med. Chem. 18 (32) (2011) 5011—5021, https://doi.org/10.2174/092986711797535155.

[41] A. Leonardi, U. Rosani, P. Brun, Ocular surface expression of SARS-CoV-2 receptors, Ocul. Immunol. Inflamm. 28 (5) (2020) 735—738, https://doi.org/10.1080/09273948.2020.1772314.

[42] K.A.A. Douglas, V.P. Douglas, M.M. Moschos, Ocular manifestation of COVID-19 (SARS-CoV-2): a critical review of current literature, vivo 34 (2020) 1619—1628, https://doi.org/10.21873/invivo.11952.

[43] C.J. Ko, C.C. Huang, H.Y. Lin, C.P. Juan, S.W. Lan, H.Y. Shyu, S.R. Wu, P.W. Hsiao, H.P. Huang, C.T. Shun, M.S. Lee, Androgen-induced TMPRSS2 activates matriptase and promotes extracellular matrix degradation, prostate cancer cell invasion, tumor growth and metastasis, Am. Assoc. Canc. Res. (2015), https://doi.org/10.1158/0008-5472.CAN-14-3297.

[44] M. Hoffman, H. Kleine-Weber, S. Schroeder, N. Krüger, T. Herrler, S. Erichsen, T.S. Schiergens, G. Herrler, N.-H. Wu, A. Nitsche, M.A. Müller, C. Drosten, S. Pölmann, SARS-CoV-2 cell entry depends on ACE-2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor, Front. Oncol. 10 (2020) 271—280, https://doi.org/10.3389/fonc.2020.01448.

[45] K.H. Stopsack, L.A. Mucci, E.S. Antonarakis, P.S. Nelson, P.W. Kantoff, TMPRSS2 and COVID-19: Serendipity or Opportunity for Intervention? American Association for Cancer Research, 2020 https://doi.org/10.1158/2159-8290.CD-20-0451.

[46] N.I. Yoshikawa, T. Okamura, Y. Shimizu, H. Hasegawa, M. Takeda, N. Nagata, TMPRSS2 contributes to virus spread and immunopathology in the airways of murine models after coronavirus infection, J. Virol. (2019), https://doi.org/10.1128/JVI.01815-18.

[47] R. Zang, M.F.G. Castro, B.T. McCune, Q. Zeng, P.W. Rothlauf, N.M. Sonnek, Z. Liu, K.F. Brulois, X. Wang, H.B. Greenberg, M.S. Diamond, M.A. Ciorba, S.P.J. Whelan, S. Ding, TMPRSS2 and TMPRSS4 promote SARS-CoV-2 infection of human small intestinal enterocytes, Sci. Immunol. (2020), https://doi.org/10.1126/sciimmunol.abc3582.

[48] Y. Kang, T. Chen, D. Mui, V. Ferrari, D. Jagasia, M. Scherrer-Crosbie, Y. Chen, Y. Han, Cardiovascular manifestations and treatment considerations in Covid-19, Heart (2020), https://doi.org/10.1136/heartjnl-2020-317056, 0.

[49] J.A. Perez-Bermejo, S. Kang, S.J. Rockwood, C.R. Simoneau, D.A. Joy, A.C. Silva, G.N. Ramadoss, W.R. Flanigan, P. Fozouni, H. Li, P. Chen, K. Nakamura, J.D. Whitman, P.J. Hanson, B.M. McManus, M. Ott, B.R. Conklin, T.C. McDevitt, SARS-CoV-2 infection of human iPSC-deriver cardiac cells reflects cytopathic features in hearts of patients with COVID-19, Sci. Trsnsl. Med. 13 (2021), https://doi.org/10.1126/scitranslmed.abf7872.

[50] X.Y. Zhao, X.X. Xu, H.S. Yin, et al., Clinical characteristics of patients with 2019 coronavirus disease in a non-Wuhan area of Hubei Province, China: a retrospective study, BMC Infect. Dis. (2020).

[51] Y. Zheng, H. Xu, M. Yang, et al., Epidemiological characteristics and clinical features of 32 critical and 67 noncritical cases of COVID-19 in Chengdu, J. Clin. Virol. (2020) 127.

[52] H. Fan, J. Chen, L. Zhang, et al., Retrospective Analysis of Clinical Features in 101 Death Cases with COVID-19, medRxiv, 2020, https://doi.org/10.1101/2020.03.09.20033068.

