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Faculdade de Farmácia

Immunopathology in COVID-19

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Monografia orientada pela Professora Doutora Elsa Anes, Professora Associada com Agregação em Microbiologia e Imunologia

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Resumo

Desde dezembro do ano de 2019 que o mundo enfrenta uma crise de saúde pública, por muitos nunca antes experienciada. O novo Coronavírus – SARS-CoV-2 – veio dar origem à COVID-19, infeção que se demonstrou sem percedentes e heterogénea nas suas manifestações. Tendo em conta o impacto que este vírus tem tido na vida da população a nível mundial e a facilidade com que é transmitido, revelou-se essencial compreender os mecanismos pelos quais a infeção se procede, bem como os seus principais intervenientes.

A imunopatologia assume um papel relevante na progressão desta doença e no desenvovlimento das manifestações mais graves. A evasão do vírus à resposta antiviral primária pode levar a uma exacerbação da resposta imunitária, caracterizada por uma extensa libertação de citocinas pro-inflamatórias, linfopénia e elevação dos marcadores de coagulação. Os sintomas respiratórios são os mais comuns nesta infeção, podendo variar de intensidade entre os vários individuos: desde uma simples rinorreia com tosse ligeira ao desenvolvimento de quadros de pneumonia grave ou síndrome agudo de insuficiência respiratório (ARDS), nos casos mais severos. Para além disso, a expressão disseminada do recetor do vírus, a enzima conversora da angiotensina tipo 2 (ACE2), permite a progressão da infeção pelos vários sistemas de órgãos, podendo levar a um estado de inflamação disseminada e falha múltipla de órgãos.

Assim, esta monografia pretende explorar os vários acontecimentos característicos do decorrer da infeção, seguindo o percurso natural da mesma pelo organismo humano. Serão descriminados os principais intervenientes na imunopatologia da COVID-19, bem como as manifestações clínicas resultantes dos vários estadios da infeção.

De momento, não existem terapêuticas antivirais para combater o vírus. O desenvolvimento de vacinas que atenuem os sintomas da doença e que reduzam a transmissibilidade do vírus tem sido amplamente impulsionado, enfrentando, no entanto, o desafio que se impõe com o aparecimento de novas variantes do vírus. É importante estarmos conscientes que as terapêuticas anti-inflamatórias terão um grande impacto no controlo desta doença, mesmo que a imunopatologia se perspetive como uma situação rara após a vacinação de toda a população.

Palavras-chave: imunopatologia; SARS-CoV-2; COVID-19; ACE2; hiper-inflamação.

Abstract

Since December 2019, the world has been facing a public health crisis that many have never experienced before. The new Coronavirus – SARS-CoV-2 – gave rise to COVID-19, an infection that proved to be without precedent and heterogeneous in its manifestations. Taking into account the impact that this virus has had on so many lives and the ease of transmission from person to person, it is essential to understand the mechanisms by which the infection proceeds, as well as its main elements.

The immunopathology plays an important role in the progression of this disease and in the development of the most severe manifestations. Virus evasion of the primary antiviral response can lead to an exacerbation of the immune response, characterized by an extensive release of pro-inflammatory cytokines, lymphopenia and elevation of coagulation markers. Respiratory symptoms are the most common in this infection and may vary in intensity among patients: from a simple rhinorrhea with a slight cough to the development of severe pneumonia or acute respiratory distress syndrome (ARDS), in the worst cases. Furthermore, the widespread expression of the virus receptor, the angiotensin-converting enzyme 2 (ACE2), allows the progression of the infection through various organ systems, which may lead to a state of widespread inflammation and multiple organ failure.

Thus, this monograph intends to explore the various events that characterize this infection, following its natural course through the human body. The main components in the immunopathology of COVID-19 will be discriminated, as well as the clinical manifestations resulting from the various stages of the infection.

There are currently no antiviral therapies to eliminate the virus. The development of vaccines that alleviate the symptoms of the disease and reduce the transmissibility of the virus has been widely promoted, facing, however, the challenge imposed by the emergence of new variants of the virus. It is important to be aware that anti-inflammatory therapies will have a great impact on the control of this disease, even though immunopathology may become a rare situation after vaccination of the entire population.

Keywords: immunopathology; SARS-CoV-2; COVID-19; ACE2; hyperinflammation.

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Abbreviations

α-SMA – Smooth muscle α-actin

3CLpro – 3-chymotrypsin-like protease

ACE2 – Angiotensin-Converting Enzyme 2

ADE – Antibody-Dependent Enhancement

AKI – Acute Kidney Injury

ALI – Acute Lung Injury

AM – Alveolar macrophages

Ang1-7 – Angiotensin 1-7

Ang1-9 – Angiotensin 1-9

Ang I – Angiotensin I

Ang II – Angiotensin II

AP-1 – Activator Protein-1

APLN – Apelin

ARDS – Acute Respiratory Distress Syndrome

AT1R – Ang II type 1 receptor

AT2R – Ang II type 2 receptor

ATP – Adenosine triphosphate

B1R – Bradykinin receptor 1

B2R – Bradykinin receptor 2

BALF – Bronchoalveolar Lavage Fluid

BK – Bradykinin

BCG – Bacille Calmette-Guerin

CAM – Cell Adhesion Molecule

CCL – C-C Motif Chemokine Ligand

CDC – Centers for Disease Control and Prevention

CK-MB – Creatinine Kinase-MB

CNS – Central Nervous System

COVID-19 – Coronavirus Disease 2019

CRP – C-Reactive Protein

CSSE – Center for Systems Science and Engineering

CXCL – C-X-C motif chemokine ligand

DAD – Diffuse Alveolar Damage

DAMP – Damage-Associated Molecular Pattern

DC – Dendritic Cell

DMVs – Double-membrane vesicles

DNA – Deoxyribonucleic acid

DPP4 – Dipeptidyl peptidase 4

dsRNA – double stranded RNA

ER – Endoplasmic Reticulum

ERGIC – Endoplasmic Reticulum-Golgi Intermediate Compartment

FDA – U.S. Food and Drug Administration

G-CSF – Granulocyte Colony-Stimulating Factor

GM-CSF – Granulocyte–Macrophage Colony-Stimulating Factor

HA – Hyaluronic acid

He – Hemagglutinin-esterase

HIF – Hypoxia Inducible Transcription Factor

ICAM-1 – Intercellular CAM-1

ICU – Intensive Care Unit

IFN – Interferon

Ig – Immunoglobulin

IL – Interleukin

IP-10 – Interferon γ-inducible Protein 10

IRF – Interferon Regulatory Factor

ISG – Interferon-Stimulated Genes

JNK – c-Jun N-terminal kinase

LT – Leukotrienes

M1 macrophages – Monocyte-derived macrophages

M2 macrophages – Pro-fibrotic macrophages

MAVS – Mitochondrial antiviral-signalling protein

MCP-1 – Monocyte Chemoattractant rotein-1

MDA 5 – Melanoma Differentiation-Associated gene 5

MDSC – Myeloid Derived Suppressor Cell

MERS-CoV – Middle East Respiratory Syndrome-Coronavirus

MIP-1α – Macrophage Inflammatory Protein-1α

MIP-1β – Macrophage Inflammatory Protein-1β

MMR – Measles-Mumps-Rubella

Mpro – Main protease

mRNA – RNA messenger

MHC – Major Histocompatibility Complex

Nab – Neutralizing antibodies

NETs – Neutrophil Extracellular Traps

NETosis – NET apoptosis

NF-κB – Nuclear Factor-κB

NK – Natural Killer

NLR – Neutrophil-Lymphocyte Ratio

NO – Nitric Oxide

NOS – Nitric Oxide Synthase

Nsps – Non-structural proteins

NTD – N-Terminal Domain

ORF – Open Reading Frame

PAI-1 – Plasminogen Activator Inhibitor

PAMP – Pathogen-Associated Molecular Pattern

pDC – plasmacytoid Dendritic Cell

PECAM-1 – Platelet Endothelial CAM-1

PG – Prostaglandin

PLpro – Papain-like protease

PRR – Pattern Recognition Receptor

RAAS – Renin-Angiotensin-Aldosterone-System

RAR – Rapidly Adapting Receptor

RBD – Receptor Binding Domain

RdRp – RNA-dependent RNA polymerase

RIG-I – Retinoic acid-Inducible Gene I

RNA – Ribonucleic Acid

ROS – Reactive Oxygen Species

RTC – Replication and Transcription Complex

rRT-PCR – real-time Reverse transcription polymerase chain reaction

SARS-CoV – Severe Acute Respiratory Syndrome-Coronavirus

SARS-CoV-2 – Severe Acute Respiratory Syndrome-Coronavirus 2

scRNA-seq – single cell RNA sequencing

scTCR-seq – single cell T-Cell Receptor sequencing

sgRNA – sub-genomic RNA

SP – Surfactant-protein

ssRNA – single stranded RNA

T1P – Type I Pneumocytes

T2P – Type II Pneumocytes

TF – Tissue Factor

TFPI – Tissue Factor Pathway Inhibitor

TGF-β1 – Transforming Growth Factor-β1

Th – T helper cell

TLR – Toll-like Receptor

TMPRSS2 – Transmembrane Protease Serine 2

TNF – tumour necrosis factor

TNF-α – tumour necrosis factor-α

tPA – Tissue plasminogen activator

Treg – Regulatory T cell

TX – Thromboxane

uPA – Urokinase plasminogen activator

VLS – Vascular Leakage Syndrome

VCAM-1 – Vascular CAM-1

VOC – Variant of Concern

vWF – von Willebrand Factor

WHO –World Health Organization

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1. Introduction

In December 2019, Severe Acute Respiratory Syndrome-Coronavirus 2 (SARS-CoV-2) was firstly identified in Wuhan, China, as the etiological agent of Coronavirus Disease 2019 (COVID-19). SARS-CoV-2 is a novel strain in the severe acute respiratory syndrome-related coronavirus species belonging to the family *Coronaviridae*, the same as its predecessors Severe Acute Respiratory Syndrome-Coronavirus (SARS-CoV) and the Middle East Respiratory Syndrome-Coronavirus (MERS-CoV). Despite the genomic similarities between the three, COVID-19 pandemic has caused considerably greater impact in terms of morbidities and mortality (1–3).

Because of the wide geographic spread and impact of SARS-CoV-2 in such a short time, with a devastating effect on an extremely large proportion of the world population, the World Health Organization (WHO) declared COVID-19 as a global health pandemic on 11th March 2020. Since then, SARS-CoV-2 continues to deeply affect worldwide population and health systems, with many countries enduring a second or a third wave of outbreaks.

The infection is rapidly transmitted from person-to-person, through liquid droplets by cough, sneeze, hand to mouth or eye contact and contaminated surfaces. It is noteworthy that a great societal SARS-CoV-2 penetration arises from increased viral spread through asymptomatic and pre-symptomatic carriers, who many times are not tested due to lack of signals or clinical manifestations, therefore serving as a nidus for rapid disease spread. According to Centers for Disease Control and Prevention (CDC), the incubation period for COVID-19 may extend to 14 days, with a median time of 4 to 5 days from viral exposure to symptoms onset. Clinically, COVID-19 manifestations are heterogeneous, with patients ranging from being asymptomatic or having mild respiratory symptoms, to requiring hospitalization with mechanical ventilation due to severe pneumonia or acute respiratory distress syndrome (ARDS), the primary cause of SARS-CoV-2 mortality. Over the course of the disease, many COVID-19 patients will experience symptoms that vary with disease severity, including fever or chills, cough (mainly dry), shortness of breath or difficulty in breathing, fatigue, muscle or joint aches, headache, loss of smell (anosmia), loss of taste (ageusia), congestion or runny nose (rhinorrhea), nausea or vomiting, diarrhea. In the worst cases, the disease can progress into a critical stage, resulting in respiratory failure, shock, or multiorgan system dysfunction, ultimately leading to death (4).

By binding to its cellular receptor, the Angiotensin-Converting Enzyme 2 (ACE2), SARS-CoV-2 can trigger in the host an innate and adaptative immune responses. However, an uncontrolled inflammatory reaction and an impaired adaptative immune response may lead to tissue damage, both locally and systemically (5).

At the moment, there is no approved medicine for the treatment of COVID-19. Therefore, current clinical management consists in prevention or treatment of the comorbidities arising from the infection. In fact, immunity acquired through vaccination is the ideal protection method in public health issues. One of the most challenging aspects about developing an anti-SARS-CoV-2 vaccine is achieving efficacy and clinical safety in such a short time. Another obstacle being faced regarding the vaccine development is indeed the rapid emergence of mutations in the genes encoding surface glycoproteins, which are responsible for the antigenic immune response, thus lessening the vaccines effectiveness (6).

Supplemental oxygen or, when deemed necessary, mechanical ventilation or extracorporeal membrane oxygenation are part of the respiratory control measures. Several repurposed drugs are being used as strategic treatments with the purpose to limit the adverse effects of the pathogenesis, although most of them with mixed results. These include antiviral drugs, antimalarial drugs, anthelmintic drugs, systemic corticosteroids, IL-6 antagonists, antibiotics, anticoagulants, anti-inflammatory drugs and even convalescent plasma. These are most often weighted to be used in severe situations (2,6–10).

1.1. Objectives

Due to the high impact and mortality that this infection is causing at a global level, it is fundamental to understand SARS-CoV-2 infectious mechanisms as well as the immunopathogenesis that is inherent to the host immune response. This knowledge is the base for the development of therapeutic options and vaccines that will allow us to overcome severe symptoms and ideally, eradicate the virus. Therefore, the aim of this literature review is to explore what is known about the immunopathogenesis of COVID-19, focusing on the main cells and mediators of inflammation. In addition, this monograph provides an overview of the clinical manifestations that result from the inflammatory process, from the mildest symptoms of the disease to the most critical cases involving extrapulmonary reactions.

