

Immunopathological mechanisms involved in SARS-CoV-2 infection

Mecanismos imunopatológicos envolvidos na infecção por SARS-CoV-2

Sávio Breno P. Brito¹; Isaque O. Braga¹; Marília M. Moraes²; Carolina C. Cunha³; Sydney C. Leão¹; Iukary Takenami¹

1. Universidade Federal do Vale do São Francisco (UNIVASF), Paulo Afonso, Bahia, Brazil. 2. Escola Paulista de Medicina (UNIFESP), São Paulo, São Paulo, Brazil.
3. Instituto de Infectologia Emílio Ribas, São Paulo, São Paulo, Brazil.

ABSTRACT

Introduction: Coronavirus disease 2019 (Covid-19) has become a global health emergency. Although many scientific advances have been achieved over the last few months, the knowledge about how the etiologic agent SARS-CoV-2 interacts with the host's immune system, regulating the pathogenesis and the outcome of the disease, is not yet fully understood. **Objective:** This is a narrative literature review to discuss the available evidence on modes of transmission and the main immunopathological mechanisms involved in SARS-CoV-2 infection. **Material and method:** A search in the indexed journals was carried out in the PubMed/MEDLINE database, during the period from January to May 2020. The following descriptors were used in the research: *Covid-19, SARS-CoV-2, transmission, immunity, immunopathogenesis, and pathogenesis*. **Results and discussion:** Transmission of SARS-CoV-2 occurs through contact with airway secretions from a symptomatic patient. However, scientific evidence encourages transmission by asymptomatic and/or oligosymptomatic patients. Immunopathological studies suggest that the hypercytokinaemia promotes lung tissue damage and, subsequently, organ and system involvement, leading to decompensation, organ dysfunction and death. Regarding the humoral response, the high titers of B lymphocytes and antibodies, traditionally, correlated with the protection of the host, may be associated with the severity of the disease by SARS-CoV-2. **Conclusion:** The host's immune response is determinant in the pathogenesis of Covid-19. However, further *in vivo* and/or *in vitro* studies are needed to elucidate the pathogenic mechanisms of SARS-CoV-2 and, thus, assist in the development of a vaccine and/or therapeutic target.

Key words: betacoronavirus; transmission of infectious disease; coronavirus infections; clinical pathology.

RESUMO

Introdução: A doença do coronavírus 2019 (Covid-19) tornou-se uma emergência de saúde mundial. Embora muitos avanços científicos tenham sido alcançados nos últimos meses, o conhecimento sobre como o agente etiológico SARS-CoV-2 interage com o sistema imunológico do hospedeiro, regulando a patogênese e o resultado da doença, ainda não está totalmente esclarecido. **Objetivo:** Revisão da literatura com o objetivo de discutir as evidências disponíveis sobre as formas de transmissão e os principais mecanismos imunopatológicos envolvidos na infecção pelo SARS-CoV-2. **Material e método:** Realizou-se uma busca de periódicos indexados na base de dados PubMed/MEDLINE, durante o período de janeiro a maio de 2020. Os seguintes descritores foram utilizados na pesquisa: Covid-19, SARS-CoV-2, transmissão, imunidade, imunopatogênese e patogênese. **Resultados e discussão:** A transmissão do SARS-CoV-2 ocorre pelo contato com secreções das vias aéreas de um paciente sintomático. No entanto, evidências científicas fomentam a transmissão por pacientes assintomáticos e/ou oligossintomáticos. Os estudos imunopatológicos sugerem que a hipercitocinemia promove lesão do tecido pulmonar e, posteriormente, comprometimento de órgãos e sistemas, levando a descompensação, disfunção orgânica e óbito. Sobre a resposta humoral, os altos títulos de linfócitos B e anticorpos, tradicionalmente correlacionados com a proteção do hospedeiro, podem estar associados à gravidade da doença pelo SARS-CoV-2. **Conclusão:** A resposta imunológica do hospedeiro é determinante na patogênese da Covid-19. No entanto, mais estudos *in vivo* e/ou *in vitro* são necessários para elucidar os mecanismos de patogenicidade do SARS-CoV-2 e, assim, auxiliar no desenvolvimento de uma vacina e/ou alvo terapêutico.

Unitermos: betacoronavírus; transmissão de doença infecciosa; infecções por coronavírus; patologia clínica.