[53] H. Han, L. Yang, R. Liu, F. Liu, F. Liu, K.L. Wu, J. Li, X.H. Liu, C.L. Zhu, Prominent changes in blood coagulation of patients with SARS-CoV-2 infection, Clin. Chem. Lab. Med. 58 (7) (2020) 1116—1120, https://doi.org/10.1515/cclm-2020-0188.

[54] J.F. Llitjos, M. Leclerc, C. Chochois, J.M. Monsallier, M. Ramakers, M. Auvray, K. Merouani, High incidence of venous thromboembolic events in anti-coagulated severe COVID-19 patients, J. Thromb. Haemostasis 18 (7) (2020) 1743—1746, https://doi.org/10.1111/jth.14869.

[55] N. Tang, D. Li, X. Wang, Z. Sun, Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia, J. Thromb. Haemostasis 18 (4) (2020) 844—847, https://doi.org/10.1111/jth.14768.

[56] E. Vazquez-Garza, C. Jerjes-Sanchez, A. Navarrete, J. Joya-Harrison,

D. Rodriguez, Venous thromboembolism: thrombosis, inflammation, and immunothrombosis for clinicians, J. Thromb. Thrombolysis 44 (3) (2017) 377–385, https://doi.org/10.1007/s11239-017-1528-7.

[57] E.W. Davie, J.D. Kulman, An overview of the structure and function of thrombin, Semin. Thromb. Hemost. 32 (Suppl. 1) (2006) 3–15, https://doi.org/10.1055/s-2006-939550.

[58] E. Di Cera, Thrombin, Mol. Aspect. Med. 29 (4) (2008) 203–254, https://doi.org/10.1016/j.mam.2008.01.001.

[59] G.G. de Ridder, R.L. Lundblad, S.V. Pizzo, Actions of thrombin in the interstitium, J. Thromb. Haemostasis 14 (1) (2016) 40–47, https://doi.org/10.1111/jth.13191.

[60] J.A. Huntington, Thrombin plasticity, Biochim. Biophys. Acta Protein Proteonomics 1824 (1) (2012) 246–252, https://doi.org/10.1016/j.bbapap.2011.07.005.

[61] K.F. Aliter, R.A. Al-Horani, Thrombin inhibition by argatroban: potential therapeutic benefits in COVID-19, Cardiovasc. Drugs Ther. (2020), https://doi.org/10.1007/s10557-020-07066-x.

[62] M.G. Lazzaroni, S. Piantoni, S. Masneri, E. Garrafa, G. Martini, A. Tincani, L. Andreoli, F. Franceschini, Coagulation dysfunction in COVID-19: the interplay between inflammation, viral infection and the coagulation system, Blood Rev. (2020), https://doi.org/10.1016/j.blre.2020.100745 xxxx.

[63] D. Chen, A. Dorling, Critical roles for thrombin in acute and chronic inflammation, J. Thromb. Haemostasis 7 (Suppl. 1) (2009) 122–126, https://doi.org/10.1111/j.1538-7836.2009.03413.x.

[64] J.J.N. Posma, J.J. Posthuma, H.M.H. Spronk, Coagulation and non-coagulation effects of thrombin, J. Thromb. Haemostasis 14 (10) (2016) 1908–1916, https://doi.org/10.1111/jth.13441.

[65] M. Levi, T. Van Der Poll, Inflammation and coagulation, Crit. Care Med. 38 (Suppl. 2) (2010), https://doi.org/10.1097/CCM.0b013e3181c98d21.

[66] B. Engelmann, S. Massberg, Thrombosis as an intravascular effector of innate immunity, Nat. Rev. Immunol. 13 (1) (2013) 34–45, https://doi.org/10.1038/nri3345.

[67] F. Frantzeskaki, A. Armaganidis, S.E. Orfanos, Immunothrombosis in acute respiratory distress syndrome: cross talks between inflammation and coagulation, Respiration 93 (3) (2017) 212–225, https://doi.org/10.1159/000453002.

[68] S. Antoniak, A.P. Owens, M. Baunacke, J.C. Williams, R.D. Lee, A. Weithäuser, P.A. Sheridan, R. Malz, J.P. Luyendyk, D.A. Esserman, J.A. Trejo, D. Kirchhofer, B.C. Blaxall, R. Pawlinski, M.A. Beck, U. Rauch, N. Mackman, PAR-1 contributes to the innate immune response during viral infection, J. Clin. Invest. 123 (3) (2013) 1310–1322, https://doi.org/10.1172/JCI66125.