The development of the thematic follows the progression of the infection, from the entry of the virus through the upper airways, following the remaining stages of the infection as the

virus spreads in the human body. In each stage, the main players in the inflammatory process will be described, as well as their activation and relevance to the disease progression.

1.2. Methods

Regarding the scientific research, several browsers were used, namely PubMed, ScienceDirect and Google Scholar. The articles and publications reviewed were chosen taking into account their publication date. Given the recent emergence of the virus, most of the information consulted was published in 2020 and 2021. Nevertheless, articles published in previous years were also considered concerning inflammatory processes and respective mediators, which are transversal to many infection processes. The research was mostly done in English, due to the extensive worldwide investigation about this emerging virus. Key terms used were essentially the following: SARS-CoV-2; COVID-19; immunopathogenesis; hyperinflammation; cytokines; immune response; interferons; clinical features and outcomes; risk factors; to state some. Another choice criterion was the review of articles, primarily selecting the peer-reviewed ones.

In addition to the mentioned research platforms, important information was also obtained from websites and documents provided from recognized entities, namely the World Health Organization (WHO), the Centers for Disease Control and Prevention (CDC), Center for Systems Science and Engineering (CSSE), the COVID-19 Vaccine Tracker and the U.S. Food and Drug Administration (FDA).

2. Severe Acute Respiratory Syndrome Coronavirus 2

2.1. SARS-CoV-2 structure

SARS-CoV-2 is a member of the family *Coronaviridae*, from the genera *Betacoronavirus*. It is an enveloped virus with a positive sense single-stranded RNA as its genome. Its virion consists of four structural proteins: N (nucleocapsid), which is integrated with the genome, E (envelope), M (membrane) and S (spike), the three arranged in the lipid bilayer that encloses the viral genetic material. In addition, SARS-CoV-2 also contains sixteen non-structural proteins (Nsp1-16) (11).

The Nucleocapsid protein (N) is a phosphorylated protein that is involved in the encapsulation of the genomic material by binding the viral genome network of proteins to the Replication and Transcription Complex (RTC). It also enables the binding of viral RNA in a classic ‘beads in a string’ conformation model.

The Envelope protein (E) is a transmembrane protein and functions as an ion channel, allowing the release of the viral genomic material to the host cell.

The Membrane protein (M) is the most abundant protein present in Coronavirus and consists of a NH_2 domain at the extracellular region and a COOH terminal at the intracellular cytoplasm. Its presence is crucial to incorporate essential viral components into new virions during morphogenesis, and its main function is to develop virus-specific humoral response.

Finally, Spike glycoprotein (S) has a homotrimer conformation, with two domains in each subunit: S1 and S2. By binding with the host cellular receptors, it is essential for the entry of the virus into the host cells. Coronavirus S proteins are heavily glycosylated, a feature that promotes immune evasion by sheltering epitopes from neutralizing antibodies (11–13).

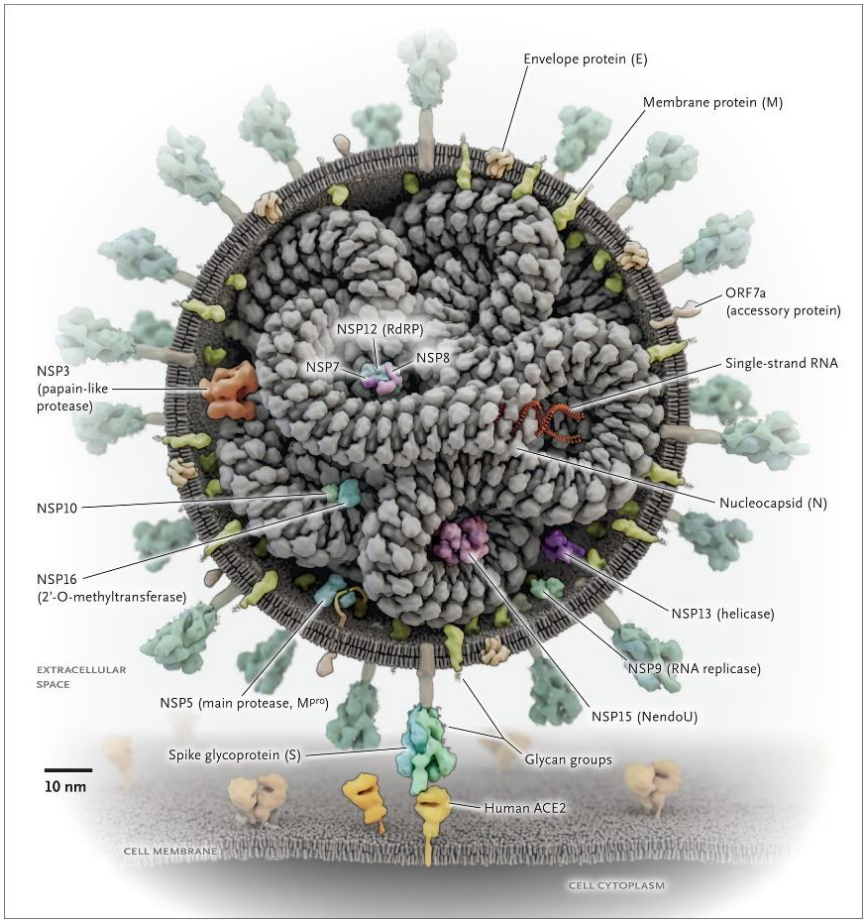

Figure 1. SARS-CoV-2 virion and its proteins.

Main components of SARS-CoV-2 virion. From (14).

2.2. SARS-CoV-2 life cycle

Virus particles that reach the upper aerial mucosae may interact via the viral Spike protein (S) with the angiotensin-converting enzyme 2 (ACE2), the SARS-CoV-2 entry receptor expressed in epithelial cells. This interaction leads to a series of fusion with the cell membranes or endocytic events that culminates with virus internalization into the host cell and the starting of the infection. Spike is a homotrimeric protein, with each subunit consisting of two domains – S1 – the globular domain – and S2 – the biomembrane-anchored stalk domain. S1 contains the Receptor-Binding Domain (RBD), the key segment involved in the attachment to the ACE2 receptor, while S2 mediates membrane fusion, enabling the virus to enter the host cell (15,16).

Following receptor binding, SARS-CoV-2 can utilize a wide array of host proteases including cathepsin L, cathepsin B, trypsin, factor X, elastase, furin, and Transmembrane

Protease Serine 2 (TMPRSS2) for S protein priming and activation, in order to allow the fusion to occur (17,18). This proteolytic cleavage takes place at two sites. The first occurs at the S1/S2 boundary, separating the two domains (although still non-covalently associated) and structurally changing S2 domain into a perfusion conformation. The second one occurs at the S2' site, which finally drives viral and host membranes fusion, through endocytosis or direct membrane fusion. This way, the ss-positive sense N-coated RNA is released inside the cell (12).

By taking the command on the host cell ribosomal machinery, the genomic RNA serves as a transcript and undergoes immediate translation of two opening reading frames: ORF1a and ORF1b. The resulting translation products are the viral polyproteins pp1a and pp1ab. Sixteen non-structural proteins (nsps), each one with specific functions, are co-translationally and post-translationally released from pp1a (nsp1-11) and pp1ab (nsp1-10; nsp12-16). This happens upon proteolytic cleavage of the polyproteins by two cysteine proteases: papain-like protease (PLpro), located within nsp3, and 3-chymotrypsin-like protease (3CLpro or Mpro), encoded by nsp5. Nsp2-16 will compose the viral Replication and Transcription Complex (RTC). The establishment of the viral RTC in double-membrane vesicles (DMVs), derived from rough endoplasmic reticulum (ER), is crucial for virus replication and thus a promising target for antivirals against SARS-CoV-2 (2,13).

Nsp12 encodes the RNA-dependent RNA polymerase (RdRp), which synthesizes a full-length negative RNA strand that functions as template for the generation of new positive-sense genomic and sub-genomic RNA (sgRNA) strands, the latter serving as mRNA for the structural and accessory proteins. The RdRp residing in the nsp12 is the centrepiece of the RTC, thus being a crucial enzyme in the SARS-CoV-2 life cycle. It is, therefore, suggested as a promising drug target as it mediates both the replication of the viral genome as well as the transcription of sgRNA (13,16,19).

Following replication and sub-genomic RNA synthesis, S, E and M proteins are translated in the endoplasmic reticulum (ER), while the N protein is translated by cytosolic ribosomes. S, E and M proteins then move along the secretory pathway in an endoplasmic reticulum-Golgi intermediate compartment (ERGIC), where they will assembly with the genomic RNA coated with the N proteins. M proteins play an important role in the virion particles assembly once it mediates protein-protein interaction. E proteins also contribute to the assembly process by interacting with the M proteins, suggesting the essential role of these two proteins to form the coronavirus envelope. The assembled viral components further undergo maturation in the

Golgi vesicle to form the mature virion with the lipid envelope. Finally, the enveloped virion is then exported from the cell by exocytosis, ready to bind other host cells (2,12).

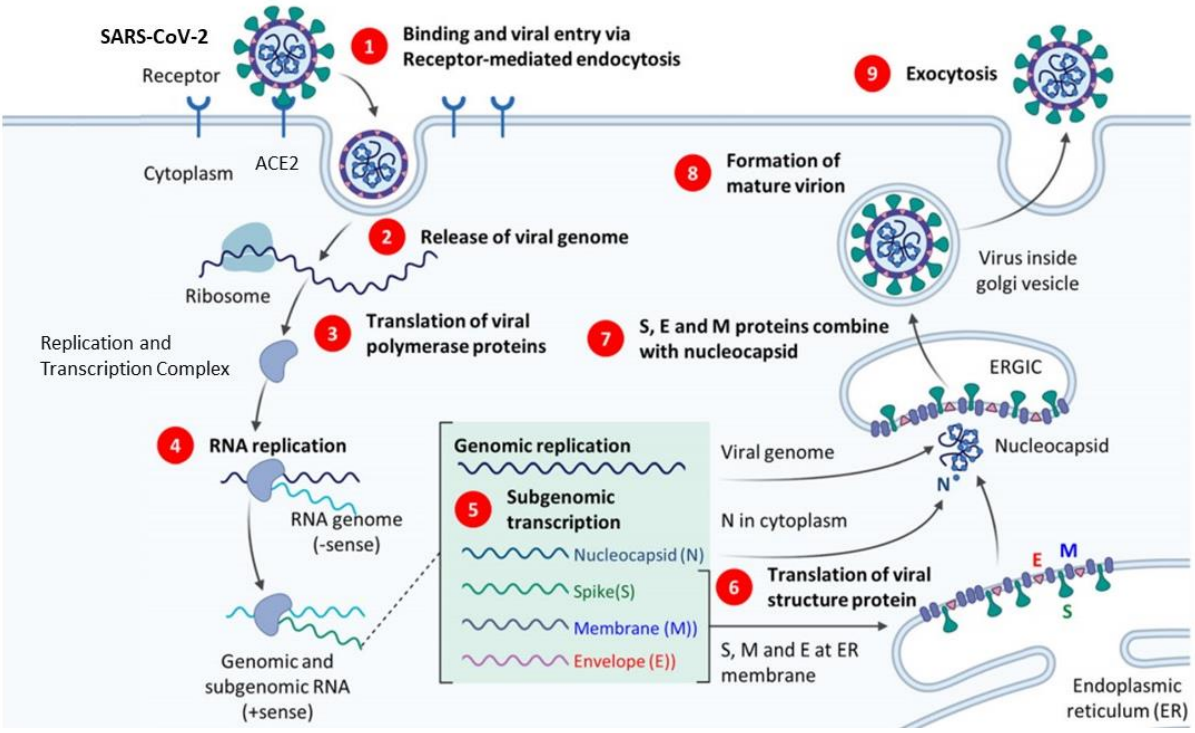

Figure 2. Main steps in SARS-CoV-2 life cycle.

The process starts with virus S protein binding to its receptor ACE2, allowing viral entry into the host cells by endocytosis or direct membrane fusion (1), with genomic RNA release (2). Viral polyproteins are translated and undergo proteolysis by 3CLpro and PLpro, resulting in non-structural proteins essential to form the viral Replication and Transcription Complex (RTC) (3). Then, the genomic and sub-genomic RNA replication and transcription take place (4 and 5), as well as the translation of the structural and accessory proteins (6). Viral particles undergo assembly in the ERGIC (7) and the formation of the mature virion takes place in the Golgi complex (8). Finally, the enveloped virion is expelled from the cell by exocytosis (9). Adapted from (20).

2.3. ACE2 as the entry receptor for SARS-CoV-2

The expression and tissue distribution of viral entry receptors will consequently influence its tropism and pathogenicity. Different coronaviruses bind different receptors. Regarding SARS-CoV-2, Zhou et al. (21) first conducted virus infectivity experiments on HeLa cells expressing or not expressing human ACE2, verifying that SARS-CoV-2 could only enter HeLa cells expressing ACE2. In addition, they concluded that SARS-CoV-2 could not enter cells expressing other Coronavirus receptors, including aminopeptidase N and dipeptidyl peptidase 4 (DPP4), confirming that ACE2 is the cell receptor for SARS-CoV-2.

Further studies on this matter, have concluded that ACE2 is highly specific for SARS-CoV-2, as it binds with an affinity about 10-20 fold higher than does with SARS-CoV, which possibly explains why SARS-CoV-2 appears to be more threatening than SARS-CoV, with a higher rate of transmission (22,23). The mechanistic explanation behind the enhanced binding affinity is not entirely clear, as ACE2 engagement is structurally similar between SARS-CoV and SARS-CoV-2 S proteins. However, there is a unique salt-bridge interaction formed between SARS-CoV-2 S protein and ACE2, which may contribute to the enhanced affinity (24). Additionally, Chen et al. (25) used molecular models (molecular docking) to show that this strong interaction between SARS-CoV-2 S protein and the ACE2 receptor is due to the SARS-CoV-2 Receptor Binding Domain penetration into a deep hydrophobic pocket in ACE2.