RESUMEN

Introducción: La enfermedad por coronavirus 2019 (Covid-2019) se ha convertido en una emergencia sanitaria mundial. A pesar de los muchos avances científicos recientes, el conocimiento acerca de cómo el agente etiológico SARS-CoV-2 interactúa con el sistema inmunitario del huésped, regulando la patogénesis y el resultado de la enfermedad, todavía no está totalmente claro. **Objetivo:** Revisión de la literatura a fin de discutir la evidencia disponible sobre las formas de transmisión y los principales mecanismos inmunopatológicos involucrados en la infección por SARS-CoV-2. **Material y métodos:** Se realizó una búsqueda por periódicos indexados en la base de datos PubMed/MEDLINE, desde enero hasta mayo de 2020. Se han utilizado las siguientes palabras clave en la investigación: Covid-19, SARS-CoV-2, transmission, immunity, immunopathogenesis y pathogenesis. **Resultados y discusión:** La transmisión del SARS-CoV-2 ocurre por el contacto con secreciones de las vías respiratorias de un paciente sintomático. Sin embargo, evidencias científicas indican la transmisión por pacientes asintomáticos y/o oligosintomáticos. Estudios inmunopatológicos sugieren que la tormenta de citocinas causa lesión en pulmones y, posteriormente, daño en órganos y sistemas, que conduce a la descompensación, disfunción orgánica y muerte. Sobre la inmunidad humoral, los títulos elevados de linfocitos B y anticuerpos, tradicionalmente relacionados con la protección del huésped, pueden estar asociados a la gravedad de la enfermedad por SARS-CoV-2. **Conclusión:** La respuesta inmunológica del huésped es determinante para la patogénesis de la Covid-19. No obstante, se necesitan más estudios in vivo y/o in vitro para aclarar los mecanismos de patogenicidad del SARS-CoV-2 y, así, ayudar en el desarrollo de una vacuna y/u objetivo terapéutico.

Palabras clave: betacoronavirus; transmisión de enfermedad infecciosa; infecciones por coronavirus; patología clínica.

INTRODUCTION

Coronavirus disease 2019 (Covid-19) is an acute respiratory disease caused by a new member of the coronavirus (CoV) family, called SARS-CoV-2⁽¹⁾. Since it was identified in Hubei province of China in December 2019, the virus has spread to at least 216 countries, territories or areas located on the five continents. Until May 29, 2020, from the 5,701,337 reported cases, 357,688 (6.27%) resulted in death^(2,3).

This lethality rate is relatively low when compared to other diseases, such as severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS), both caused by members of the coronavirus (CoV) genus, called SARS-CoV and MERS-CoV, respectively. However, the lethality rate of Covid-19 is around 10 times higher than that of seasonal influenza^(4,5). Although the data reflects emergency public health and health surveillance scenarios, they should be interpreted with caution, as not all individuals have been tested for the disease.

SARS-CoV-2 shows little-known evolution. However, it is known that patients over 65 years of age and/or in the presence of comorbidities that directly and/or indirectly affect the host's immune system have an even higher mortality rate⁽⁶⁻⁸⁾. A meta-analysis carried out by Emami *et al.* (2020)⁽⁶⁾ demonstrated that 16.37%, 12.11%, and 7.87% of hospitalized patients showed systemic arterial hypertension, cardiovascular disease, and diabetes mellitus, respectively, which are the main comorbidities present in patients admitted for Covid-19.

The incubation period for Covid-19 is, on average, three to seven days, and can last up to 14 days⁽⁹⁾. The most common symptoms are fever (ranging from 44% to 89% of cases), cough (68%), and fatigue (38%)⁽¹⁰⁾. There may be respiratory impairment and dyspnea, wheezing, acute respiratory distress syndrome (ARDS) and, in more severe cases, multiple organ failure and death^(11,12). Furthermore, it is believed that death may be associated with sepsis and/or acute myocardial injury^(13,14). However, the presence of one or more symptoms is dependent on the SARS-CoV-2 and host interaction, that is, the patient's immune response is decisive for the disease phenotype and may predispose the progression to more severe forms of Covid-19, which can vary from 15.7% to 17.6% of cases^(7,10,12,15).