[69] E. Boilard, G. Par, M. Rousseau, N. Cloutier, I. Dubuc, L. Tania, P. Borgeat, L. Flamand, In Fl Uenza Virus H 1 N 1 activates platelets through Fc G RIIA signaling and thrombin generation 123 (18) (2016) 2854–2864, https://doi.org/10.1182/blood-2013-07-515536.

[70] P. Skendros, A. Mitsios, A. Chrysanthopoulou, D.C. Mastellos, S. Metallidis, P. Rafailidis, M. Ntinopoulou, E. Sertaridou, V. Tsironidou, C. Tsigalou, M. Tektonidou, T. Konstantinidis, C. Papagoras, I. Mitroulis, G. Germanidis, J.D. Lambris, K. Ritis, Complement and tissue factor–enriched neutrophil extracellular traps are key drivers in COVID-19 immunothrombosis, J. Clin. Invest. 130 (11) (2020) 6151–6157, https://doi.org/10.1172/JCI141374.

[71] W.B. Mitchell, Thromboinflammation in COVID-19 acute lung injury, Paediatr. Respir. Rev. 35 (2020) 20–24, https://doi.org/10.1016/j.prrv.2020.06.004.

[72] M. Ranucci, C. Sitzia, E. Baryshnikova, U. Di Dedda, R. Cardani, F. Martelli, M. Corsi Romanelli, Covid-19-Associated coagulopathy: biomarkers of thrombin generation and fibrinolysis leading the outcome, J. Clin. Med. 9 (11) (2020) 3487, https://doi.org/10.3390/jcm9113487.

[73] D. White, S. MacDonald, T. Edwards, C. Bridgeman, M. Hayman, M. Sharp, S. Cox-Morton, E. Duff, S. Mahajan, C. Moore, M. Kirk, R. Williams, M. Besser, W. Thomas, Evaluation of COVID-19 coagulopathy; laboratory characterization using thrombin generation and nonconventional haemostasis assays, Int. J. Lit. Humanit. (2020) 1–8, https://doi.org/10.1111/ijlh.13329. July.

[74] L. Ayerbe, C. Risco, S. Ayis, The association between treatment with heparin and survival in patients with Covid-19, J. Thromb. Thrombolysis 50 (2) (2020) 298–301, https://doi.org/10.1007/s11239-020-02162-z.

[75] N. Tang, H. Bai, X. Chen, J. Gong, D. Li, Z. Sun, Anticoagulant treatment is associated with decreased mortality in severe coronavirus disease 2019 patients with coagulopathy, J. Thromb. Haemostasis 18 (5) (2020) 1094–1099, https://doi.org/10.1111/jth.14817.

[76] Y. feng Lu, L. ya Pan, W.W. Zhang, F. Cheng, S.S. Hu, X. Zhang, H. yin Jiang, A meta-analysis of the incidence of venous thromboembolic events and impact of anticoagulation on mortality in patients with COVID-19, Int. J. Infect. Dis. 100 (2020) 34–41, https://doi.org/10.1016/j.ijid.2020.08.023.

[77] E. Maldonado, D. Tao, K. Mackey, Antithrombotic therapies in COVID-19 disease: a systematic review, J. Gen. Intern. Med. 35 (9) (2020) 2698–2706, https://doi.org/10.1007/s11606-020-05906-y.

[78] C. Nougier, R. Benoit, M. Simon, H. Desmurs-Clavel, G. Marcotte, L. Argaud, J.S. David, A. Bonnet, C. Negrier, Y. Dargaud, Hypofibrinolytic state and high thrombin generation may play a major role in SARS-COV2 associated thrombosis, J. Thromb. Haemostasis 18 (9) (2020) 2215–2219, https://doi.org/10.1111/jth.15016.

[79] A.E. Tsantes, F. Frantzeskaki, A.G. Tsantes, E. Rapti, M. Rizos, S.I. Kokoris, E. Paramythiotou, G. Katsadiotis, V. Karali, A. Flevari, E. Chrysanthopoulou, E. Maratou, E. Kyriakou, A. Gialeraki, S. Bonovas, G. Dimopoulos, I. Tsangaris,

A. Armaganidis, The haemostatic profile in critically ill COVID-19 patients receiving therapeutic anticoagulant therapy: an observational study, Medicine 99 (47) (2020), e23365, https://doi.org/10.1097/MD.0000000000023365.