ACE2 is a cellular functional receptor for SARS-CoV-2. In the host it works as a type I transmembrane protein that functions as a monocarboxypeptidase with a single catalytic domain exposed to the circulation that hydrolyzes various peptides, including angiotensin II (Ang II) and angiotensin I (Ang I), generating angiotensin 1-7 (Ang1-7) and angiotensin 1-9 (Ang1-9), respectively (26).

In terms of human body distribution, ACE2 is expressed in vascular endothelium as well as in nearly all organs in varying degrees, starting with nasal epithelium, oral mucosa, brain, lungs, heart, kidney, small intestine, colon, and testis. In the respiratory system, both immunohistochemical method and single-cell RNA-seq analysis revealed that ACE2 is mainly expressed on type II alveolar epithelial cells, while weakly expressed on the surface of epithelial cells in the nasopharynx, oral and nasal mucosa. This suggests greater vulnerability in the lungs compared to the rest of the respiratory tree, making them prone to a more severe infection (27,28).

Further investigation is required to understand the regulatory mechanisms of ACE2 expression after SARS-CoV-2 infection. On the one hand, viral invasion may induce a decrease

in ACE2 levels through adaptative protective mechanisms in the host, which will consequently cause impairment in the renin-angiotensin-aldosterone-system (RAAS) homeostasis, leading to lung and heart injury. On the other hand, viral infection activates the immune system, promoting the expression of a variety of cytokines able to upregulate ACE2 expression by mechanisms such as the c-Jun N-terminal kinase (JNK) pathway, providing more receptors for viral entry and thus facilitating the spread of SARS-CoV-2. Therefore, either downregulation or upregulation of ACE2 may have adverse consequences for the human body, making ACE2 a potential target for therapeutic options against COVID-19 (29,30).

3. Immunopathology in COVID-19

3.1. Upper airways infection and the role of type I and type III IFNs in the innate immune response

After inhalation, SARS-CoV-2 infects the mouth, tongue, nasal- and larynx- epithelial cells. The viral replication is detected by the infected cells through pattern recognition receptors (PRRs) that serve as sentinels when sense pathogen-associated molecular patterns (PAMPs), such as unique viral genomic structures or viral replication intermediates. Upon PAMPs binding, PRRs recruit adaptor proteins crucial to activate downstream transcription factors, including interferon regulatory factor (IRF), Nuclear Factor-κB (NF-κB), and Activator Protein-1 (AP-1), resulting in the production of type I and type III antiviral Interferons (IFNs) and different chemokines. Meanwhile, other proinflammatory products derived from myeloid derived suppressor cells (MDSCs), dendritic cells (DCs), macrophages, and natural killer (NK) cells are also released in patients with SARS-CoV-2 (31).

Regarding RNA viruses such as Coronaviruses, both viral genomic RNA and its replication intermediates, are recognized by either endosomal receptors, as toll-like receptors (TLRs), mainly TLR3 and TLR7/8, and by the cytosolic receptors, such as retinoic acid-inducible gene I (RIG-I) and melanoma differentiation-associated gene 5 (MDA5) (31,32). The effects that each one produce on the immune response against the pathogen is described in Table 1.

Table 1. Main pattern recognition receptors in detecting RNA viruses and respective effects on immune response.

PPRs	Expression cells	Recognized PAMPs	Effects on immune response
TLR3	Endothelial cells (33) and Dendritic Cells (DCs) (34) Epithelial cells (35)	dsRNA (36)	Activation of IRF3 and NF- κB pathways, leading to the production of IFN-I and pro-inflammatory cytokines (36)
TLR7	Macrophages and DCs (36) Epithelial cells (35)	ssRNA (36)	Production of IL-1, IL-6, MCP-1, MIP-1α, TNF-α, and IFN- α (36)
TLR8			
RIG-I-like receptors	Epithelial cells and alveolar macrophages (37,38)	Short dsRNA and ssRNA specific motifs (37)	Promote the interaction of the viral RNA with the Mitochondrial antiviral-signalling protein (MAVS), consequently activating NF-κB and IRF3/IRF7 (39,40)
MDA-5	Epithelial cells (37,38)(37,41)	Long dsRNA (37)	

dsRNA: double-stranded RNA; IRF: interferon regulatory factor; MAVS: Mitochondrial antiviral-signalling protein; MCP-1: monocyte chemoattractant protein-1; MDA-5: melanoma differentiation-associated gene 5; MIP-1α: macrophage inflammatory protein-1α; NF-kB: Nuclear Factor-kB; RIG-I: Retinoic acid-inducible gene I; ssRNA: single-stranded RNA; TLR: toll-like receptor; TNF: tumour necrosis factor.

Based on the experience obtained from other respiratory viral infections, caused by pathogens such as SARS-CoV, MERS-CoV, Influenza viruses and Rhinoviruses, it is possible to infer a potential sequence of events regarding the response given by interferons (IFNs) to SARS-CoV-2 infection.

During the incubation stage of COVID-19, an efficient IFN response may be required to limit initial viral spread and prevent disease progression to moderate and severe stages. The infected epithelial cells may produce type I and type III IFNs (IFN-I and IFN-III) that can provide a local antiviral response through influencing various types of surrounding cells. IFN-III are produced first, and act as the first line of defence at the epithelial barrier, without triggering inflammation. When viral load is low, this initial antiviral response may suffice to

confront infection, causing viral clearance without expression of clinical symptoms or limiting them to mild respiratory manifestations such as cough (mostly dry), dyspnea and fatigue. However, when viral load is high in the first place or escapes IFN-III control, expression of IFN-I is upregulated, providing the second line of defence. IFN-I response potentiates viral resistance beyond the respiratory epithelium and stimulates pro-inflammatory responses, which are essential for host protection but are also responsible for the immunopathology inherent to COVID-19 (41).

The timing of IFN response can be affected by both viral and host factors and this influences the disease severity. When the viral burden is low, IFNs can clear the infection effectively, if induced early. However, if the viral load is high, the virus may strongly supress IFN response due to its evasion mechanisms, causing IFN delayed action. This late onset of IFN not only fails do control virus but may also lead to hyperinflammation and lung injury (42).

Notably, all IFNs share the unique ability to activate large sets of genes, collectively known as interferon-stimulated genes (ISGs) that inhibit viral replication, degrade viral nucleic acids, and induce viral resistance in uninfected neighbouring cells. Both type I and type III IFNs stimulate the response of a wide range of cell types (41), detailed in Table 2.

However, IFN-I exhibit powerful proinflammatory properties, characterized by the up regulation of various cytokines and chemokines, such as TNF-α, IL-1β, and IL-6. Thus, despite providing a strong defence line against the virus invasion, they may also exacerbate viral-associated complications by promoting excessive inflammatory responses. On the other hand, IFN-III lacks this impact, inducing a more sustained expression of ISGs without affecting the production of inflammatory mediators. With this being said, IFN-III may be regarded as regulators of the antiviral system, acting first in an attempt to prevent unnecessarily excessive inflammation associated with IFN-I activity (32).

Table 2. Type I and type III IFNs main characteristics and respective effects regarding human innate immunity. Adapted from (32).

Characteristics	IFN-I	IFN-III
Total elements	IFN-α and IFN-β, IFN-δ, IFN-k, IFN-ε, IFN-τ, IFN-ζ, and IFN-ω	IFN-λ1 (IL-29), IFN- λ2 (IL-28A), IFN- λ3 (IL-28B) and IFN- λ4
Main elements	IFN-α and IFN-β	IFN-λ1, IFN- λ2 and IFN- λ3
Receptor expression	All cells - leukocytes, fibroblasts, endothelial cells	Epithelial cells and some leukocytes. Macrophages, CD8+ T cells, NK cells, Treg cells, DCs, and hepatocytes
Effects on Neutrophils	Promote survival of neutrophils and the production of proinflammatory cytokines, as well as its activation Promote NETosis	Reduce ROS production by neutrophils Reduce degranulation Reduce migration
Effects on Dendritic Cells (DCs)	Cause conventional DC-induced Th1 cell differentiation Enhance MHC I and II expression and CD80, CD86, and CD40 expression in DCs	Cause DC-induced Treg cell differentiation Enhance CD80, CD86, and CD40 expression in pDCs Cause IFN-I and TNF-α production in human pDCs
Effects on Natural Killer cells (NK)	Increase NK cell-mediated cytotoxicity	May increase the IFN-γ secretion from NK cells
Other immunological effects	Induce NOS expression in macrophages Stimulate the migration of monocytes Increase perforin, granzyme B, and IFN-g production in CD8+ CTLs	May suppress human Th1 cell and Th2 cell activity

CTL: Cytotoxic T Lymphocyte; MHC: Major Histocompatibility Complex; NK cells: natural killer cells; NOS: nitric oxide synthase; pDCs: plasmacytoid dendritic cells; ROS: reactive oxygen species; Th cell: T helper cell; Treg cells: regulatory T cells.

When considering IFN administration as a possible therapeutic option, there are some aspects to take into account. IFN-III is effective to achieve a sustained antiviral response that limits viral spread both in the upper airways and in the lungs, whereas IFN-I should be restricted to the early phase of infection, since the objective is to facilitate viral clearance while limiting systemic inflammation induced by its antiviral response. Therefore, prophylactic treatment or early administration, prior to viral load peak, may provide host protection without an exacerbated immunopathology (42).

3.2. Lower airways infection due to immune response failure in the upper airways

Normal human airway is lined by a pseudostratified epithelium from the nose to terminal bronchioles, containing predominantly basal cells, ciliated cells, goblet cells, club cells and neuroendocrine cells. These cells play an active role in eliminating impurities and microorganisms from the respiratory tract.

Together with the mucins secreted by goblet cells and by mucous and serous cells from the submucosal gland, ciliated cells lead to the trapping of the virus with consequent clearance from the airways. Mucins attached to the airway epithelial microvilli and cilia generate an osmotic barrier that not only preserves the periciliary layer but also maintains the coordinated ciliary motility. In turn, secreted oligomeric mucin proteins retain water and form viscoelastic gels. The gel-forming mucins polymerize, allowing the trapped particles in the gel matrix to be cleared by mucociliary or cough transport. These synergetic actions between mucins and ciliated cells ensure mucociliary clearance. In addition, mucins also have a contribution in innate immunity, including direct interactions with dendritic cells and the activation of inflammatory cascade pathways (43).

During SARS-CoV-2 infection, both ciliated and goblet cells tend to suffer metaplasia, compromising the innate immune response. In addition to this damaged epithelium, Coronaviruses can interfere with innate antiviral immunity, mainly targeting 1) innate sensing, 2) IFN production, 3) IFN signalling and 4) ISG effector function (42). For example, SARS-CoV genome codes for the non-structural protein 1 (Nsp1), which antagonises IFN-I and IFN-III signalling. Similarly, SARS-CoV-2 ORF6, ORF8 proteins and its nucleocapsid also inhibit IFN-I signalling *in vitro* (44,45). This way, the virus may evade the antiviral IFN response and replicate in epithelial cells leading to higher viral loads. This high viral load may then reach

the low respiratory tract and access the alveoli space, where type I and type II pneumocytes are major host cells for new rounds of infection.

Regarding the alveolar epithelium, it is lined by squamous type I pneumocytes (T1P) and by cuboidal type II pneumocytes (T2P). T1P are thin cells, covering around 95% of the alveolar surface area and are closely juxtaposed with the capillary plexus, in order to maintain alveoli gas exchange. T2P, known as the defenders of the integrity and function of the alveoli, are responsible for the production of pulmonary surfactant, a lipid/protein complex manly composed by phospholipids (~80%) – phosphatidylcholine, phosphatidylglycerol –, neutral lipids (~10%) – mainly cholesterol –, and proteins (~10%). Among these proteins, surfactant-specific ones (6-8%) assume essential functions to sustain the proper structure and activity of the surfactant layer. Surfactant-protein B (SP-B) and SP-C are small highly hydrophobic and cationic proteins required for the formation and stabilization of the interfacial film during respiratory dynamics. Simultaneously, SP-A and SP-D are hydrophilic proteins with the ability to recognize and bind to pathogens, a characteristic of the collectin family. Generating pulmonary surfactant is essential for reducing surface tension in alveoli and preventing them to collapse upon every breath. On the other hand, accumulation of surfactant in the alveolar airspaces blocks gas exchange. Thus, the regulation of surfactant synthesis, secretion, and metabolism is essential for air breathing and, ultimately, survival (46,47).

It has been shown, using single cell RNA sequencing, that ACE2 is most abundant in alveolar T2P, making them a particular target during SARS-CoV-2 infection. Additionally, the alveolus is also inhabited by several immune cells, including alveolar macrophages (AM), interstitial macrophages, and dendritic cells, which will be recruited to eliminate the virus.

As a cytopathic virus, SARS-CoV-2 induces death and injury of the infected cells and tissue, as part of its replicative cycle. In humans, the immune responses activate pyroptosis in some infected cells, a highly inflammatory form of programmed cell death with associated vascular leakage. This event is a trigger for the subsequent inflammatory response (48).

Following cell pyroptosis, PRRs, alveolar macrophages and epithelial cells sense the viral RNA as well as the products released by the dead cells, called damage-associated molecular patterns (DAMPs). These include ATP, host DNA, proteins like ASC oligomer and IL-1β (36). IL-1β is an important cytokine released during pyroptosis and was demonstrated to be elevated in SARS-CoV-2 patients (49).

