

EVALUATION OF CYTOKINE IL-6 PROFILE IN PATIENTS HOSPITALIZED WITH COVID-19 IN THE 15TH REGIONAL HEALTH OF PARANA

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Abstract

COVID-19 is a viral infection caused by SARS-CoV-2 coronavirus, discovered in Wuhan, China, in 2019. It is a potentially serious disease with high transmissibility, characterized by worsening due to the exacerbated immune response, coordinated from pro-inflammatory cytokines signaling, leading to tissue damage. Objective: to evaluate the immunological profile of patients affected by COVID-19 through protein measurement that modulates immune response and has a great influence on cytokine storms. Materials and methods: the serum concentration analysis of the cytokine IL-6 was performed in patients with the disease. This procedure was developed through the duplicate capture ELISA technique, following manufacturer's standards, with the serum used from University Hospital of Maringa (HUM) patients who were hospitalized in the ward (G2), and who were in the intensive care unit but died (G3), as well as negative samples for COVID-19 (G1-control) from blood donors stored in the Maringa Regional Blood Center. Data were analyzed using GraphPad Prism software, version 7.0. Results: an increase in IL-6 was observed in more severe patients (G3). Conclusion: the cytokine IL-6 has been shown to be important prognostic markers of worsening of COVID-19.

Keywords: COVID-19, Cytokine Storm, Immune Response

1. Introduction

Covid-19 is a potentially severe respiratory infection that has high transmissibility. This disease is caused by SARS-CoV-2, classified as a betacoronavirus, being first analyzed in December 2019 in bronchoalveolar lavage samples from pneumonia patients in the city of Wuhan, China. It is a virus belonging to Coronaviridae family, being the seventh coronavirus known to infect humans. In Brazil, more than 36 million cases have been confirmed and more



than 698 thousand deaths have been verified, therefore, the disease has a mortality rate of about 2% in this country [1].

The infection evolution in patients consists of an initial phase, related to viral replication in the airways accompanied by mild symptoms, and also consists of a pulmonary phase, which involves the activation of the immune response. As a result of the exacerbated immune response, the process of hyperinflammation occurs, a phase in which there is a predominance of respiratory symptoms [2].

Studies have shown that the exacerbated innate immune response is capable of causing lethal pneumonia in patients infected with SARS-CoV-2, and this data alerts to the need for a more detailed analysis of the innate immune process [3].

The severity of this disease is directly related to cytokine storm, characterized by increased pro-inflammatory interleukins IL-2, IL-7, IL-6, IL-17, Interferon-gamma (INF γ) and Tumor Necrosis Factor alpha (TNF- α). This process is responsible for a hyperinflammatory response that generates tissue damage [4].

This cytokine storm in COVID-19 can cause, due to the inflammatory process, a picture of lung injury, which can evolve to coagulopathy. In addition to the lung, kidney, liver and heart injuries are also observed [3]

Thus, the disease's high transmissibility, along with pro-inflammatory cytokines high production and, consequently, an exacerbated inflammatory process related to respiratory complications, constitute the basis of this disease [5]. At first, the disease inflammatory complications were justified due to an infectious process secondary to viral infection by SARS-CoV-2. Later studies have shown that respiratory complications are related to the inflammatory process due to the serum increase in pro-inflammatory cytokines in SARS-CoV-2 infection [2]. Therefore, the cytokine profile analysis in different clinical stages of patients with COVID-19 is important to elucidate the patients immunological profile who develop a more severe disease form, and thus assist in choosing the most appropriate treatment approach for these cases, and even in supportive treatments development.



2. Material and methods

This study was approved by the Permanent Committee on Ethics in Research (Copep/UEM) CAAE: 38443420.6.0000.0104 and all patients and/or guardians signed the informed consent form (ICF) authorizing the collection and use of serum for research.

COVID-19 positive samples in the RT-PCR test were collected from November 2020 to February 2021, from University Hospital of Maringa (HUM) patients, who were hospitalized in the ward (group 2) and who were in the intensive care unit (ICU) but died (group 3). The viral etiological agent research was carried out by Central Laboratory of the State of Parana (LACEN-Paraná) as a HUM protocol for the COVID-19 cases investigation, or by LEPAC according to authorization from the government of the State of Parana.