In the current scenario, in the midst of a major health crisis caused by the Covid-19 pandemic, understanding the mechanisms of action of the immune system facing SARS-CoV-2 infection and its repercussions on the progression and pathology of the disease are important, because they provide subsidies to support possible vaccine and/or therapeutic targets to combat the disease. Therefore, the aim of this study was to gather and discuss the available evidence on the main transmission and immunopathological mechanisms involved in SARS-CoV-2 infection.

MATERIAL AND METHOD

This is a narrative literature review. Although this type of review guarantees less systematicity of studies, it is of central

importance in the constant learning process, since it offers the reader an opportunity to update and complement the most diverse knowledge quickly and effectively⁽¹⁶⁾.

The search strategy included the survey of articles indexed in the PubMed/MEDLINE database, on the modes of transmission and the immunopathological mechanisms involved in SARS-CoV-2 infection. The following descriptors were used: *Covid-19*, *SARS-CoV-2*, *transmission*, *immunity*, *immunopathogenesis*, and *pathogenesis* in national and/or international journals, published between January and May 2020. In addition, we sought to complement the survey of articles through manual citation searching from the primary studies identified in the PubMed/MEDLINE database.

Initially, an article screening by reading the title and abstract was performed. Articles unavailable in full online, studies involving animal models, as well as journals that did not address the theme of the review were excluded. Finally, the articles were selected based on critical reflective reading and relevance to the topic addressed. Based on this selection, a narrative review was carried out, whose discussion was divided into the following topics: i) transmission of SARS-CoV-2; ii) pathogenesis of Covid-19; e iii) innate, cellular and humoral immunity against SARS-CoV-2.

RESULTS AND DISCUSSION

Transmission of SARS-CoV-2

Although SARS-CoV-2 is not a highly virulent pathogen, it has spread rapidly across different continents with sustained transmission from person to person. According to the World Health Organization (WHO), the basic reproduction number (R_0) for Covid-19 can range from 1.4 to 2.5⁽¹⁷⁾. Another analysis carried out by Liu *et al.* (2020)⁽¹⁸⁾, using 12 studies published in February 2020, showed that R_0 can vary from 1.5 to 6.5. Although there are divergences between the values obtained, an exponential transmission behavior was observed, which raised in the scientific community the hypothesis of transmission among asymptomatic infected people^(14, 19-21).

For Yang, Gui and Xiong (2020)⁽²²⁾, the role of the symptomatic infected in the spread of the virus is well established. Symptomatic patients with Covid-19 eliminate, through speech, coughing and/or sneezing, respiratory droplets containing SARS-CoV-2, which can settle on surfaces such as steel and plastic, and may survive for up to 72 hours⁽²³⁾. Cascella *et al.* (2020)⁽²⁴⁾ demonstrated that direct contagion requires a proximity 1 to 1.5 m between the patient and the susceptible host. Therefore, closed and crowded environments facilitate the spread of the virus.

The hypothesis of transmission from asymptomatic individuals has gained a growing body of scientific evidence^(20, 25-27). Arons *et al.* (2020)⁽²⁸⁾ demonstrated that 56.3% (27/48) of the patients who obtained positive results in the molecular diagnostic test, known as real-time/qualitative reverse-transcription polymerase chain reaction with amplification (RT-qPCR), were totally asymptomatic. Although it is not possible to document the exact moment of transmission, it is plausible to consider that the high viral load detected in the blood of these asymptomatic patients could predict a possible risk of contagion. According to Wei *et al.* (2020)⁽²⁰⁾, 80% of asymptomatic infected patients in Singapore transmitted SARS-CoV-2 when they were in a pre-symptomatic stage, one to three days before the first signs and symptoms appeared. Such studies must be interpreted with caution, as they have certain limitations, such as the impossibility of ensuring that the infection has not occurred from an unknown source, and the memory bias. At the time, the patient himself reported the onset of signs and symptoms, believing he was asymptomatic, when, in fact, he could be oligosymptomatic.

Finally, the detection of asymptomatic patients for a better assessment of individuals infected by Covid-19 is difficult to reach, since the available diagnostic tests are primarily aimed at critically ill patients, health professionals, and public safety workers⁽²⁰⁾. Thus, the real impact of oligosymptomatic and asymptomatic patients on virus transmission is still unknown. In view of this uncertainty, the largest large-scale testing study, using rapid tests to detect anti-SARS-CoV-2 antibodies, is being carried out in Brazil, by the Universidade Federal de Pelotas (UFPeL), with the objective of verifying the percentage of asymptomatic individuals and the real lethality rate of the disease in the country^(29, 30).