The inflammatory signal is then disseminated to the neigbouring cells, involving increased secretion of pro-inflammatory cytokines and chemokines by the macrophages and dendritic cells, such as IL-6, IFN-γ, macrophage inflammatory protein 1 (MIP-1α, also known as CCL3; and MIP-1β, also known as CCL4), monocyte chemoattractant protein-1 (MCP-1, also known as CCL2) and interferon γ-inducible protein 10 (IP-10, also known as CXCL10) (48). These proteins attract monocytes, macrophages and T lymphocytes – CD4+ and CD8+ – from the blood to the site of infection, promoting further inflammation and establishing a pro-inflammatory feedback loop. CD8+ T cells directly attack infected cells and kill them, whereas CD4+ T cells play an essential role in priming both CD8+ T cells and B cells. CD4+ T cells are also responsible for cytokine production, such as INF-γ, TNF and IL-2, driving immune cell recruitment (48). The recruitment of immune cells from the blood to the lungs, followed by infiltration of lymphocytes into the airways may explain the lymphopenia, mainly pronounced in the CD8+ T cell compartment, and the increased neutrophil-lymphocyte ratio (NLR), a well-known marker of systemic inflammation and infection, present in severe COVID-19 patients (48,50).

In most individuals, with an efficient immune response (right side of figure 3), the cytotoxic effect of virus-specific T cells recruited to the site of infection will eliminate the infected cells before the virus can replicate. Additionally, the proliferation of lymphocytes B with the release of neutralizing antibodies will block viral infection. Moreover, alveolar macrophages will recognize neutralized viruses and apoptotic cells, clearing them by phagocytosis. Thus, the host can reach viral clearance with minimal lung damage, the immune response recedes and patients recover by the second week of infection (48).

Alternatively, in a defective immune response (left side of figure 3), virus will block the signalling pathways to the neighbouring cells, resulting in supressed IFN response with local cell destruction and an exacerbated release of PAMPs and DAMPS. The further accumulation of immune cells in the lungs allied to the overproduction of pro-inflammatory cytokines, leads to widespread lung inflammation. Patients with this clinical condition typically exhibit higher blood plasma levels of IL-1β, IL-2, IL-7, IL-8, IL-10, granulocyte colony-stimulating factor (G-CSF), granulocyte–macrophage colony-stimulating factor (GM-CSF), IP-10, MCP-1, MIP-1α and tumour necrosis factor (TNF). In addition, IL-6 continuously increases over time. This acute phase response is characterized by an elevated serum ferritin, C-reactive protein (CRP) and pro-coagulant factors (48,51).

Humoral response is also an important component against viral infections. SARS-CoV-2 infection induces a strong B cell activation, maturation and consequently antibody production. Neutralizing antibodies (Nab), block the viral S protein interaction with the host, inhibiting viral entrance in the cells. In addition, neutralizing antibodies can interact with other immune components, potentiating the antiviral response. However, viral entry via Fc receptors can lead to 1) viral replication inside myeloid cells – productive infection – or 2) viral destruction, with no viral release – unproductive infection. Although there is no evidence of SARS-CoV-2 replication inside myeloid cells, its entry mainly through Fc-γ-RII (CD32) may activate endosomal TLRs and induce the release of pro-inflammatory cytokines, resulting in a phenomenon known as antibody-dependent enhancement (ADE). Whether an antibody efficiently neutralizes the virus or results in ADE with consequent acute inflammation is dependent on multiple factors, including the specificity, concentration, affinity and isotype of the antibody. This process is independent of ACE2 expression, although it is also a way that allows viral entry into host cells. Regarding SARS-CoV-2, it is possible that this antibody-dependent enhancement occurs derived from a suboptimal humoral response, resulting in low titters of anti-S IgG or production of non-neutralizing antibodies by B cell (52–54).

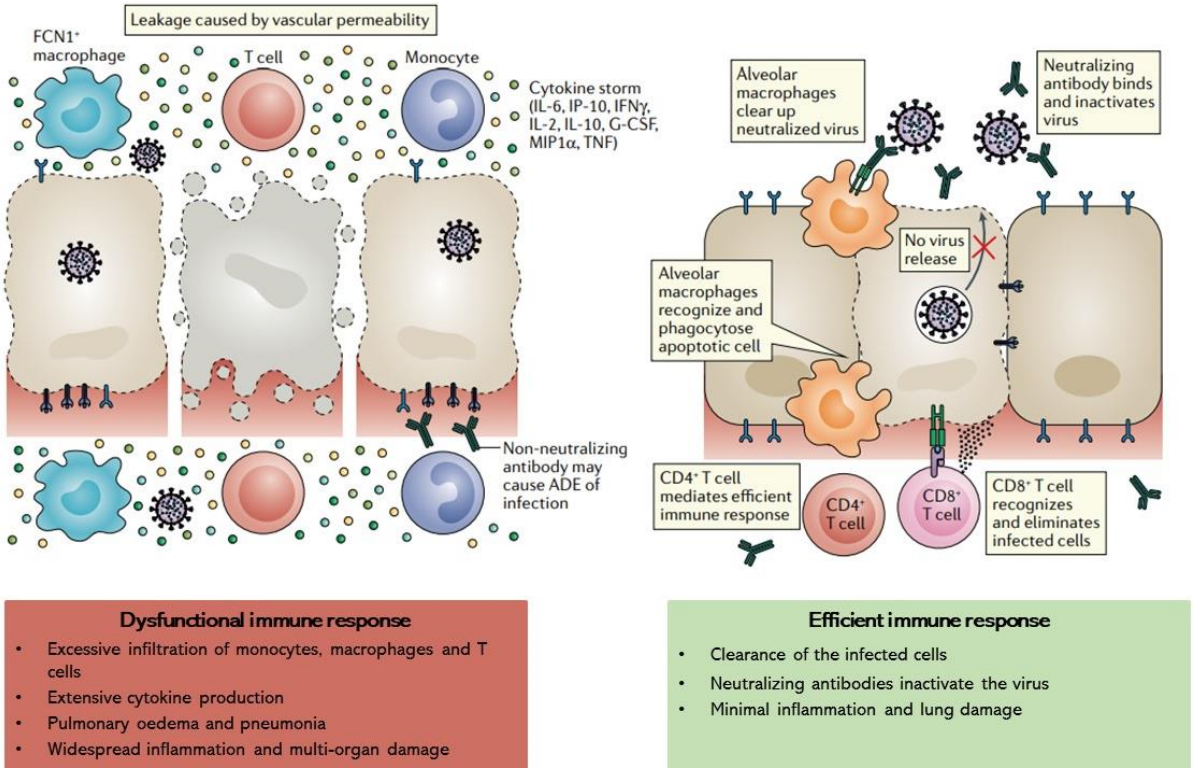

Figure 3. Different host responses to SARS-CoV-2 infection.

Schematic comparison between two different host immune responses to viral infection in the alveolar epithelium. Right side shows a healthy immune system, with the production of neutralizing antibodies by B cells and viral inactivation. Subsequently, T cells will recognize and eliminate the infected cells while alveolar macrophages will phagocytise the infected cells, resulting in an efficient viral clearance with minimal lung damage. On the other hand, left side presents an excessive infiltration of immune cells as a result of a cytokine storm, a state that leads to pulmonary edema and widespread inflammation. Adapted from (48)**.**

In terms of lung pathogenesis, Liao et al. (55) applied single cell RNA sequencing (scRNA-seq) and single-cell TCR-seq (scTCR-seq) to characterize the lung bronchoalveolar lavage fluid (BALF) cells from COVID-19 patients, comparing control samples with mild and severe cases. The results revealed an increased recruitment of immune cells to the lung, mainly macrophages, T cells and NK cells, in response to SARS-CoV-2 infection. Progression of severe COVID-19 was also characterized by an imbalance in lung macrophage populations, manifested by substantially increased monocyte-derived macrophages (M1 macrophages), pro-fibrotic macrophages (M2 macrophages), and almost a complete loss of lung alveolar macrophages (AM). Another relevant aspect to take from this analysis is the presence of a

significantly large population of CD8+ T effectors in the mild group of patients, revealing itself with the highest expansion levels among different T cell subsets. This fact evidenced their specificity for SARS-CoV-2, making it possible to infer that CD8+ T cells response may hold an important role in viral control in COVID-19 (55).

The pro-inflammatory cytokines released will cause the enlargement of the pores that separate the capillary cells, thus increasing the permeability of the alveolar capillaries, leading to a widespread edema, firstly interstitial and eventually an alveolar edema. Uncontrolled inflammatory cell infiltration may also increase an excessive secretion of proteases and reactive oxygen species (ROS), which combined with the direct harmful action of the virus will contribute to diffuse alveolar damage (DAD), with alveolar cells desquamation, hyaline membrane formation and pulmonary edema. As a consequence, there is a significant impairment of gas exchange in the lung, leading to difficulties in breathing and low blood oxygen levels. Additionally, this situation leaves the lung more vulnerable to secondary infections (48).

Most of the infected patients develop severe pneumonia with alveolar exudates, intralobular involvement and chest computed tomography typically characterized by ground-glass opacities, bilateral patchy shadowing and bronchovascular thickening within lesions (56). The outcome of these patients usually progresses to acute respiratory distress syndrome (ARDS), accounting for a large percentage of the morbidity and mortality associated with this disease (57–60).

3.3. Phagocytic cells and local coagulation

After the initial inflammatory response to alveolar damage and the first wave of cytokines, it is worth noting the role that monocytes and neutrophils play in the additional release of cytokines and in the local clotting process.

Endothelial cells are essential to maintain hemostasis in the coagulation system. Therefore, their dysfunction is the main cause of microcirculation alterations by moving the vascular balance more towards vasoconstriction with subsequent platelet activation, thrombosis, and inflammation with associated tissue edema and procoagulant state. One of the main signalling pathways promoting thrombo-inflammation is the NF-κB pathway (61,62).

Following induction of the NF-κB pathway, mononuclear cells will secrete pro-inflammatory cytokines, with emphasis on TNF-α, IL-1β, IL-6, and IL-8, that activate vascular

endothelial cells to increase the expression of cell adhesion molecules (CAMs), mainly vascular CAM-1 (VCAM-1), intercellular CAM-1 (ICAM-1), and platelet endothelial CAM-1 (PECAM-1). Levels of these adhesion molecules were elevated in patients with mild disease and strongly elevated in severe cases. Later studies also found that COVID-19 patients who were admitted to the intensive care unit (ICU) had increased soluble E-selectin and P-selectin plasma levels. These will attract immune cells such as monocytes and neutrophils, mediating their extravasation to the tissues, namely to the alveoli. In addition, IP-10, a chemokine secreted by alveolar pro-inflammatory macrophages, recruits more monocytes. In the alveoli, monocytes differentiate into monocyte-derived macrophages (M1 macrophages), which will secrete more IL-8**,** recruiting and activating more neutrophils (61,63–68).

Activated neutrophils exert their action through the formation of neutrophil extracellular traps (NETs) by a process called NETosis, a specific type of programmed cellular death. NETs are composed of a chromatin network associated with nuclear histones and granular antimicrobial proteins. Therefore, they will release DNA; reactive oxygen species (ROS); proteases, namely elastase; and eicosanoids, namely leukotrienes. Viral trapping within DNA fibres prevent its spread and promote the accumulation of antimicrobial factors at the site of infection (69).

In the alveolar capillaries, the endothelial cells can be activated either by direct viral damage, NETs' products or cytokine action, mainly TNFα, IL-1 and IL-6. This activation promotes the secretion of the previously mentioned CAMs and selectins, and the exposure of von Willebrand Factor (vWF) and Tissue Factor (TF) on epithelial cells, which in turn will attract and link cells from the bloodstream. TF is also expressed by activated monocytes and monocyte-derived microvesicles, thus activating the extrinsic coagulation pathway. TF stimulates the transformation of prothrombin into thrombin, which in turn converts circulating fibrinogen into fibrin, resulting in fibrin-based blood clots. This fibrin deposit is linked to an excess of activation of the coagulation and a defect in fibrinolysis. Fibrinolysis is the process by which plasmin breaks down fibrin in blood clots. Plasmin itself is an enzyme derived from plasminogen and is dependent on tissue plasminogen activator (tPA) and urokinase plasminogen activator (uPA). During an inflammatory situation, these two enzymes are released but are also inhibited by plasminogen activator inhibitor (PAI-1) (63,64,70).

TNF-α, IL-1 and IL-6, promote the release of PAI-1 as well as the inhibition of natural anticoagulants (tissue factor pathway inhibitor (TFPI), anti-thrombin III, proteins C and S). The inhibition of these major anticoagulant pathways associated with NETs' products will

activate the intrinsic/contact coagulation pathway, with additional platelet activation to amplify blood clotting. It is noteworthy that virus-induced NETosis acts as a double-edged sword: even though they eliminate the infected cells, they may also be harmful by itself due to the extensive release of ROS and proteases, which will destroy type I and type II pneumocytes. The damage in type I pneumocytes leads to a lack of gas exchange whereas the damage of type II pneumocytes results in decreased surfactant production. All these factors contribute to the collapse of the pulmonary alveoli (63,64).