[80] M. Artifoni, G. Danic, G. Gautier, P. Gicquel, D. Boutoille, F. Raffi, A. Néel, R. Lecomte, Systematic assessment of venous thromboembolism in COVID-19 patients receiving thromboprophylaxis: incidence and role of D-dimer as predictive factors, J. Thromb. Thrombolysis 50 (1) (2020) 211–216, https://doi.org/10.1007/s11239-020-02146-z.

[81] G. Chi, J.J. Lee, A. Jamil, V. Gunnam, H. Najafi, S. Memar Montazerin, F. Shojaei, J. Marszalek, Venous thromboembolism among hospitalized patients with COVID-19 undergoing thromboprophylaxis: a systematic review and meta-analysis, J. Clin. Med. 9 (8) (2020) 2489, https://doi.org/10.3390/jcm9082489.

[82] L. Suleiman, C. Négrier, H. Boukerche, Protein S: a multifunctional anticoagulant vitamin K-dependent protein at the crossroads of coagulation, inflammation, angiogenesis, and cancer, Crit. Rev. Oncol. Hematol. 88 (3) (2013) 637–654, https://doi.org/10.1016/j.critrevonc.2013.07.004.

[83] M. Gierula, J. Ahnström, Anticoagulant protein S—new insights on interactions and functions, J. Thromb. Haemostasis 18 (11) (2020) 2801–2811, https://doi.org/10.1111/jth.15025.

[84] Y. Zhang, W. Cao, W. Jiang, M. Xiao, Y. Li, N. Tang, Z. Liu, X. Yan, Y. Zhao, T. Li, T. Zhu, Profile of natural anticoagulant, coagulant factor and anti-phospholipid antibody in critically ill COVID-19 patients, J. Thromb. Thrombolysis 50 (3) (2020) 580–586, https://doi.org/10.1007/s11239-020-02182-9.

[85] L.E. Stoichitoiu, L. Pinte, M.I. Balea, V. Nedelcu, C. Badea, C. Baicus, Anticoagulant protein S in COVID-19: low activity, and associated with outcome, Roman. J. Int. Med. = Revue Roumaine de Medecine Interne 58 (4) (2020) 251–258, https://doi.org/10.2478/rjim-2020-0024.

[86] A.A. Hasan, B. Hocher, Role of soluble and membrane-bound dipeptidyl peptidase-4 in diabetic nephropathy, J. Mol. Endocrinol. 59 (1) (2017) R1–R10, https://doi.org/10.1530/JME-17-0005.

[87] A.M. Lambeir, C. Durinx, S. Scharpé, I. De Meester, Dipeptidyl-peptidase IV from bench to bedside: an update on structural properties, functions, and clinical aspects of the enzyme DPP IV, Crit. Rev. Clin. Lab Sci. 40 (3) (2003) 209–294, https://doi.org/10.1080/713609354.

[88] E.E. Mulvihill, D.J. Drucker, Pharmacology, physiology, and mechanisms of action of dipeptidyl peptidase-4 inhibitors, Endocr. Rev. 35 (6) (2014) 992–1019, https://doi.org/10.1210/er.2014-1035.

[89] H. Du, D.W. Wang, C. Chen, The potential effects of DPP-4 inhibitors on cardiovascular system in COVID-19 patients, J. Cell Mol. Med. 24 (18) (2020) 10274–10278, https://doi.org/10.1111/jcmm.15674.

[90] D.J. Drucker, The biology of incretin hormones, Cell Metabol. 3 (3) (2006) 153–165, https://doi.org/10.1016/j.cmet.2006.01.004.

[91] N.H. Kim, T. Yu, D.H. Lee, The nonglycemic actions of dipeptidyl peptidase-4 inhibitors, BioMed Res. Int. (2014), https://doi.org/10.1155/2014/368703, 2014.

[92] C.F. Deacon, Physiology and pharmacology of DPP-4 in glucose homeostasis and the treatment of type 2 diabetes, Front. Endocrinol. 10 (February) (2019), https://doi.org/10.3389/fendo.2019.00080.

[93] N. Zhou, Y. Zhang, J.C. Zhang, L. Feng, J.K. Bao, The receptor binding domain of MERS-CoV: the dawn of vaccine and treatment development, J. Formos. Med. Assoc. 113 (3) (2014) 143–147, https://doi.org/10.1016/j.jfma.2013.11.006.