In addition to these, other transmission possibilities are being investigated, such as fecal-oral or by blood transfusion transmission, and vertical transmission^(9, 31-34). Regarding the latter, a study by Alzamora *et al.* (2020)⁽³³⁾ highlights a case of vertical transmission that, although the exact moment of SARS-CoV-2 infection is not known, it is believed to have occurred at birth or after the delivery of the newborn. According to the review published by Yang e Liu (2020)⁽³⁴⁾, SARS-CoV-2 infection in three neonates (3/83) occurred at 16, 36 and 72 hours after birth. The infection was diagnosed using the RT-qPCR technique, using swabs from the nasopharyngeal region as samples. However, the lack of positive tests on samples of amniotic fluid, placenta or umbilical cord blood strengthens the hypothesis that vertical intrauterine transmission is impossible and shows the need for further studies to better understand this transmission^(33, 34). On the other hand, other possible modes of transmission have been ruled out. According to studies by Song *et al.* (2020)⁽³⁵⁾ and Paoli *et al.*

(2020)⁽³⁶⁾, no SARS-CoV- viral particles were found in the semen of one and of 13 patients, respectively, diagnosed with Covid-19.

Pathogenesis of Covid-19

After SARS-CoV-2 enters through the airways, the virus adheres to the mucosa of the upper respiratory epithelium, through the recognition and binding of the viral surface protein, called protein S, to the tissue receptor, called angiotensin-converting enzyme 2 (ACE2)^(9, 37, 38), a protein that mediates the entry of the virus into the target cell. The tropism for these cells affects the manifestation of symptoms, mostly respiratory. However, the presence of this receptor in other tissues, such as cardiac, renal, and intestinal tissues, also contributes to other clinical manifestations⁽⁹⁾.

After recognition, the viral envelope merges with the host cytoplasmic membrane, allowing it to enter into the cell's cytosol. It is possible that, like SARS-CoV, SARS-CoV-2 may also use the endocytic route of the target cell. Once in the endosome, it goes to the cytoplasm and releases the positive-sense single-stranded ribonucleic acid (RNA) viral, allowing the production of polyproteins and protein structures, which initiates the viral replication process. The viral particles are transported, aggregated the endoplasmic reticulum (ER), and sent to the Golgi complex through the ER-Golgi intermediate compartment. Finally, vesicles containing the viral particles fuse with the cytoplasmic membrane, promoting the release by budding^(12, 39-41). This replication process occurs with greater intensity in type I and II respiratory epithelial cells, which are located in the lower respiratory tract and have a large amount of ACE2 on the cell surface⁽¹²⁾.

These new viral particles can then invade the bloodstream, providing the peak of viremia and hematogenous spread. SARS-CoV-2 can, at that moment, infect several other tissues of the host, such as liver, kidney, heart, striated muscle, endocrine glands, and any other cell that has ACE2 expression on its surface^(9, 12). The pathological consequences caused by SARS-CoV-2 in these organs are still inconsistent; many studies are being carried out and seek to better understand the mechanism of action of the virus and its repercussions on host organs and tissues.

Some evidence could be confirmed by the histopathological findings described in the study by Xu *et al.* (2020)⁽⁴²⁾. The necropsy performed on a 45-year-old Chinese patient, whose cause of death was severe infection by Covid-19, showed significant changes in the lung and other organs, such as the liver and heart. In the right lung, desquamation of pneumocytes was observed and, in the left, pulmonary edema; both lungs with hyaline membrane formation, suggestive of ARDS. The bronchi and bronchioles presented necrotic buds, exudate and an excess of mucus. At the

cellular level, the presence of inflammatory cells was observed in the interstitium, notably lymphocytes, in addition to diffuse alveolar lesions. Multinucleated giant cells were present in large numbers throughout all the alveoli; however, no viral inclusion bodies were detected.