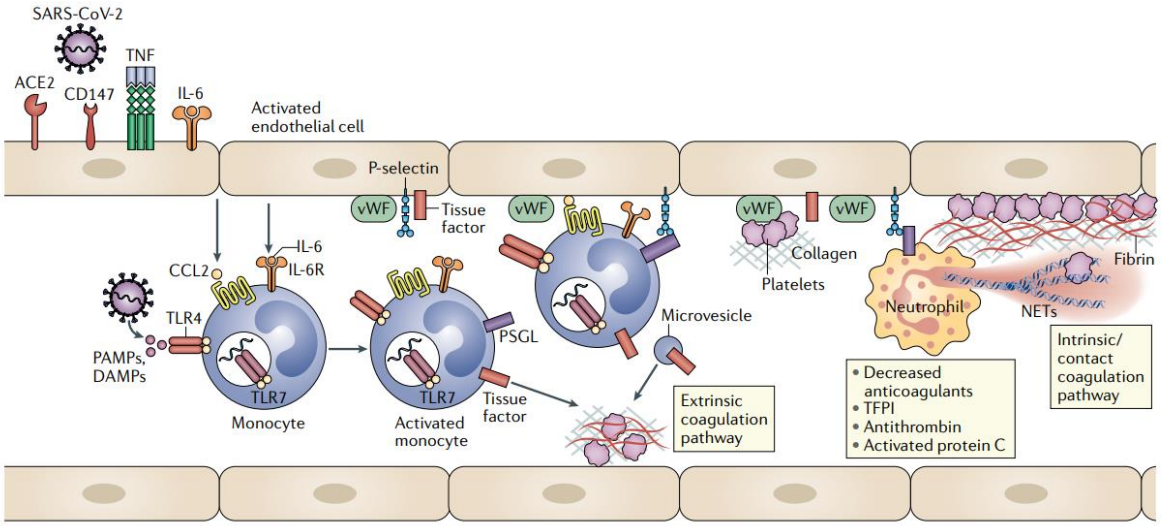

Figure 4. Key role of monocytes and neutrophils in the coagulation process.

Activated endothelial cells produce monocyte chemo-attractants and CAMs. In addition, they will also expose vWF, TF and recruit monocytes and neutrophils. Blood monocytes are activated by various stimuli, namely PAMPs, DAMPs and cytokines, consequently, inducing TF membrane expression. TF expressed by activated monocytes, monocyte-derived microvesicles and endothelial cells will activate the extrinsic coagulation pathway, with fibrin deposition and blood clotting. On the other hand, neutrophils release NETs, which will activate the intrinsic coagulation pathway and platelets, amplifying clots formation.

CCL2: CC-chemokine ligand 2; NETs: neutrophil extracellular traps; TF: tissue factor; TFPI: tissue factor pathway inhibitor; TLR: Toll-like receptor; TNF: tumour necrosis factor; vWF: von Willebrand factor. Adapted from (64).

Another aspect to take into consideration is the depth of hypoxemia, a state that may stimulate thrombosis through not only increasing blood viscosity but also activating hypoxia inducible transcription factors (HIFs) signalling pathway. These pathways lead to the transcription of multiple pro-thrombotic factors such as PAI-1 or TF while inhibiting the synthesis of natural anticoagulants (63,70).

The synergistic effects of these events result in the formation of microthrombi in the pulmonary bloodstream, a clinical entity called pulmonary emboly. Patients in this situation show signs of endothelial alterations such as high levels of D-dimers (in line with the high levels of D-dimers in angioedema), elevated prothrombin time, increased ferritin levels and an increased activity of von Willebrand factor and TF. In addition, angiotensin II levels are also high in the plasma of COVID-19 patients, associated with viral load and lung damage. Angiotensin II leads to microvascular permeability, transcription of TF, platelets activation and can also induce endothelial cells to release components of the complement system (61,62,71,72).

Regarding the local coagulation, a retrospective clinical evidence in Chinese patients has shown that the use of a low molecular weight heparin was related to a better outcome but only in cases characterized by an increase in D-dimers concomitant with altered coagulation parameters. It is a therapeutic option that must be carefully considered since heparin is associated with adverse effects when D-dimers levels are not high or when there is no alteration in the coagulation parameters (62). The last situation can be verified, for example, when patients develop angioedema, in which high levels of D-dimers are present but no alteration in the coagulation parameters is verified, highlighting the role of vasoactive peptides, such as bradykinin, which will be discussed in the following sections (73).

3.4. ACE2 dysfunction and the role of RAAS in COVID-19

Renin-Angiotensin-Aldosterone System (RAAS) is one of the most important hormonal networks in human body that regulates hemodynamic stability. In response to hypotension, renin, also known as angiotensinogenase, hydrolyses angiotensinogen into angiotensin I (Ang I). Angiotensin converting enzyme (ACE) converts Ang I into angiotensin II (Ang II), which has the possibility to bind to two different receptors – Ang II type 1 receptor (AT1R) or angiotensin II type 2 receptor (AT2R). Through AT1R, Ang II generates vasoconstriction,

inflammatory effects and sodium retention, while through AT2R it leads to vasodilation and natriuresis. On the other hand, ACE2 directly hydrolyses Ang II into Ang (1–7), or firstly Ang I into Ang (1–9), which can later be hydrolysed to Ang (1–7) by ACE or other enzymatic molecules. Ang (1-7) binds to the MAS receptor, exerting the opposite effects of Ang II, and Ang1-9 binds to the ATR2 receptor.

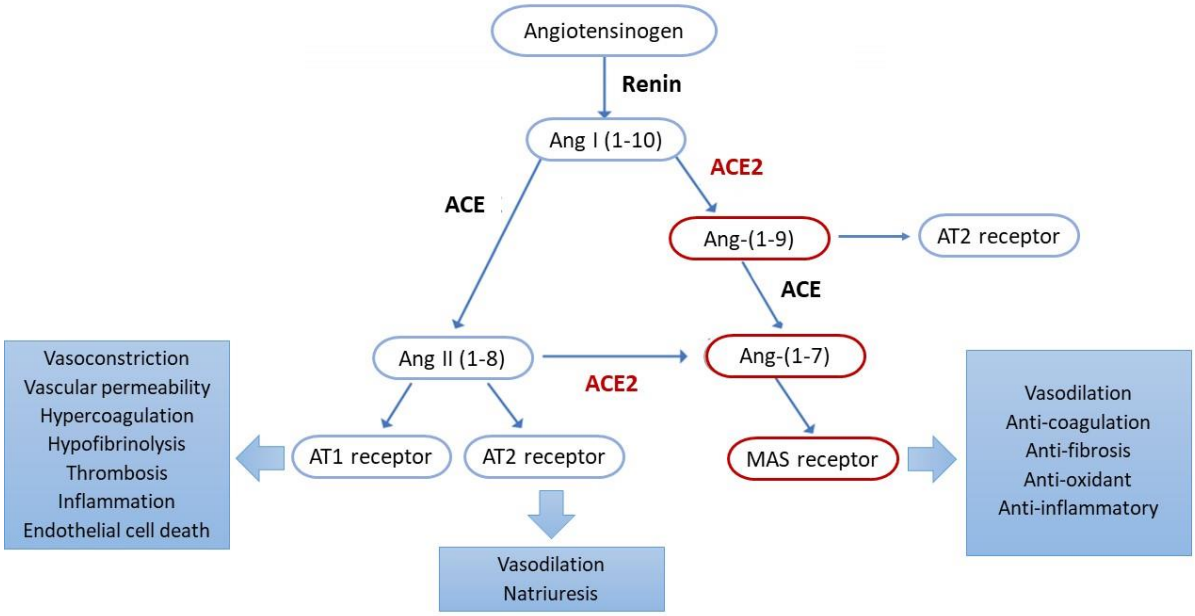

Figure 5. Metabolic pathway of the Renin-Angiotensin-Aldosterone-System.

The two main axes of the RAAS: ACE-Ang II-AT1R regulatory axis associated with pro-inflammation, pro-fibrosis, vasoconstriction and proliferation; and ACE2-Ang-(1-7)-MAS counter regulatory axis associated with anti-inflammation, anti-fibrosis, vasodilatation and antioxidant effects. Ang: angiotensin; ACE: angiotensin-converting enzyme; ACE2: angiotensin-converting enzyme 2; AT1 receptor: angiotensin II type 1 receptor; AT2 receptor: angiotensin II type 2 receptor. Adapted from (29,72,74).

ACE2 is an essential component of the infection, since it is the gateway of SARS-CoV-2 in human body. For that reason, understanding its regulation during each stage of the infection is crucial to acknowledge its role in SARS-CoV-2 pathogenesis.

In the acute phase of the infection, SARS-CoV-2 causes ACE2 downregulation by binding to it, which results in endocytosis of ACE2 alongside viral particles into endosomes,

reducing surface ACE2 expression (26). This endocytic event also upregulates ADAM17 activity, a disintegrin and metalloprotease 17, which in turn cleaves ACE2 on cell membrane surfaces. The lack of ACE2 removes an important pathway for the cells to degrade Ang I and Ang II into cardioprotective peptides: Ang (1-7) and Ang (1-9), thus lacking its anti-inflammatory benefits and its preventive effect of fibrosis (29,75).

The positive feedback pathways that facilitate further down regulation of ACE2 expression represent particular concern, since it perpetuates tissue damage, imbalance of RAAS homeostasis and increased Ang II levels. Besides promoting vasoconstriction, inflammation, blood coagulation and cell proliferation through AT1 receptors, Ang II also upregulates ADAM17 activity, contributing once again to further cleavage of cell surface attached ACE2 (29). An increase in the ratio of Ang II to Ang (1-7) following the removal of ACE2, contributes to hypertension, cardiac hypertrophy and enhanced susceptibility to heart failure. It also facilitates the progression of inflammatory and hyper-coagulation processes in the lungs, loss of its function, and generation of a cytokine storm, contributing to widespread inflammation observed in COVID-19 (11,75). In addition, accumulation of Ang II induces the expression of plasminogen activator inhibitor-1 (PAI-1) in endothelial cells, which in turn inhibits tissue plasminogen activator (tPA) and urokinase plasminogen activator (uPA), two proteins that mediate fibrinolysis. The increased levels of PAI-1 compared to tPA/uPA observed in COVID-19 results in hypofibrinolysis, which likely leads to vascular microthrombosis and fibrin deposits in the alveoli (72). Therefore, potential therapeutic strategies may include blocking of the receptor binding domain (RBD) of the viral S-protein, preventing its binding to ACE2 (18).

On the other hand, due to inflammatory widespread response, upregulation of ACE2 was detected in Bronchoalveolar Lavage (BAL) samples from patients with severe COVID-19 symptoms, whereas ACE was downregulated, likely leading to a shift in the RAAS toward the Ang (1-9) production axis. In addition, both AT1 receptor and AT2 receptor are upregulated in those samples. In this context, bradykinin (BK) is a peptide influenced by this impairment in RAAS, since Ang(1-9) augments bradykinin receptor signalling, presumably by Bradykinin receptor 2 (B2R) re-sensitization. There is evidence that ACE has a higher affinity for BK than for Angiotensin. Consequently, under low levels of ACE, the BK-directed hypotensive axis predominates, which may lead to severe hypotension and ultimately, shock (76,77).

Given the relevance of this system to human body homeostasis, factors that affect RAAS balance should be further investigated and may open new therapeutic perspectives. An

example is Vitamin D, another well known regulator of RAAS, since its receptor supresses renin expression. Garvin et al. (77) BAL gene expression revealed down regulation of Vitamin D receptors as well as an up-regulation in its catabolizing enzymes, suggesting a consequent increase in renin levels. Furthermore, vitamin D deficiencies have been associated with COVID-19 severity.

3.5. Bradykinin storm

Bradykinin (BK) is another potent regulator of blood pressure and is tightly integrated with the RAAS, not only because Ang (1-9) augments BK receptor signalling but also because both ACE and ACE2 have roles in inactivating the ligands for the BK receptors. ACE degrades BK in its inactivated peptides, while ACE2 is able to inactivate Lys des-Arg9-BK and des-Arg9 - BK, two potent ligands of the bradykinin receptor 1 (B1R) in the lungs. Similar to AT2 receptor stimulation, bradykinin also induces vasodilation, natriuresis and hypotension through bradykinin receptor 2 (B2R) (73,77,78).

In addition to its role in pressure and fluid homeostasis, bradykinin is a normal component of the inflammatory response after injury and induces pain through the stimulation of the B1R, as well as neutrophil recruitment and vascular permeability. It is also noteworthy that many of the COVID-19 reported symptoms such as myalgia, fatigue, nausea, vomiting, diarrhea, anorexia, headaches and decreased cognitive functions are common to other hyper-bradykinin known situations linked to hyper-permeabilization, such as angioedema (77).

In normal conditions, serine protease kallikrein converts kininogen to bradykinin, which will be available to bind B2R on endothelial cells. This binding stimulates the release of tPA/uPA to mediate protective vasodilatation and fibrinolysis, thus counteracting the effects of Ang II on the PAI-1 to tPA/uPA ratio.

During SARS-CoV-2 acute infection, despite the increased activity of kallikrein that results in upregulation of bradykinin, ACE2 downregulation leads to an imbalance to the Ang II RAAS axis at the same time. Ang II can convert bradykinin to its inactive peptides through aldosterone-induced ACE expression and increase PAI-1, thus inhibiting bradykinin's protective effects through tPA/uPA. Furthermore, ACE2 would normally inactivate des-Arg(9)-BK and Lys-des-Arg(9)-BK, two products that result from the action of carboxypeptidases in bradykinin and cause detrimental vascular effects. In COVID-19, ACE2 depletion makes these two kinins more available to bind to the endothelial B1R, leading to

edema, inflammation and oxidative stress (72). A schematic representation of this situation is seen in Figure 6.