[94] S. Shao, Q.Q. Xu, X. Yu, R. Pan, Y. Chen, Dipeptidyl peptidase 4 inhibitors and their potential immune modulatory functions, Pharmacol. Ther. 209 (2020), https://doi.org/10.1016/j.pharmthera.2020.107503.

[95] A. Smelcerovic, G. Kocic, M. Gajic, K. Tomovic, V. Djordjevic, D. Stankovic-Djordjevic, M. Anderluh, DPP-4 inhibitors in the prevention/treatment of pulmonary fibrosis, heart and kidney injury caused by COVID-19—a therapeutic approach of choice in type 2 diabetic patients? Front. Pharmacol. 11 (August) (2020) 9–12, https://doi.org/10.3389/fphar.2020.01185.

[96] B.S. Solerte, D.A. Sabatino, M. Galli, P. Fiorina, Dipeptidyl Peptidase-4 (DPP4) Inhibition in COVID-19, vol. 4, 2020, 0123456789.

[97] R. Strollo, E. Maddaloni, C. Dauriz, R. Pedone, R. Buzzetti, P. Pozzilli, Use of DPP4 inhibitors in Italy does not correlate with diabetes prevalence among COVID-19 deaths, Diabetes Res. Clin. Pract. 171 (2020), 108444, https://doi.org/10.1016/j.diabres.2020.108444.

[98] S. Lim, J.H. Bae, H.S. Kwon, M.A. Nauck, COVID-19 and diabetes mellitus: from pathophysiology to clinical management, Nat. Rev. Endocrinol. 17 (2020), https://doi.org/10.1038/s41574-020-00435-4. January.

[99] N. Vankadari, J.A. Wilce, Emerging WuHan (COVID-19) coronavirus: glycan shield and structure prediction of spike glycoprotein and its interaction with human CD26, Emerg. Microb. Infect. 9 (1) (2020) 601–604, https://doi.org/10.1080/22221751.2020.1739565.

[100] M.F. Bassendine, S.H. Bridge, G.W. McCaughan, M.D. Gorrell, COVID-19 and comorbidities: a role for dipeptidyl peptidase 4 (DPP4) in disease severity? J. Diabetes 12 (9) (2020) 649–658, https://doi.org/10.1111/1753-0407.13052.

[101] N. Mozafari, S. Azadi, S. Mehdi-Alamdarlou, H. Ashrafi, A. Azadi, Inflammation: a bridge between diabetes and COVID-19, and possible management with sitagliptin, Med. Hypotheses 143 (May) (2020), https://doi.org/10.1016/j.mehy.2020.110111.

[102] P. Eleftheriou, D. Amanatidou, A. Petrou, A. Athina, In Silico Evaluation of the E ff ectivity of Approved Protease Inhibitors against the Main Protease of the COVID19. In Silico Evaluation of the Effectivity of Approved Protease

Inhibitors against the Main Protease of the Novel SARS-CoV-2 Virus, vol. 1, 2020, pp. 1–20.

[103] M. Letko, A. Marzi, V. Munster, Functional assessment of cell entry and receptor usage for SARS-CoV-2 and other lineage B betacoronaviruses, Nat. Microbiol. 5 (4) (2020) 562–569, https://doi.org/10.1038/s41564-020-0688-y.

[104] C.R. Xi, A. Di Fazio, N.A. Nadvi, K. Patel, M. Sui, W. Xiang, H.E. Zhang, C. Deshpande, J.K.K. Low, X.T. Wang, Y. Chen, C.L.D. Mcmillan, A. Isaacs, B. Osborne, J. Ana, W. Mccaughan, J.P. Mackay, W.B. Church, M.D. Gorrell, *A Novel Purification Procedure for Active Recombinant Human DPP4 and the Inability of DPP4 to Bind SARS-CoV-2*, 1–16, 2020.

[105] T. Klein, H. Nar, G. Schnapp, O. Hucke, T.C. Hardman, Action of dipeptidyl peptidase-4 inhibitors on SARS-CoV-2 main protease, ChemMedChem (2020) 1–3, https://doi.org/10.1002/cmdc.202000921.

[106] M.A. Nauck, J.J. Meier, Reduced covid-19 mortality with sitagliptin treatment? Weighing the dissemination of potentially lifesaving findings against the assurance of high scientific standards, Diabetes Care 43 (12) (2020) 2906–2909, https://doi.org/10.2337/dci20-0062.