Another study described the pulmonary necroscopic findings in a series of 38 cases in northern Italy⁽⁴³⁾. Macroscopically, the lungs looked like organs, surprisingly, edematous and congested with irregular involvement. The histopathological study revealed changes corresponding to the exudative and intermediate phases of diffuse alveolar damage, which leads to the clinical features of ARDS, with uneven involvement of the fragments. Changes corresponding to the exudative phase of the disease were present in all patients, with capillary congestion, interstitial edema, dilated alveolar spaces, hyaline membranes composed of fibrin and serum proteins, and loss of pneumocytes. Hyperplasia and atypia of type II pneumocytes, proliferation of myofibroblasts, alveolar granulation tissue, and obliterating fibrosis were observed in approximately half of the patients. There was an inflammatory component with a low number of CD45+ T lymphocytes in the interstitium and a large number of CD68 + macrophages in the alveolar lumen. A peculiar finding found in 33 of the 38 cases was the presence of platelet-rich thrombi in the small arterial vessels (diameter < 1 mm). There was also an increase in the number of CD61 + megakaryocytes in the pulmonary capillaries. In addition, the electron microscope study showed the presence of viral particles with the typical SARS-CoV-2 morphology in the pneumocyte cytoplasm. The study highlights pulmonary thromboembolism as one of the potential causes of death for infected patients. According to Carsana *et al.* (2020)⁽⁴³⁾, there is local pulmonary thrombosis in pulmonary arterioles with diameters below 0.1 cm, which leads patients to pulmonary arterial hypertension. Taken together, these findings suggest that SARS-CoV-2 induces, by some immunological mechanism, systemic endothelial dysfunction, leading to thrombus formation. This result justifies the use of oral, subcutaneous, and intravenous anticoagulants such as low molecular weight heparin or unfractionated heparin, from the early stages of Covid-19, to prevent pulmonary thromboembolism⁽⁴⁴⁾.

Another study published by Yao *et al.* (2020)⁽⁴⁵⁾ describes the anatomopathological findings of the heart after minimally invasive necropsy performed in three patients diagnosed with Covid-19. Among the reported findings, in some cardiomyocytes, hypertrophy, degeneration, and necrosis were identified, in addition to hyperemia, mild interstitial edema, and inflammatory infiltrate. It was demonstrated by immunohistochemical staining

that the inflammatory cells in the myocardium were composed mainly of macrophages and CD4+ T lymphocytes; components of SARS-CoV-2 were not detected in the tissue. On the other hand, a German study, published by Wichmann *et al.* (2020)⁽⁴⁶⁾, reported that only one out of 12 patients had a condition compatible with viral myocarditis.

Nevertheless, some studies have also evaluated the histopathology of the pharynx, kidneys, and liver. In the pharynx, the study by Wichmann *et al.* (2020)⁽⁴⁶⁾ demonstrated hyperemia, with a dense lymphocytic infiltrate, which is similar to that of chronic pharyngitis, which could justify the upper respiratory symptoms, such as cough and sore throat found in early cases of Covid-19. In the kidneys, a Chinese study showed diffuse proximal tubular lesion with brush border loss, non-isometric vacuolar degeneration, and even frank tubular necrosis. Occasional hemosiderin granules have been identified. There were prominent erythrocyte aggregates obstructing the lumen of capillaries without platelet or fibrinoid material. Fibrinous thrombi were also found⁽⁴⁷⁾. According to Su *et al.* (2020)⁽⁴⁸⁾, liver necropsy showed microgoticular steatosis, which could be associated with dyslipidemia. Although studies in the area are still few, they contribute to a better understanding of the signs and symptoms caused by SARS-CoV-2.

Innate, cellular and humoral immunity against SARS-CoV-2

Innate immunity appears to have a central role in the defense against SARS-CoV-2^(39, 49). Through pattern recognition receptors (PRR), such as *Toll-like* receptors (TLR), *RIG-I-like* receptors (RLR), *NOD-like* receptors (NLR), among others, the recognition of the viral molecular pattern is detected. Depending on the stimulated receptor, different biological responses of the host are developed. The recognition of viral antigens by TLR, except for TLR-3, is dependent on the Toll-MyD88 pathway, leading to a signal transduction that involves the activation of the transcription factor NF- κ B. On the other hand, the activation of the TLR-3 pathway by the TRIF adapter molecule induces the production of type I interferon (IFN Type I), which limits viral replication and increases phagocytosis by macrophages and cytotoxic activity of NK cells⁽⁴⁹⁾.

However, regardless of the activation pathway, recognition culminates in the production of pro-inflammatory cytokines and chemical mediators, to provide an effective antiviral response. Different cells, monocytes-macrophages, lymphocytes and neutrophils, migrate to the pulmonary epithelium, in an attempt to restrain SARS-CoV-2. When this attempt to limit infection is

exacerbated, there are non-specific oxidative and inflammatory effects that result in secondary damage to functional and uninfected tissues^(12, 50).