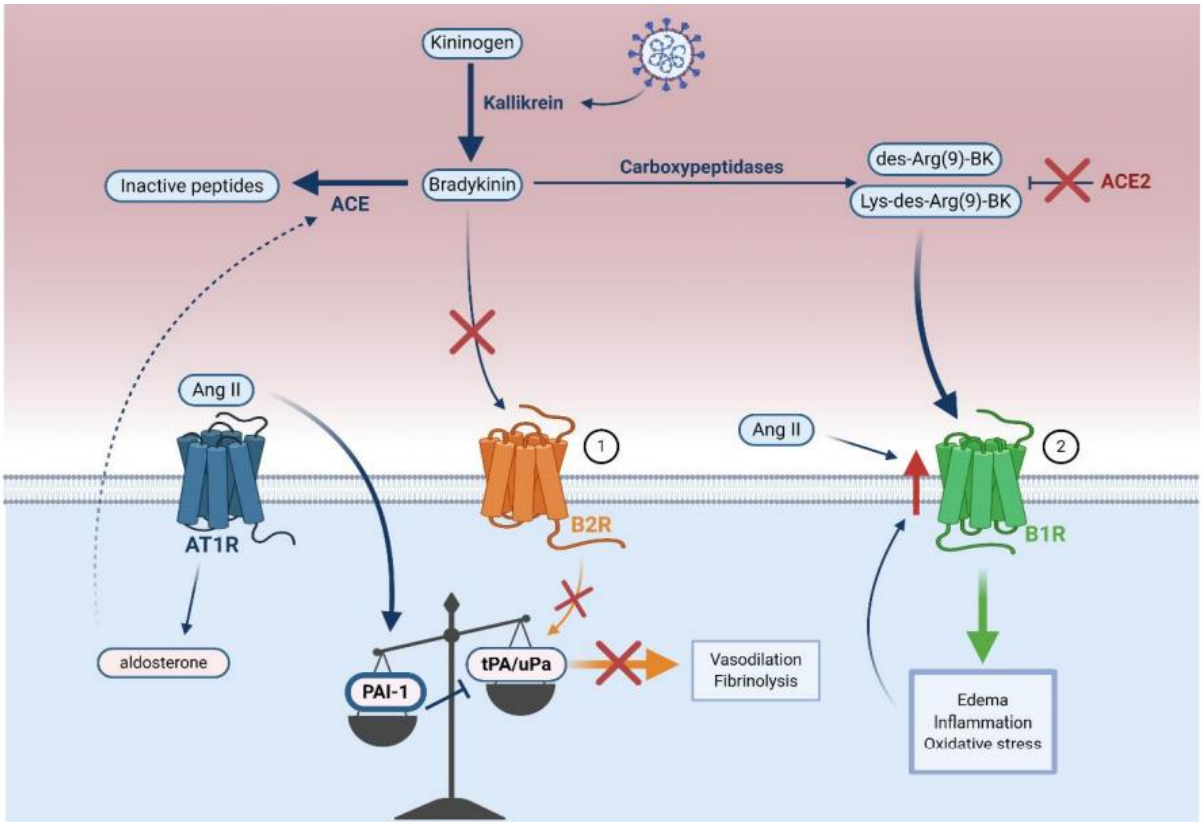

Figure 6. Effects of ACE and ACE2 on kallikrein-bradykinin pathway.

ACE mediates the conversion of bradykinin into its inactive peptides and increases the expression of PAI-1 through Ang II effects. This results in less bradykinin binding to B2R, leading to an imbalance in PAI-1 to tPA/uPA ratio and decreased vasodilatation with hypofibrinolysis. On the other hand, des-Arg(9)-BK and Lys-des-Arg(9)-BK are present in higher levels, due to the ACE2 downregulation which would normally inhibit their binding to the B1R. Ang II accumulation coupled with an increased B1R activity results in edema, inflammation and oxidative stress events in the alveolar endothelium. Adapted from (72).

However, as the inflammatory process spreads throughout the lung, RAAS may shift its main axis, also affecting bradykinin effects. Garvin et al. (77) analysed gene expression data from cells in bronchoalveolar lavage fluid (BALF) from severe COVID-19 patients and detected that, atypically, most of the enzymes that degrade bradykinin, including ACE, are downregulated in COVID-19 BALF compared to controls, whereas there is an upregulation in

bradykinin receptors (B1R and B2R), a typical result under pro-inflammatory conditions. This happens because, once SARS-CoV-2 reaches the host cells and starts to replicate its proteins, these will act to inactivate the host's first line of defence, most likely supressing NF-kB (reduced in BALF samples). This directly affects the RAAS since NF-kB normally induces ACE transcription by binding to its promoter (73).

As mentioned above, downregulation of ACE will cause the production of BK-augmenting peptide Ang (1-9). Expansion of this imbalance will increase BK levels, exacerbating its effects, namely pain and increased vascular permeability through B1R sensitization (77).

Furthermore, excess BK can lead to hypokalemia, as it increases urinary K+ excretion, which is associated with arrhythmia and sudden cardiac death. As what happens with K+, BK also increases urinary Na+ excretion, both resulting from B2R stimulation. Additionally, IL-2 is induced by bradykinin in the lung, also contributing to vascular leakage syndrome (VLS). This cytokine was reported to be highly upregulated in symptomatic but not asymptomatic COVID-19 patients and was detected in higher levels in BALF samples compared to controls (77,79).

In addition to the vascular effects, BK potently induces reflex bronchoconstriction, which makes it even harder for oxygen (O_2) to enter and carbon dioxide (CO_2) to escape from the airways. The sensory receptors responsible for this reflex action have been reported to be both rapidly adapting receptors (RARs) and C-fibers, whose afferent fibers are carried by the vagus nerve. Consequently, there is a stimulus to produce cough, mostly dry (80).

3.6. Lymphopenia

As described early in this document, during SARS-CoV-2 infection, macrophages and dendritic cells trigger an initial immune response, characterized by cytokine release and lymphocytosis. However, further inflammatory activity causes the destruction of lymphocytes, resulting in lymphopenia, another hallmark of the disease and a condition reported in many patients with COVID-19 (51,53). In addition, the excessive recruitment and accumulation of lymphocytes in the lung is one of the potential explanations for lymphopenia, a hypothesis supported by post mortem examination of pulmonary lymphoid infiltrates and BAL from mild and severe COVID-19 patients (81–83). Severe COVID-19 is associated with the T-cell rather

than B-cell compartment, supported by studies revealing decreased levels of both CD4+ T cells and CD8+ T cells as well as a lower production of IFN-γ by CD4+ T cells (84).

Even though not totally confirmed, this lymphopenia may be a consequence of the SARS-CoV-2 direct damage, causing cytotoxicity to T-cell populations (number 1 in figure 7) (85).

It is also suggested that elevated serum cytokines may play a role in depleting T-lymphocytes, since IL-6 inhibition proved to improve lymphopenia in severe disease in a subset of COVID-19 patients (86). In addition, there is evidence that main cytokine storm players can contribute to the development of lymphopenia. This is highlighted by the role of TNF-α, whose activity induces apoptosis in lymphocytes, and IL-6, which produces inhibitory effects on hematopoietic progenitor cells (number 2 in figure 7) (32,87).

Moreover, spleen atrophy and lymph node necrosis were observed in COVID-19 patients, supporting the idea that SARS-CoV-2 directly damages these lymphatic organs, further inducing lymphopenia (number 3 in figure 7) (5,88).

Finally, increased levels of lactic acid were detected in the blood of severe COVID-19 patients, which possibly inhibits lymphocyte proliferation (number 4 in figure 7) (88,89).

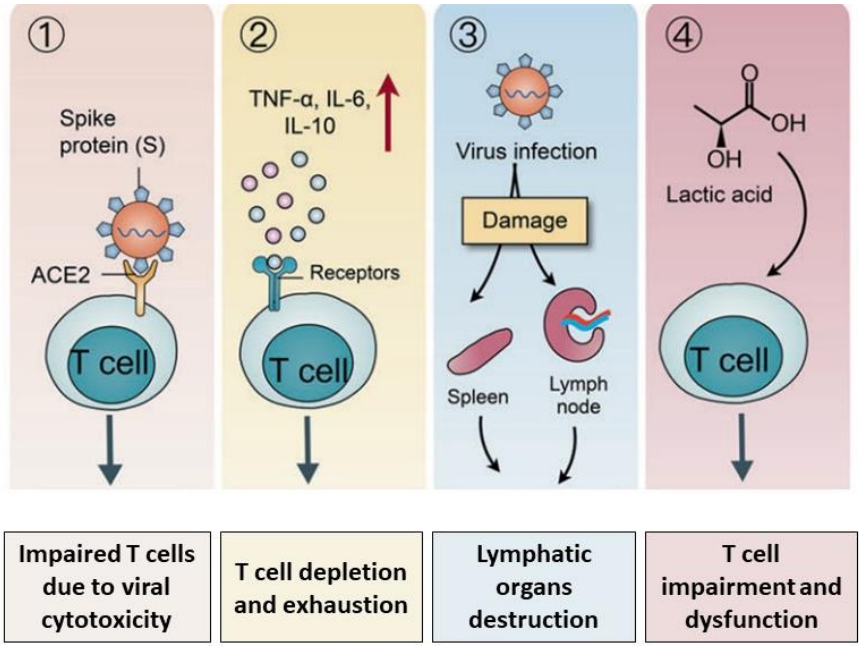

Figure 7. Mechanisms underlying lymphopenia in COVID-19.

A schematic presentation of some of the hypothesis that may explain the development of lymphopenia during SARS-CoV-2 infection. These include direct viral cytotoxicity in T-cells (1), T cell recruitment and exhaustion due to excessive cytokine production (2), damaged lymphatic organs (3) and T-cell dysfunction due to high levels of lactic acid (4). Adapted from (90).

3.7. Second wave of cytokine production: cytokine storm

SARS-CoV-2 infection leads to severe tissue damage, which stimulates cell programmed death with DAMPs release. Both primary infection – release of PAMPs – and cell debris accumulation – release of DAMPs – activate the endoplasmic reticulum (ER) stress response and causes up-regulation of enzymes that subsequently produce eicosanoids such as prostaglandins (PGs), leukotrienes (LTs) and thromboxanes (TXs). Associated with these eicosanoids release is cytokine production, as the host immune response. Normally, ER stress response downregulates protein synthesis to supress further viral replication in host cells. However, prolonged immune responses induce cytokine storms that mediate widespread inflammation and ultimately lead to a multi-organ damage and dysfunction (32,91).

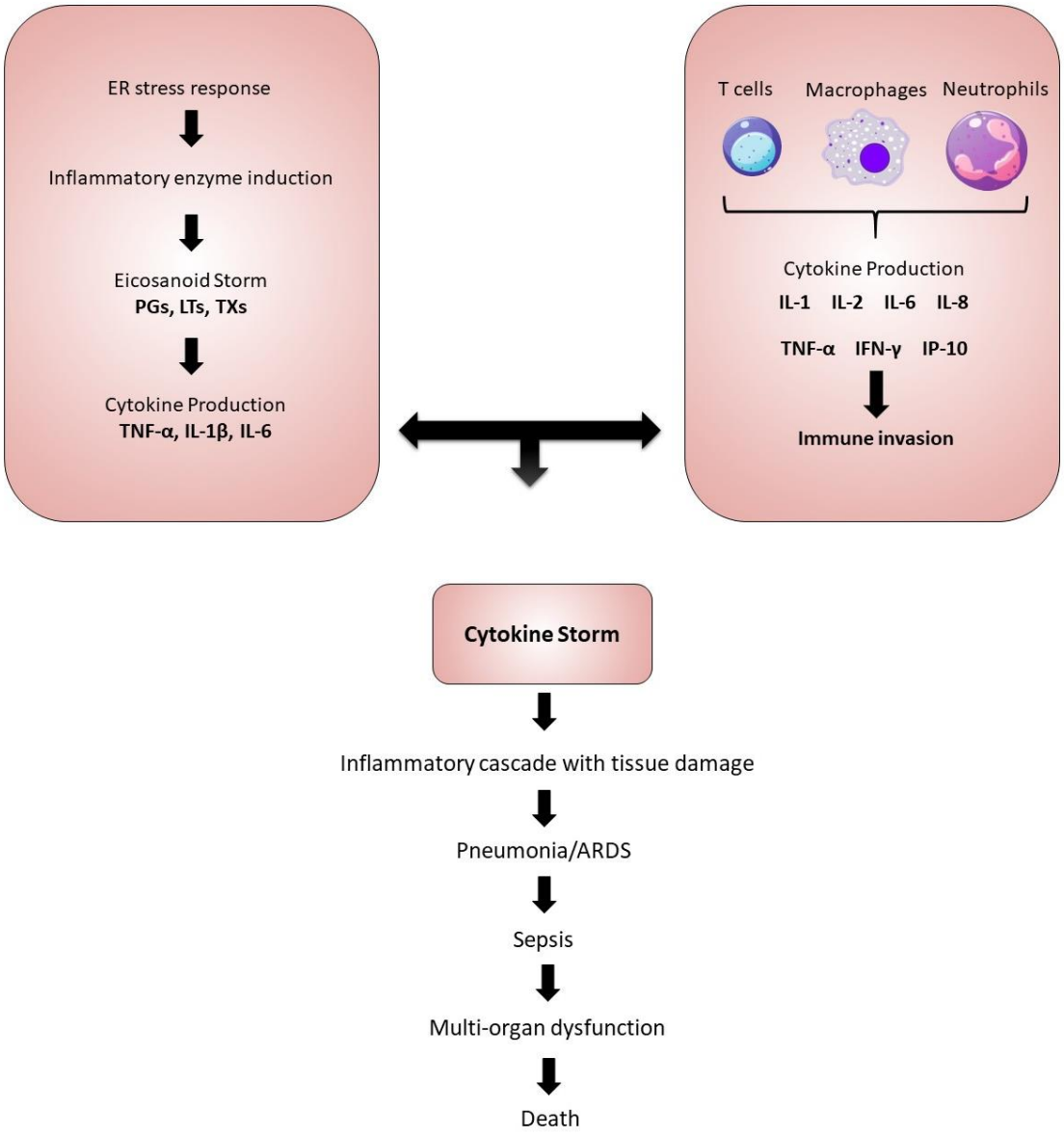

Figure 8. Cytokine storm and ultimate consequences.

Cytokine storm in COVID-19 is a result of excessive eicosanoids and cytokine release during the antiviral immune response launched by the host. An eicosanoid storm is triggered by an endoplasmic reticulum stress response, while the recruitment of T-cells, neutrophils and macrophages is a result from innate sensing of viral particles and products released from infected cells. These two events lead to an overproduction of various cytokines that, because of a prolonged pro-inflammatory response, cause a widespread inflammation with multi-organ dysfunction in the worst cases and, consequently, death. Adapted from (51).