[107] K. Schlicht, N. Rohmann, C. Geisler, T. Hollstein, C. Knappe, K. Hartmann, J. Schwarz, F. Tran, D. Schunk, R. Junker, T. Bahmer, P. Rosenstiel, D. Schulte, K. Türk, A. Franke, S. Schreiber, M. Laudes, Circulating levels of soluble Dipeptidylpeptidase-4 are reduced in human subjects hospitalized for severe COVID-19 infections, Int. J. Obes. 44 (11) (2020) 2335–2338, https://doi.org/10.1038/s41366-020-00689-y.

[108] A.J. Scheen, DPP-4 Inhibition and COVID-19: from Initial Concerns to Recent Expectations. Diabetes & Metabolism, 2020, p. 124187, https://doi.org/10.1016/j.jhazmat.2020.124187. Retrieved from.

[109] B. Cariou, S. Hadjadj, M. Wargny, M. Pichelin, A. Al-Salameh, I. Allix, C. Amadou, G. Arnault, F. Baudoux, B. Bauduceau, S. Borot, M. Bourgeon-Ghittori, O. Bourron, D. Boutoille, F. Cazenave-Roblot, C. Chaumeil, E. Cosson, S. Coudol, P. Darmon, P. Gourdy, Phenotypic characteristics and prognosis of inpatients with COVID-19 and diabetes: the CORONADO study, Diabetologia 63 (8) (2020) 1500–1515, https://doi.org/10.1007/s00125-020-05180-x.

[110] G.P. Fadini, M.L. Morieri, E. Longato, B.M. Bonora, S. Pinelli, E. Selmin, G. Voltan, D. Falaguasta, S. Tresso, G. Costantini, G. Sparacino, B. Di Camillo, L. Tramontan, A.M. Cattelan, A. Vianello, P. Fioretto, R. Vettor, A. Avogaro, Exposure to dipeptidyl-peptidase-4 inhibitors and COVID-19 among people with type 2 diabetes: a case-control study, Diabetes Obes. Metabol. 22 (10) (2020) 1946–1950, https://doi.org/10.1111/dom.14097.

[111] M.K. Kim, J.H. Jeon, S.W. Kim, J.S. Moon, N.H. Cho, E. Han, J.H. You, J.Y. Lee, M. Hyun, J.S. Park, Y.S. Kwon, Y.K. Choi, K.T. Kwon, S.Y. Lee, E.J. Jeon, J.W. Kim, H.L. Hong, H.H. Kwon, C.Y. Jung, J.H. Lee, The clinical characteristics and outcomes of patients with moderate-to-severe coronavirus disease 2019 infection and diabetes in Daegu, South Korea, Diabetes Metabol. J. 44 (2020) 602–613, https://doi.org/10.4093/dmj.2020.0146.

[112] J.-H. Zhou, B. Wu, W.-X. Wang, F. Lei, X. Cheng, J.-J. Qin, J.-J. Cai, X.-J. Zhang, F. Zhou, Y.-M. Liu, H.-M. Li, L.-H. Zhu, Z.-G. She, X. Zhang, J. Yang, H.-L. Li, No significant association between dipeptidyl peptidase-4 inhibitors and adverse outcomes of COVID-19, World J. Clin. Cases 8 (22) (2020) 5576–5588, https://doi.org/10.12998/wjcc.v8.i22.5576.

[113] S.B. Solerte, F. D'Addio, R. Trevisan, E. Lovati, A. Rossi, I. Pastore, M. Dell'Acqua, E. Ippolito, C. Scaranna, R. Bellante, S. Galliani, A.R. Dodesini, G. Lepore, F. Geni, R.M. Fiorina, E. Catena, A. Corsico, R. Colombo, M. Mirani, P. Fiorina, Sitagliptin treatment at the time of hospitalization was associated with reduced mortality in patients with type 2 diabetes and COVID-19: a multicenter, case-control, retrospective, observational study, Diabetes Care 43 (12) (2020) 2999–3006, https://doi.org/10.2337/dc20-1521.

[114] M. Mirani, G. Favacchio, F. Carrone, N. Betella, E. Biamonte, E. Morenghi, G. Mazziotti, A.G. Lania, Impact of comorbidities and glycemia at admission and dipeptidyl peptidase 4 inhibitors in patients with type 2 diabetes with covid-19: a case series from an academic hospital in lombardy, Italy, Diabetes Care 43 (12) (2020) 3042–3049, https://doi.org/10.2337/dc20-1340.