In acquired immunity, it is observed that, after the virus enters the target cell, viral peptides are presented by the major histocompatibility complex [(MHC) or human leukocyte antigen (HLA)] class I to CD8+ T lymphocytes, which exercise their cytotoxic function, leading to cell death due to apoptosis of the infected cell. The presentation of viral antigens can also be mediated by antigen-presenting cells through MHC class molecules, promoting the activation of CD4+ T cells (cross presentation)^(51, 52). This will result in the production and release of interleukin (IL)-12, a cytokine that will co-stimulate the production of lymphocytes with a Th1 profile.

IL-12, together with IFN- α , increases the expression of MHC class I and the activation of NK cells, which allows the action of antiviral mechanisms and the eradication of cells infected by SARS-CoV-2. Alongside these events, there is a large production of cytokines that recruit neutrophils and monocytes to the infection site and activate several other pro-inflammatory cytokines and chemokines such as IL-1, IL-6, IL-8, IL-21, tumor necrosis factor (TNF)- β , and monocyte chemoattractant protein 1 (MCP-1)⁽⁵¹⁾. Due to the importance of HLA and ACE2 molecules, some studies suggest that polymorphisms in HLA genes, host immune response genes and/or changes in ACE2 receptor expression, may be correlated with susceptibility and resistance to SARS-CoV-2⁽⁵³⁻⁵⁶⁾. Although studies in the area are scarce, conducting scientific research may clarify whether the levels or functionality of these molecules contribute to the severity of the disease observed.

Even if the individual develops an immune response against SARS-CoV-2, we observe that, in some cases, patients quickly evolve to more critical stages, such as ARDS. Evidence published by Chen *et al.* (2020)⁽⁵⁰⁾ suggest that a subgroup of critically ill patients who progress to ARDS have a syndrome characterized by a “cytokine storm”. This condition, also known as hypercytokinaemia, triggers a hyperinflammatory response in the host, responsible for the organ dysfunction observed in these patients. It is known that immune hyperactivation occurs when effector cells, mainly NK and CD8+ T lymphocytes, are not able to eliminate infected cells and, consequently, antigens, resulting in a persistence of these that lead to the excessive production of pro-inflammatory cytokines, such as IFN- α , IFN- γ , IL-1 β , IL-6, IL-12, IL-18, IL-33, TNF- α , transforming growth factor (TGF)- β , granulocyte-macrophages colony-stimulating factor (GM-CSF), and chemokines such as CCL2, CCL3, CCL5, CXCL8, CXCL9, CXCL10, IP-10^(12, 50, 51, 57, 58). Although the exact mechanism that triggers hypercytokinemia is unknown, the exacerbated increase in pro-inflammatory cytokines

results in pulmonary and vascular injury, which promotes the production of exudate, edema and fibrosis. Thus, an exacerbated pulmonary inflammation occurs that leads to ARDS and severe immune dysregulation, which may have as one of its mechanisms the extensive stimulation of T lymphocyte apoptosis^(12, 57, 59). Interestingly, this condition reported in patients with Covid-19 is also described in infections by SARS-CoV and MERS-CoV⁽³⁹⁾.

Another characteristic evidenced in critically ill patients was the presence of high levels of cytokines IL-6, IL-10, and TNF- α during the third phase of the disease (8th to 10th day) and, respective decrease during the recovery process^(13, 50, 59). The role of these cytokines in the inflammatory process corroborates the findings by Chen *et al.* (2020)⁽¹¹⁾, who point out that patients requiring ICU admission have significantly higher levels of IL-6, IL-10, and TNF- α and reduced CD4+, CD8+ T lymphocytes and regulatory T lymphocytes (Treg). Thus, the increase in serum levels of these cytokines associated with lymphopenia may indicate more severe modes of Covid-19^(11, 59).

Although low lymphocyte counts are common in viral infections, a study by Huang *et al.* (2020)⁽⁶⁰⁾ demonstrated that 85% of patients admitted to the ICU by Covid-19 had lymphopenia with a lymphocyte count $< 1 \times 10^3/\text{mm}^3$. These results are also evidenced in histological sections⁽⁶¹⁾, demonstrating that lymphopenia is not a condition observed only in peripheral blood, but also at the anatomical site of infection.