3.7.1. Acute Respiratory Distress Syndrome

In a study conducted by Guan et al (92), 1099 patients with COVID-19 were assessed regarding their clinical features and disease. They concluded that the most common complications were pneumonia (91.1%) followed by acute respiratory distress syndrome (ARDS) (3.4%). Severe pneumonia developed after exposure to coronavirus is usually related to rapid replication of the virus and extensive production and accumulation of inflammatory cells, leading to acute lung injury (ALI) and acute respiratory distress syndrome (ARDS), in the worst cases (32).

ARDS occurs as a consequence of an acute systemic inflammatory response, which results in lung damage, either directly or indirectly. It is a condition characterized by reduced lung compliance and severe hypoxemia, with chest images that suggest bilateral pulmonary effusion in the absence of a cardiogenic or hydrostatic aetiology. Regarding ARDS pathogenesis, it is possible to identify three stages of disease progression and severity (75,93).

3.7.1.1. Phase I of ARDS: the exudative phase

Phase I, termed the exudative phase of ARDS, concerns the lung's initial response to injury and corresponds to the first ten days of viral infection. This stage is characterized by inflammation of the alveolar epithelial cells, due to the innate immune cell-mediated damage of the alveolar barriers - endothelial and epithelial - that increase vascular permeability and cause the accumulation of protein-rich edema fluid within the pulmonary tissue and alveoli (75).

The exacerbation of IFN-I and IFN-III released by infected epithelial cells induce cell apoptosis. Although it is already known that the immune response fails to launch balanced levels of IFN at the right time in SARS-CoV-2 infection, the levels of cytokines are consistently high. IL-1β and IL-18 secretion activates alveolar macrophages (AM), which will phagocytose apoptotic cells, promoting viral clearance. Endothelial and epithelial damaged cells also release chemokines such as CCL2, CCL3 and CXCL10, which in turn attract myeloid mononuclear cells that release more inflammatory cytokines, creating a positive feedback loop. Meanwhile, more pro-inflammatory and chemotactic cytokines are secreted in response to the macrophage activity, including IL-6 and IL-8. The reactions from lung epithelial cells and alveolar macrophages work synergistically to recruit other immune cells to the lung parenchyma. These include the invasion of neutrophils, natural killer (NK) cells and monocytes, the latter

differentiating into monocyte-derived macrophages (M1 macrophages) and dendritic cells (85,94).

Neutrophils induce nonspecific epithelial cell necrosis by secreting effector compounds such as NETs. In a similar way, NK cells exert both pro-necrotic and -apoptotic effects by secreting cytotoxic granzymes and perforins (85).

Monocyte-derived macrophages contribute to infected epithelial cell phagocytosis, as well as to the secretion of TNF-α and nitric oxide (NO) to activate cell apoptosis (85).

Dendritic cells are essential to link the innate and adaptative immunity through sampling viral antigens from the lung alveoli and migrating to lymph nodes. There, they act as antigen-presenting cells for naïve CD8+ and CD4+ T-cells, activating them to expand and mature in an antigen specific clonal expansion, after which they are mobilised to the lung. Once in the lung, CD8+ T-cells are co-stimulated by CD4+ T cells to induce the lysis of cells presenting antigens. CD4+ T cells also secret additional cytokines such as IFN-γ.(85).

Despite being an attempt to activate the clearance of the virus, these responses also contribute to uncontrolled lung injury due to epithelial cell destruction and prolonged inflammation. Ultimately, there is hyaline membrane deposition, a hallmark of diffuse alveolar damage (DAD) (58).

This hyaline membrane is essentially formed by two main components: fibrin and hyaluronic acid. Fibrin, an insoluble protein formed during immune response, leaks into the interstitial space, where it accumulates. Hyaluronic acid (HA) is a polysaccharide found in most connective tissues that is able to trap around 1000 times its weight in water, forming a hydrogel. The increased expression of genes involved in the HA synthesis combined with the vascular hyperpermeability mediated by the bradykinin storm leads to the formation of a viscous hydrogel that will accumulate in the alveoli and reduce the efficiency of gas exchange. This event is the cause of the ground-glass opacities seen in the chest computed tomography of COVID-19 severe patients (77).

As a consequence, surfactant function and pulmonary clearance remain deficient and increase surface tension throughout the lung, leading to alveolar collapse and a significant reduction in oxygen levels in the blood - hypoxemia (75,93).

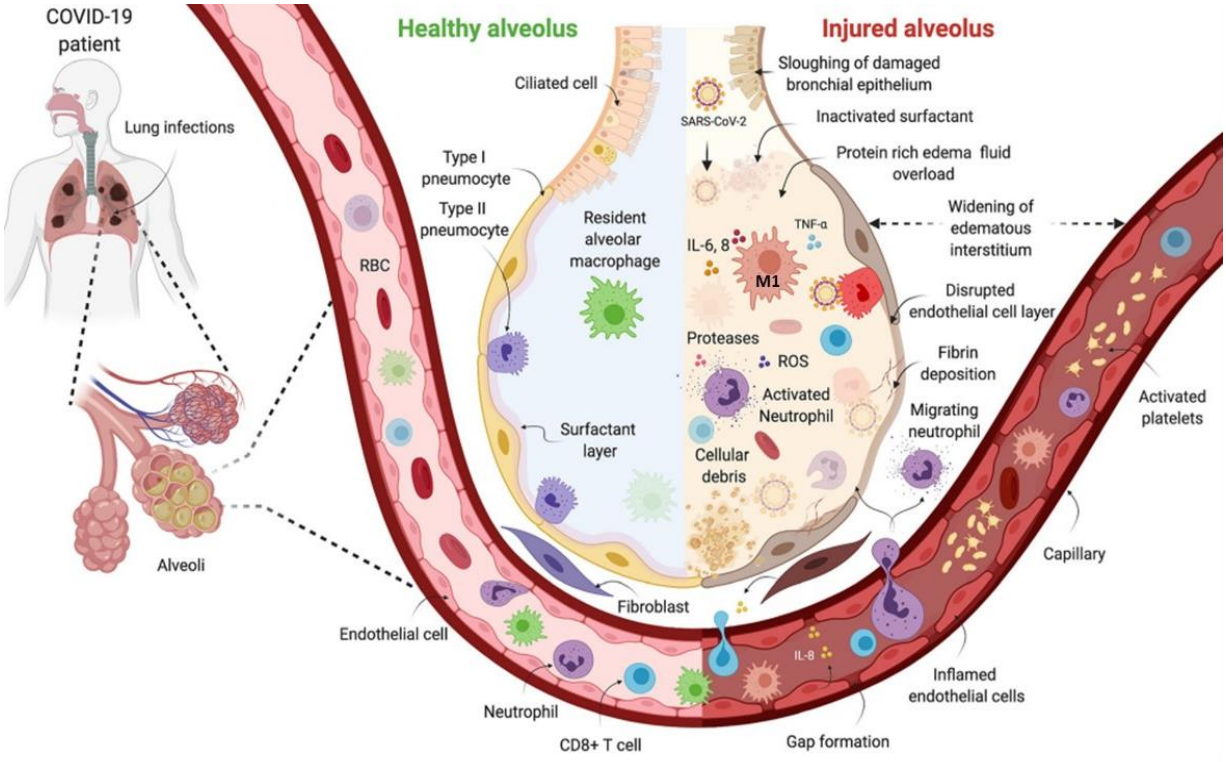

Figure 9. SARS-CoV-2 induced ARDS.

In the alveoli, SARS-CoV-2 primarily infects type 2 pneumocytes that express ACE2. Pyroptosis of the infected cells induced by viral replication induces the accumulation of ROS, cellular debris, proteases and proinflammatory cytokines that will be recognized by alveolar macrophages, triggering the production of more cytokines and chemokines: cytokine storm. Activated monocytes, CD4+ and CD8+ T cells, neutrophils and NK cells are recruited and leak through the pulmonary capillaries to the lung parenchyma and interstitum. M1 macrophages and CD4+ T cells exacerbate inflammation by producing additional cytokines. M2 macrophages contribute to fibrin deposition in the alveoli. This way, a pro-inflammatory feedback loop is established and leads to ARDS. Direct viral damage on endothelial cells may also trigger endotheliitis in the pulmonary vasculature. Edema results from protein accumulation in interstitial space allied to vasoconstriction via platelet activation, further decreasing oxygen exchange capacity. Adapted from (2).

3.7.1.2. Phase II of ARDS: the proliferative phase

Phase II, the proliferative stage, occurring around weeks 2-5 of the disease, is characterized by an extensive fibroblast and myofibroblast recruitment and proliferation, with subsequent matrix deposition. Along with the fibrotic deposition, there is also the reepithelization by airway progenitor cells and type II pneumocytes (T2P) proliferation. The latter then differentiates in type I pneumocytes (T1P) (58). Additionally, thrombotic events in pulmonary small arteries may occur in this phase due to neutrophil extracellular traps influence (81,95).

Over time, the repair process begins, mediated by host components such as FOXP3+ regulatory T cells (Tregs). The contribute of Tregs in the recovery phase has been identified in many ways, including the negative regulation of CD4+ T cells, which promotes immune self-tolerance, as well as the inhibition of the excessive pro-inflammatory effects of macrophages, while simultaneously enhancing their phagocytic activity. Moreover, Tregs also stimulate the secretion of keratinocyte growth factor and amphiregulin, a ligand of the epidermal growth factor receptor, promoting lung epithelial cell proliferation (20,41).

These processes modulate the host immune response and play an essential role in restoring lung parenchymal homeostasis. Consequently, their potential malfunction may partially explain the wide spectrum of outcomes among patients with SARS-CoV-2-induced ARDS (85).

3.7.1.3. Phase III of ARDS: the fibrotic phase

Lastly, phase III, the fibrotic stage of ARDS is not usual in many patients. It is characterized by extensive damage throughout the cellular basement membrane and by delayed re-epithelialization of the lung tissue, with subsequent development of interstitial and intra-alveolar fibrosis. This stage is linked to prolonged mechanical ventilation and increased mortality (75).

Patients reaching this severe stage have their lung architecture and function altered by the progressive enlargement of fibroblasts population and extracellular matrix. Both transforming growth factor-β1 (TGF-β1) and collagen may play critical roles in airway remodelling. This airway remodelling has also been associated with elevated plasma levels of Ang II, which can trigger the production of TFG-β1 and collagen deposition (96).

Within this context, TFG-β1 and ROS are the main contributors to this process. TFG-β1 is expressed in high levels during fibrosis and acts as a potent pro-fibrogenic cytokine. In

addition, TFG-β1 favors a redox imbalance by increasing ROS levels while supressing antioxidant enzymes. On the other hand, ROS induces TFG-β1 fibrogenic activity. During pulmonary viral infection fibrosis, the oxidative stress rises in epithelial cells, inducing the production and release of TFG-β, which results in excessive migration, proliferation and activation of fibroblasts as well as its differentiation into myofibroblasts. The latter are able to synthesize smooth muscle α-actin (α-SMA) and produce extracellular matrix, which is composed by collagen and fibronectin (96).

Furthermore, Ang II-induced collagen expression also depends on TFG-β, which consequently causes extracellular matrix accumulation and inflammation. Additionally, activated fibroblasts induce further alveolar epithelial cells injury and death, creating a vicious circle of interactions between profibrotic cells and fibroblasts, maintained by TFG-β, and ultimately leading to the formation of non-functional scar tissue. In the alveoli, TFG-β will induce alveolar macrophages to secrete cytokines like IL-4, IL-6, and IL-13, thus enhancing the development of fibrosis (96).

Depending on the infection degree and lungs involvement, patients can reach a state where gas exchange becomes impossible, requiring invasive mechanical ventilation and/or extracorporeal membrane oxygenation (97).

ALI/ARDS may be the first organ dysfunction, occurring in the early stage of a systemic inflammation syndrome that causes multiple organ dysfunction and leads COVID-19 patients to a terminal state (98).

3.7.2. Systemic multi-organ dysfunction

This literature review explores the systemic inflammatory response caused by SARS-CoV-2, which triggers a series of damage initially observed in the lung, with many patients experiencing respiratory manifestations such as respiratory distress and pneumonia. Additional severe pathologies are now widely recognized in many other organs including heart, spleen, lymph nodes, brain, liver, kidneys, eyes and vasculature, due to an extensive widespread inflammatory response. Table 3 describes the clinical manifestations reported so far in COVID-19 cases.

Key mechanisms that may be in the origin of multi-organ injury upon SARS-CoV-2 infection include direct viral toxicity, endothelial dysfunction and inflammation with

thrombois, dysregulation of the immune response linked to hypercytokinemia and impairment of the renin-angiotensin-aldosterone system (RAAS) (99).

Table 3. Clinical manifestations reported by COVID-19 patients. Adapted from (2,51,99).

Affected organ system	Clinical manifestations
Cardiovascular	Cardiac inflammation Cardiomyopathy Myocardial ischemia Arrythmias Acute cor pulmonale Blood clots Cardiogenic shock
Renal	Acute Kidney injury Proteinuria Haematuria
Gastrointestinal	Altered intestinal flora Abdominal pain Diarrhea Anorexia Nausea/vomiting
Hepatobiliary	Raised Aminotransferases Elevated Bilirubin
Neurologic	Headache Dizziness Confusion/impaired consciousness Encephalopathy Myalgia Anosmia Ageusia Shock Guillain-Barré syndrome
Dermatologic	Urticaria Erythematous rash Vesicles Chilblain-like lesions Petechiae

3.7.2.1. Cardiovascular system

SARS-CoV-2 can cause both direct and indirect cardiovascular sequelae. This negative effect includes myocardial injury, acute coronary syndromes, cardiomyopathy, acute cor pulmonale, arrhythmias, thrombotic complications and ultimately, shock (99).