[115] Y. Luan, W. Xu, The structure and main functions of aminopeptidase N, Curr. Med. Chem. 14 (6) (2007) 639–647, https://doi.org/10.2174/092986707780059571.

[116] P. Mina-Osorio, The moonlighting enzyme CD13: old and new functions to target, Trends Mol. Med. 14 (8) (2008) 361–371, https://doi.org/10.1016/j.molmed.2008.06.003.

[117] R.L. Morgan, N. Behbahani-Nejad, J. Endres, M.A. Amin, N.J. Lepore, Y. Du, A. Urquhart, K.C. Chung, D.A. Fox, Localization, shedding, regulation and function of aminopeptidase N/CD13 on fibroblast like synoviocytes, PLoS One 11 (9) (2016), https://doi.org/10.1371/journal.pone.0162008.

[118] J.S. Zotz, F. Wölbing, C. Lassnig, M. Kauffmann, U. Schulte, A. Kolb, B. Whitelaw, M. Müller, T. Biedermann, M. Huber, CD13/aminopeptidase N is a negative regulator of mast cell activation, FASEB (Fed. Am. Soc. Exp. Biol.) J. 30 (6) (2016) 2225–2235, https://doi.org/10.1096/fj.201600278.

[119] C. Lu, M.A. Amin, D.A. Fox, CD13/aminopeptidase N is a potential therapeutic target for inflammatory disorders, Physiol. Behav. 204 (1) (2018) 3–11, https://doi.org/10.1038/s41395-018-0061-4.

[120] B.J. Bosch, S.L. Smits, B.L. Haagmans, Membrane ectopeptidases targeted by human coronaviruses, Curr. Opin. Virol. 6 (1) (2014) 55–60, https://doi.org/10.1016/j.coviro.2014.03.011.

[121] C.L. Yeager, R.A. Ashmun, R.K. Williams, C.B. Cardellichio, L.H. Shapiro, A.T. Look, K.V. Holmes, Human aminopeptidase N is a receptor for human coronavirus 229E, Nature 357 (6377) (1992) 420–422, https://doi.org/10.1038/357420a0.

[122] Leonardi, U. Rosani, P. Brun, Ocular Surface Expression of SARS-CoV-2 Receptors, Ocular Immunology and Inflammation, 2020, https://doi.org/10.1080/09273948.2020.1772314.

[123] J. Park, D. Jeong, Y.W. Chung, D.H. Kim, J.H. Cheon, J.H. Ryu, Quantitative proteomic analysis of the expression of SARS-CoV-2 receptors in the gut of patients with chronic enterocolitis, Yonsei Med. J. 61 (10) (2020) 891–894, https://doi.org/10.3349/ymj.2020.61.10.891.

[124] F. Qi, S. Qian, S. Zhang, Z. Zhang, Single cell RNA sequencing of 13 human tissues identify cell types and receptors of human coronaviruses, Biochem. Biophys. Res. Commun. 526 (1) (2020) 135–140, https://doi.org/10.1016/j.bbrc.2020.03.044.

[125] C.K.V. Devarakonda, E. Meredith, L.H. Shapiro, Coronavirus Receptors as Immune Modulators, 2021, https://doi.org/10.4049/jimmunol.2001062.

[126] S. Sookoian, C.J. Pirola, Liver enzymes, metabolomics and genome-wide association studies: from systems biology to the personalized medicine, Jan 21, World J. Gastroenterol. 21 (3) (2015) 711–725, https://doi.org/10.3748/wjg.v21.i3.711.

[127] N. Patel, D. Rai, Shivam, S. Shahane, U. Mishra, Lipases: sources, production, purification, and applications, Recent Pat. Biotechnol. 13 (1) (2019) 45–56, https://doi.org/10.2174/1872208312666181029093333.

[128] K.P. Patel, P.A. Patel, R.R. Vunnam, A.T. Hewlett, R. Jain, R. Jing, S.R. Vunnam, Gastrointestinal, hepatobiliary, and pancreatic manifestations of COVID-19, Jul, J. Clin. Virol. 128 (2020) 104386, https://doi.org/10.1016/j.jcv.2020.104386. Epub 2020 Apr 29.

[129] A. Farhana, S.L. Lappin, Biochemistry, Lactate Dehydrogenase, May 7. In: StatPearls [Internet], StatPearls Publishing, Treasure Island (FL, 2021, 2021 Jan–. PMID: 32491468.