It is reasonable to assume that peripheral lymphopenia results from the sequestration of lymphocytes to the pulmonary focus, which, when activated, excessively produce pro-inflammatory cytokines that cause the “cytokine storm”, but which eventually die during the infectious process. Thus, after the “cytokine storm”, the host is “adrift” in relation to SARS-CoV-2 and other microorganisms, such as bacterial infections. However, it is important to consider that, in addition to the low CD4+ T lymphocyte count, impairment of the lung parenchyma promoted by the exacerbated inflammatory process also contributes to the susceptibility to the underlying infections. Taken together, the data suggest that the main cause of death is ARDS, resulting from the “cytokine storm” caused by an exacerbated immune reaction of the host against the viral agent. Therefore, patients with severe Covid-19 should be screened for hyperinflammation using laboratory markers, such as increasing levels of C-reactive protein (CRP), D-dimer, ferritin, IL-6, and CD4+ T lymphocyte count^(10, 11). Even though many of these markers are not recommended by the Brazilian Unified Health System [Sistema Único de Saúde (SUS)], they represent important tools to monitor the inflammatory state and the severity of the patient.

Regarding the humoral response, it is observed that serum levels of B lymphocytes are increased in critically ill patients^(11, 59). In a series of cases with patients diagnosed with congenital agammaglobulinemia, a disease characterized by low levels of B lymphocytes and hypogammaglobulinemia, it was found that, despite having been infected by SARS-CoV-2, they had interstitial pneumonia, but with good evolution, with no need for ICU care and/or mechanical ventilation⁽⁶²⁾. Another study by Matricardi *et al.* (2020)⁽⁶³⁾ reported clinically recovered patients with low levels of circulating antibodies in the blood. Although incipient, the results suggest a possible causal relationship between humoral response and disease severity. The high titers of B lymphocytes and antibodies, traditionally correlated with host protection, could be associated with the severity of the disease by Covid-19 and, therefore, represent a worse clinical prognosis for the patient⁽¹¹⁾. This would partly explain why children are less susceptible to SARS-CoV-2, since they have physiological hypogammaglobulinemia. However, the reason why Covid-19 is less severe in children is still unknown. Three theories try to elucidate the behavior of SARS-CoV-2 infection in children, adults and the elderly. The first one is based on the difference in ACE2 expression in the cells of children and adults/elderly. It is believed that the prevalence of ACE2 may be lower in the pediatric population. In addition, sex could also affect the ACE2 expression. Circulating ACE2 levels are higher in men than in women. These results corroborate the epidemiological data that show that the severity and lethality are higher in males than in females^(55, 56). The second theory correlates with the qualitative difference between the immune response of children, adults and the elderly against SARS-CoV-2. Continuous exposure to antigens during aging, together with thymic involution, results in a shift in the distribution from immature T lymphocytes to memory T lymphocytes and effector T lymphocytes; this process is accompanied by the loss of co-stimulatory molecules, such as CD27+ and CD28+, resulting in greater susceptibility to infections⁽⁶⁶⁾. In addition, the aging process is associated with increased production of pro-inflammatory cytokines, which are correlated with ARDS. Finally, the latter theory is based on the simultaneous presence of other viruses in the lung tissue and/or in the airway mucosa, a situation that is quite recurrent in young children. This condition could result in a competition process between SARS-CoV-2 and the other viruses that previously colonized the region, which would be limiting the development and expansion of SARS-CoV-2^(55, 56).

Similar to common acute viral infections, SARS-CoV-2 has a typical pattern of antibody production, so that the initial humoral response is mediated by class M immunoglobulins (IgM) followed by the production of class G immunoglobulins

(IgG)⁽⁶⁴⁾. According to Long *et al.* (2020)⁽⁶⁵⁾, almost all patients have seroconverted by days after the onset of symptoms. The proportion of individuals positive for IgG and IgM was 100% and 94.1%, between the 17th-19th and 20th-22nd days, respectively, after the onset of symptoms. Another study by Haveri *et al.* (2020)⁽⁶⁶⁾, showed that on the 9th day after the onset of symptoms, it was already possible to detect the presence of antibodies of the IgM and IgG class. From this period, the IgM and IgG titers rise exponentially, reaching titers equal to or greater than 1:320 or 1:1,280, respectively, at the 20th day. According to the study by Long *et al.* (2020)⁽⁶⁵⁾, in which 262 patients with Covid-19 were analyzed in three different hospitals, the positive IgG specific for the virus occurred in 100%, between the 17th and the 19th day after the onset of symptoms, while IgM showed positivity in 94.1% of the patients, between the 20th and the 22nd day after the onset of symptoms. Although the studies present small divergences in relation to the moment of detection of antibodies in peripheral blood, it is observed that the production of antibodies is slow in relation to the moment of infection.