Remaining serums collected for routine examinations during hospitalization were used, stored in a freezer at -20°C for subsequent cytokine measurement. The samples used were from the period in which patients presented a worsening of their clinical conditions.

COVID-19 negative samples (group 1) were acquired from blood donors, proven healthy, in June 2021 from the HUM/UEM Blood Center.

The cytokine IL-6 profile was evaluated (ELISA Kit, Elabscience). At the dosing time, the serum was removed from freezer at -20°C and left at room temperature for thawing and later use in the test. The technique used for cytokines measurement was capture ELISA, being developed in duplicate according to the manufacturer's guidance. Considering the capacity of the Elisa plate (96 wells), 20 different serum samples from each group were used. The plate was read in an ELISA plate reader (Asys-Expert plus).

All data were analyzed using GraphPad Prism software version 7.0 (GraphPad Software Inc., La Jolla, CA, USA) and the results comparison for the cytokines were expressed as arithmetic means and standard error of the mean, using "one-way ANOVA".

3. Results and discussion

Regarding the serum concentration (pg/mL) of IL-6 there was an exacerbated increase in the G3 group (~85 pg/mL) in relation to the other groups, and G2 (~50 pg/mL) presented a



concentration significantly higher than G1 (~20 pg/mL). Thus, Figure 1 shows a proportional increase in IL-6 concentration with patient severity.

IL-6 is in high contractions due to the immune response attempt to eliminate the virus, because the cytokine activates the proliferation and differentiation of B cells and neutrophils, but also stimulates the proliferation of Th2 lymphocytes, the main producers of IL-6, consequently, causing positive feedback and further increasing the production of IL-6.

This mechanism favors the inflammatory process, which in COVID-19 is exacerbated and generates tissue damage in the respiratory system and other tissues [4], because IL-6 activates neutrophils that release reactive oxygen species (ROS) and leukotrienes, and that in addition to causing lesions in the epithelial cells of the pulmonary alveoli (pneumocytes), induce the production of mucus, bronchoconstriction and mucosal edema [6].

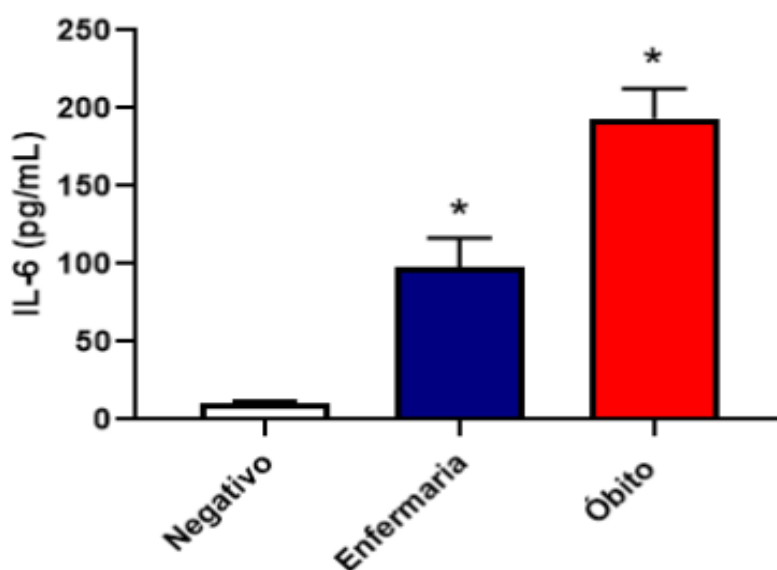


Figure 1. Result of interleukins 6 (IL-6) concentration (pg/mL) measurement in the blood of healthy people (G1-control), COVID-19 patients admitted to the ward (G2) and patients with COVID-19 who died (G3). *# Statistically significant comparing between groups ($p < 0.05$).



4. Conclusion

Cytokine storm was observed with an increase in IL-6 proportional to clinical severity, thus being considered an important predictor of this clinical evolution. In addition, IL-6 may be a possible therapeutic target in the treatment of the COVID-19 hyperinflammatory process.

Founding: CAPES

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