[130] B.M. Henry, G. Aggarwal, J. Wong, S. Benoit, J. Vikse, M. Plebani, G. Lippi, Lactate dehydrogenase levels predict coronavirus disease 2019 (COVID-19) severity and mortality: a pooled analysis, Sep, Am. J. Emerg. Med. 38 (9) (2020) 1722–1726, https://doi.org/10.1016/j.ajem.2020.05.073. Epub 2020 May 27.

[131] I. Serin, N.D. Sari, M.H. Dogu, S.D. Acikel, G. Babur, A. Ulusoy, M.I. Onar, E.C. Gokce, O. Altunok, F. YaylaciMert, A. Karakilic, M. Baltik, B. Gulesir, A new parameter in COVID-19 pandemic: initial lactate dehydrogenase (LDH)/Lymphocyte ratio for diagnosis and mortality, Nov, J Infect Public Health 13 (11) (2020) 1664–1670, https://doi.org/10.1016/j.jiph.2020.09.009. Epub 2020 Sep. 30.

[132] M. Gioia, C. Ciaccio, P. Calligari, G. De Simone, D. Sbardella, G. Tundo, G.F. Fasciglione, A. Di Masi, D. Di Pierro, A. Bocedi, P. Ascenzi, M. Coletta, Role of proteolytic enzymes in the COVID-19 infection and promising therapeutic approaches, Biochem. Pharmacol. (2020) 182, https://doi.org/10.1016/j.bcp.2020.114225.

[133] J. Huang, W. Song, H. Huang, Q. Sun, Pharmacological therapeutics targeting RNA-Dependent, RNA Polymerase, Proteinase and Spike protein: from Mechanistic studies to clinica trials for COVID-19, J. Clin. Med. 9 (2020) 1131, https://doi.org/10.3390/jcm9041131.

[134] T. Liu, S. Luo, P. Libby, G.-P. Shi, Cathepsin L-selective inhibitors: a potentially promising treatment for COVID-19 patients, Pharmacol. Therapeut. 213 (2020) 107587, https://doi.org/10.1016/j.pharmthera.2020.107587.

[135] I. Glowacka, S. Bertram, M.A. Muller, P. Allen, E. Soilleux, S. Pfefferle, S. Pohlmann, 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. 85 (2011) 4122–4134, https://doi.org/10.1128/JVI.02232-10.

[136] D. Meyer, F. Sielaff, M. Hammami, E. Bottcher-Friebertshauser, W. Garten, T. Steinmetzer, Identification of the first synthetic inhibitors of the type II transmembrane serine protease TMPRSS2 suitable for inhibition of influenza virus activation, Biochem. J. 452 (2013) 331–343, https://doi.org/10.1042/BJ20130101.

[137] J. Hu, C. Li, S. Wang, T. Li, H. Zhang, Genetic Variants Are Identified to Increase Risk of COVID-19 Related Mortality from UK Biobank Data, Medrxiv, 2020, https://doi.org/10.1101/2020.11.05.20226767.

[138] W.R. Fleischmann, in: S. Baron (Ed.), Medical Microbiology, fourth ed., University of Texas Medical Branch at Galveston, Galveston (TX), 1996. https://www.ncbi.nlm.nih.gov/books/NBK8439/.

[139] N.D. Grubaugh, W.P. Hanage, A.L. Rasmussen, Making sense of mutation: what D614G means for the COVID-19 pandemic remains unclear, Cell 182 (2020) 794–795, https://doi.org/10.1016/j.cell.2020.06.040.

[140] T.D. Brock, D.W. Smith, M.T. Madigan, Biology of Microorganisms, 1985, 4TH EDITION.

[141] B. Korber, W.M. Fischer, S. Gnanakaran, C.C. LaBranche, E.O. Saphire, D.C. Montefiori, Tracking changes is SARS-CoV-2 spike: evidence that D614G increases infectivity of the COVID-19 virus, Cell 182 (2020) 812–827, 10.1016%2Fj.cell.2020.06.043.

[142] T. Koyama, D. Platt, L. Parida, Variant analysis of SARS-CoV-2 genomes, Bull. World Health Organ. 98 (2020) 495–504, https://doi.org/10.2471/BLT.20.253591.

[143] T.N. Starr, A.J. Greaney, S.K. Hilton, N.P. King, D. Veesler, J.D. Bloom, Deep mutational scanning of SARS-CoV-2 receptor binding domain reveals constraints on folding and ACE-2 binding, Cell 182 (2020) 1295–1310, https://doi.org/10.1016/j.cell.2020.08.012.