Another possibility reported by Long *et al.* (2020)⁽⁶⁵⁾ was the seroconversion of the IgM antibodies subsequently to that of the IgG class in 38.5% (10/26) of patients diagnosed with Covid-19. This directly implies the results of the rapid tests that separately detect the presence of IgM and IgG. Symptomatic individuals with a positive IgG test and negative IgM may falsely interpret the result as cured, when, in fact, they represent potential spreaders of the virus, since they are infected in the acute phase. Evidence suggests that IgM antibodies against SARS-CoV-2 last for around 12 weeks, while IgG class antibodies remain, as far as is known, for long periods in the bloodstream, but in low amounts⁽⁶⁵⁾.

Thus far, many studies have been carried out to assess the presence of antibodies and their possible use as a diagnostic tool. However, the data is still limited and contradictory. Most likely, the variations observed in the studies are due to the different antibody detection techniques [immunoenzymatic test (ELISA), rapid test and chemiluminescence], different types of antigens (protein S, S1, S2, N, and E), and samples (whole blood, serum and plasma) used, in addition to the sample collection period. Given these circumstances, the possible causal relationship between antibodies and disease severity needs to be confirmed in a larger cohort of patients. Furthermore, there are no scientific records that prove the quality of the antibodies produced. However, it is reasonable to consider that neutralizing antibodies can block the entry and/or fusion of the viral particles through the recognition of specific

viral epitopes. Given this perspective, the viral fusion mediated by the SARS-CoV-2 protein S could be blocked by neutralizing anti-S antibodies, even in low amounts, preventing the virus from entering the host cell, as well as interacting with other immunological components, such as complement system proteins, phagocytes and NK cells, assisting the SARS-CoV-2 clearance⁽⁶⁴⁻⁶⁸⁾.

Likewise, research on the ability of Covid-19 to generate long-lasting immunity in the host has resonated through the scientific community. Interestingly, some patients, from South Korea and China, tested positive for the virus after clinical cure^(69, 70). If, eventually, immunity against SARS-CoV-2 is long-lasting and results in the production of neutralizing antibodies, would these cases be reinfection? Most likely not. Regarding the studies by Kang (2020)⁽⁶⁹⁾ and Lan *et al.* (2020)⁽⁷⁰⁾, patients considered clinically cured returned to test positive by the RT-qPCR test. However, as with tuberculosis⁽⁷¹⁾, it is not advisable to perform the molecular test during or after treatment of the disease, as the presence of small fragments from *Mycobacterium tuberculosis* could result in a false positive test. Thus, it is possible that, after clinical cure, the maintenance of positivity could be due to the remaining viral particles or remnants of the viral genetic material. In view of these findings, patients who maintain a positive RT-qPCR for a few weeks are not sick and, therefore, do not represent a potential risk of transmission to the community.

FINAL CONSIDERATIONS

The host's immune response is decisive in the virulence and pathogenesis of Covid-19. The available studies suggest that hypercytokinaemia that occurs in critically ill patients promotes lung tissue damage and, subsequently, the involvement of organs and systems, leading to decompensation, organ dysfunction and death. However, there is no evidence on which immunological mechanisms are associated with asymptomatic or oligosymptomatic individuals, in part because of the difficulty in identifying them in the general population. In the absence of an immune system that works properly, the imminent risk of death from hypercytokinaemia secondary to Covid-19 is real. In addition, the results suggest a possible causal relationship between humoral response and disease severity; patients with high antibody titers in the acute phase have a worse clinical prognosis. However, further *in vivo* and/or *in vitro* studies are needed to elucidate the pathogenic mechanisms of SARS-CoV-2 and, thus, assist in the development of a vaccine and/or therapeutic target.

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CORRESPONDING AUTHOR

Iukary Takenami  0000-0001-5660-7766
iukary.takenami@univasf.edu.br



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