Several clinical features were reported from COVID-19 patients who were admitted to the intensive care unit (ICU) in comparison with COVID-19 patients who were not in need of intensive care. In this context, we can highlight the higher systolic pressure of ICU patients – 145 mmHg – compared with non-ICU patients – 122 mmHg - and a significant increase in myocardial biomarkers such as creatinine kinase-MB (CK-MB) and cardiac troponin levels (49,75).

The mechanisms underlying the cardiovascular manifestations include diverse factors. First of all, ACE2 is highly expressed in cardiovascular tissue, including myocytes, fibroblasts, endothelial cells and smooth-muscle cells. This fact supports the hypothesis of a direct viral injury (99).

Another factor to consider is the vasodilating peptide apelin (APLN), a potent inotrope, which is increased in COVID-19 BAL samples, as well as its receptor and B1R. Therefore, APLN is an important extension of the RAAS in controlling cardiac contraction and blood pressure, and its increased signaling can be added to the imbalanced RAAS effects. Furthermore, APLN upregulates ACE2 expression, enhancing viral entry and infection in the cardiac tissue. High levels of APLN are associated with arrythmias. Moreover, its activity in cardiac contraction and vasodilation associated with abnormally high BK output can be another cause of cardiac events (99,100).

As aforementioned, excess BK can lead to hypokalemia, a clinical feature reported mainly in severe COVID-19 patients, which is associated with arrhythmia and sudden cardiac death, both of which also verified in COVID-19 patients (49,101,102).

3.7.2.2. Renal system

Acute kidney injury (AKI) is a frequent manifestation of COVID-19, occurring in much higher rates in critically ill patients, and is associated with mortality. In a study from USA (103), around 5500 patients were admitted with COVID-19 in a New York City hospital, approximately 37% of which had AKI with 14% of them requiring dialysis. Data on kidney function in COVID-19 patients has revealed increased proteinuria and hematuria (103,104). Moreover, hyperkalemia and acidosis are also common electrolyte abnormalities associated to renal complication, even among patients without AKI (99).

Direct viral infection and consequent kidney injury is one of the possible causes and was observed specifically in the brush border of proximal tubules, where ACE2 has its higher expression (51,75).

Histopathological findings show acute tubular injury and diffuse erythrocyte aggregation with obstruction in peritubular and glomerular capillary loops. In addition, distinctive viral particles have been visualized though electron microscopy in tubular epithelium, podocytes and glomerular capillary endothelial cells (27,105,106).

Indeed, ACE2 internalization upon virus binding can lead to an increase in sodium reabsorption, blood volume, blood pressure and kidney damage. Moreover, internalization of ACE2 alters the ratio Ang II/Ang (1-7), therefore exacerbating renal damage. As stated before, increased circulating levels of Ang II results in vasoconstriction and increased blood pressure, affecting not only the kidneys but also other organs. Also, lymphocytic endothelialitis in the kidney associated with the viral inclusion particles in the glomerular capillary endothelium suggests microvascular dysfunction as a consequence of endothelial damage. Finally, cytokine overproduction and release may also have an important contribution to the pathophysiology of AKI (107,108).

3.7.2.3. Gastrointestinal tract

Regarding gastrointestinal symptoms, the most frequently reported include anorexia, nausea and/or vomiting, diarrhea and abdominal pain.

Direct viral damage is once again a plausible hypothesis to explain gastrointestinal manifestations, since ACE2 is abundantly expressed in the gastric, duodenal, and rectal epithelia glandular cells. In addition, viral nucleocapsid protein was visualized in gastric,

duodenal and rectal epithelial cells, as well as in glandular enterocytes. Deficiency in ACE2 in intestinal epithelial cells has been associated to altered intestinal flora, amino acid malabsorption from diet and intestinal barrier dysfunction, with increased susceptibility to colitis, thus suggesting its potential role as an inflammatory regulator. Therefore, it is hypothesized that loss of ACE2 due to SARS-CoV-2 infection may contribute for the development of symptoms including gastrointestinal discomfort, diarrhea and pain. In addition, the dysfunction in gastrointestinal barrier increases the host susceptibility for opportunistic infections and exacerbated metabolic syndrome (75,99,109).

On the other hand, the local increase of prostaglandins, naturally derived from the inflammatory process, will act on the intestine smooth muscle and induce its contraction, consequently causing pain, nausea, diarrhea, vomiting.

Endothelium inflammation, either by direct viral involvement or host immune response, is also a complication transversal to all organs and, in this case, there is histopathological evidence of diffuse endothelial inflammation in the submucosa vessels of the small intestine and mesenteric ischemia from COVID-19 patients' intestine, suggesting microvascular injury in the small-bowel. Moreover, the presence of infiltrating lymphocytes and plasma cells as well as interstitial edema in the lamina propria of the stomach, duodenum and rectum of COVID-19 patients supports the presumption of tissue damage from inflammatory processes (99,109).

Xiao et al. (109) have detected viral RNA in feces from COVID-19 patients, even after viral RNA in the respiratory tract revealed a negative test result. This evidence suggests that the infected gastrointestinal cells continuously secrete infectious virions (49). Therefore, prevention of a possible fecal-oral transmission should be considered to control viral spread. Accordingly, it is recommended that rRT-PCR testing for SARS-CoV-2 from feces should also be performed and hospitalized patients with SARS-CoV-2 should maintain transmission-based precautions if their rRT-PCR feces test remain positive.

3.7.2.4. Hepatobiliary manifestations

Hepatocellular injury is seen between 14-53% of hospitalized COVID-19 patients, with abnormal levels of alanine aminotransferase and aspartate aminotransferase typically characterizing this complication. At hospital admission, elevated bilirubin has also been associated to disease severity and progression to critical illness.

ACE2 is expressed in cholangiocytes, thus allowing the entry of SARS-CoV-2, which results in direct damage of the biliary ducts. In addition, hyperinflammation and metabolic dysfunctions following cytokine storm and hypoxia, respectively, are also plausible mechanisms underlying liver damage. There is also a possibility of drug hepatotoxicity in patients taking agents such as remdesivir, lopinavir and tocilizumab (99,110,111).

3.7.2.5. Neurologic manifestations

Neurologic impairment following SARS-CoV-2 infection has been widely reported and studied. Autopsies from dead COVID-19 patients reveal brain tissue edema and partial neuronal degeneration (112). The most common reported symptoms include headache, dizziness, myalgia and/or fatigue, anosmia and ageusia. More severe manifestations include acute cerebrovascular diseases – stroke – and confusion or impaired consciousness (49,113,114). There are also few reports of demyelinating polyneuropathy – Guillain-Barré syndrome (115).

SARS-CoV-2 may reach the central nervous system through the nasal mucosa, olfactory bulb, lamina cribrosa, or even via retrograde axonal transport. Within the brain, ACE2 expression occurs mainly in the glial cells, neurons and spinal fluid (75,99).

Starting from the nasal mucosa, the epithelial cells there present a considerably high level of ACE2 expression. This fact may explain the symptoms of altered sense of taste – ageusia – and smell – anosmia – frequently reported among COVID-19 patients (116).

In addition, Bhaskar et al. (51) propose that both immune system and central nervous system (CNS) play an important role in the process. Both activated microglia and IL-1 can lead to an increased production of ROS, phagocytosis, apoptosis and cytokine expression within the CNS. Consequently, this results in neuroinflammation, oxidative stress and toxicity in the nervous cells – excitotoxicity –, thus catalysing neural tissue damage and synaptic dysfunction. In addition, IL-1β and TNF-α induce prostaglandin 2 (PG2) release in the CNS, which will cause an increase in body temperature, responsible for the common symptom of fever.

3.7.2.6. Dermatologic manifestations

Cutaneous manifestations have occasionally been reported by some COVID-19 patients, even though in a lower rate compared to the complications described above. These include erythematous rash, urticaria, chickenpox-like vesicles and chilblain-like lesions, the latter being the most commonly reported. While chilblain-like lesions are described in less-severe cases, livedoid and/or necrotic lesions are reported in more critical states of COVID-19. Exanthematous rashes and petechiae were also described in some case reports (117,118).

Interestingly, there is a condition mainly reported by children and young adults, called ‘covid-toe’, which appears to be related to the dysfunction of the underlying vasculature, probably due to thrombotic microangiopathy (81).

Potential mechanisms that may be behind these reactions and simultaneously related to SARS-CoV-2 infection include an immune hypersensitivity response, with extensive cytokine release, microthrombi deposition and vasculitis. However, before any skin lesion is attributed to the viral infection, it is important to exclude drug exposure reactions, since these manifestations frequently develop during disease progression. Treatment with hydroxychloroquine, remdesivir, tocilizumab and other experimental drugs should be firstly evaluated (99).

4. Conclusion

The uncontrolled and unforeseen spread of SARS-CoV-2 infection throughout the globe has resulted in an unprecedented public health crisis. Although the majority of the cases are asymptomatic or mild, there is still a great percentage of cases reporting severe manifestations, much of them leading to a terminal state.

Reaching the end of this research, the role that the immune response plays in the pathogenesis of this disease is unquestionable. The evasion mechanisms developed by SARS-CoV-2 to escape the host's antiviral defences gives place to an exacerbated and impaired immune response, with a huge inflammatory damage to the human body. Exuberant cytokine release, NK-cell and CD8+ T-cell impairment, bradykinin storm, fibrin clots formation and extensive macrophage activation are some of the main signatures of COVID-19.

It is possible to identify several stages of the infection. There are cases where it is limited to one week, with full recovery of the patient and very mild symptoms, only affecting the upper airways. On the other hand, if the infection reaches the lungs, it can progress to mild pneumonia, with full recovery by the end of the second week of infection. However, if this state is not controlled, after one month upon viral exposure, patients may experience the most severe manifestations of the infection, including cytokine storms, sepsis, states of disseminated intravascular coagulation, acute respiratory distress syndrome and multi organ failure. Eventually, patients at this stage can either recover or, in the worst scenarios, their situation may further worsen, with the development of secondary bacterial infections and eventually succumb to death.

Another aspect to take in account when predicting the outcomes of COVID-19 are comorbidities. There is evidence that comorbidities were present in nearly half of the inpatients with COVID-19. Hypertension is the most common comorbidity reported, followed by diabetes, cardiovascular diseases (CVDs) and chronic kidney disease (CKD). In addition, the elderly, immunosuppressed individuals or those who have recently undergone surgeries are part of the most susceptible population group to severe symptoms (2,85,119).

Higher age – approximately > 60 years - is, indeed, the most pointed out risk factor for developing a severe state of COVID-19. In fact, elderly people are among the most susceptible individuals to infection and complications, not only due to prolonged exposure to environmental and host risk factors, such as tobacco smoke and comorbidities, but also because

of the weakened immune system derived from the development of maladaptive physiological processes (2,85,119).

People with hypertension have right from the start an imbalance in the RAAS system, with a higher prevalence of the ACE-Ang II axis, which as seen before, might contribute to the development of severe symptoms in COVID-19 (119).

Regarding diabetes, patients are generally accompanied by a low-grade chronic inflammation and pro-coagulant state, due to higher expression of ACE2, conditions that might facilitate the cytokine release. In addition, several chronic comorbidities associated with diabetes can further worsen the prognosis of the disease, including obesity, hypertension, coronary artery disease and chronic kidney disease (119,120).

Obesity is another risk factor for a more-severe course of COVID-19. In association with diabetes, the effects of this condition may be related to an impairment in pulmonary function, characterized by reduced lung volume and compliance and increased airway resistance. In addition, obesity-associated inflammation results in an abundant activation of both innate and adaptative immunity cells in adipose tissue, which will consequently exacerbate the hyperinflammatory response in COVID-19 (99,120,121).

Although these are reported as risk factors for the development of COVID-19, there are also young individuals with unexplained inflammatory conditions without the presence of comorbidities.

Despite the extraordinary work that has been done and that is still ongoing with the production of new vaccines, there is still a long path to walk when it comes to controlling this disease. In this context, the development of anti-inflammatory therapeutics will have a great impact on the management of the disease, although with the worldwide vaccination process, the probability of post-infection immunopathology will become a rare situation. Therapies modulating the immune response may also be useful for treating and preventing immunopathology in patients who progress to severe states. In this context, some consider IFN administration as a potential option for prophylactic treatment or early management of COVID-19. There are also certain components that contribute to the immunopathogenesis of this infection that could be promising targets for therapeutic drugs. These include ACE2, TMPRSS2, the RTC, bradykinin and IL-6, to state some.

Currently, there are no antivirals to control the infection or therapeutics to prevent morbidities associated to COVID-19. Remdesivir has been repurposed for SARS-CoV-2 initial

stages of infection with some efficacy. This antiviral acts as a nucleoside analogue, thus inhibiting the RNA-dependent RNA polymerase of coronaviruses. In addition, several repurposed drugs are being evaluated regarding their effectiveness in the treatment and alleviation of immunopathologic symptoms. Systemic corticosteroids have been widely used to reduce inflammation, as well as antibiotics to prevent or treat pneumonia. Anticoagulants are also an option, especially for patients who already have coagulation impairments. At home, patients resort to antipyretics and anti-inflammatory oral drugs to alleviate mild symptoms.

Time is also a very important factor regarding the administration of therapeutics since a controlled immune response at the right time can be enough for a course of infection without major symptoms or exacerbations, in terms of inflammation.

To conclude, the importance of the collaboration between the various health professionals is undeniable, which has proven, now more than ever, to be fundamental in combating this pandemic. Due to health professionals' effort, allied to the technological advances and scientific research methods, it is now possible to rapidly monitor the disease progression, work towards new therapeutic options and develop effective and safe vaccines. There is still a lot to improve and discover about the management of this disease, and us pharmacists play an essential role in this demanding process.

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