SARS-CoV-2 induces mitochondrial dysfunction and cell death by oxidative stress in leukocytes of COVID-19 patients

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Abstract

Background: From sepsis to COVID-19-induced multi-organ failure, inflammation and immune system activation play an important role. It has been argued that inflammation and over-activation of the immune system could be mediating a pro-oxidant microenvironment that can induce cytotoxic effects that potentiate tissue damage favoring organic deterioration. Aims: To investigate whether induction of oxidative stress by COVID-19 infection could inhibit mitochondrial function and cause cellular damage in leukocytes. Methods: We evaluated plasma levels of nitric oxide, hydrogen peroxide and protein carbonylation using spectrophotometry, in addition to evaluating mitochondrial function and cell death by fluorescence microscopy and leukocyte morphology, in COVID-19 patients at two time points: viremia and severe sepsis with multi-organ failure. Results: COVID-19 induces increased oxidative stress markers that activate cellular damage processes. In the viremia stage, was observe with an increase in peroxide (28.9%), nitric oxide (370.3%) and carbonylated proteins (61.8%), which was correlated with an increase in inhibition of mitochondrial function (66%), early apoptosis (212%) necrosis (405%), and leukocytes-reactivity. The severe sepsis stage with multi-organ failure also showed a further increase in levels of peroxide (46.4%) with a slight decrease in nitric oxide (216.2%) but with more carbonylated proteins (102%), regarding what was observe in viremia. This oxidative process was correlate with less inhibition of mitochondrial function (32.4%) and an increase in late apoptosis (463%), and morphology changes evidencing damage in the leukocytes. Conclusion: SARS-CoV-2 induced damage promotes levels of oxidative stress markers and mitochondrial dysfunction that potentiate morphological changes and cell death in leukocytes. These cellular effects could be integrating into the physiopathology of COVID- 19. These processes explain the rapid changes in the immune system, and that present an initial over-activation and early massive death due to SARS-CoV-2 infection, promoting endothelial-alveolar damage that would cause multi-organ failure.

WHAT'S KNOWN? (what is already known about this subject?)

Current research indicates that SARS-CoV-2 infection induces various processes of cellular damage mediated by inflammation derived from the activity of the immune system, and that these events potentiate cellular damage in various tissues such as the endothelium, lungs, heart, etc. In severe cases, it has been indicated that patients have leukopenia, however, the effect of the infection on the cells of the immune system and in the early stages of the disease has not been evaluated.

WHAT'S NEW? (what does this study contribute to the literature?)

Our results show the effects of SARS-CoV-2 infection on mitochondrial function and cell damage in early stages, specifically in leukocytes as a fundamental population that must mediate the damage induced by the infection, and that responds to the inductive effect. of oxidative stress. So it proposes a fundamental condition in the damage induced by the infection.

1 INTRODUCTION

COVID-19 is a viral infection induced by the coronavirus of severe respiratory syndrome (SARS-CoV-2), that represents a serious health problem worldwide; due to its association with various pathophysiological processes such as: inflammation, increased proinflammatory cytokines, and cell death, which could be closely related to redox imbalance or oxidative stress.[1] The high risk of severity and mortality from SARS-CoV-2 infection or COVID-19 disease has been associated with several clinical indicators such as: lymphocytopenia, increased D-dimer, increased serum ferritin, and high titers IL-6.[1,2]

The inflammatory response induced by SARS-CoV-2 can generate an effect of cellular oxidative stress, which induces an increase in circulatory inflammatory mediators, including cytokines, produced through redox pathway activation in patients with sepsis.[3,4]Because, oxidative stress and inhibition of mitochondrial activity may be an alternative to explain the tissue injury associated with COVID-19-induced damage to endothelial, alveolar, and cardiac cells, among other.[5,6] We consider it vitally important that health professionals can analyze and understand a little more about these pathophysiological mechanisms, to evaluate and design new protocols that allow a better prognosis for patients.

According to tissue damage, accumulation of inflammatory cells associated with endothelial tissue has been reported, as well as apoptotic bodies in alveolar cells and others.[7] Thus, it is postulated the accumulation of mononuclear cells in the lung and in the small pulmonary vessels induce congestion and therefore endothelial tissue damage associated with the lung.[8]The endothelial dysfunction is a main determinant of microvascular dysfunction, which is associated with the processes of inflammation and oxidative stress, that potentiate tissue edema associated with a pro-coagulant state.[6,9]

The SARS-CoV-2 induce an explosion of inflammatory cytokines, reactive oxygen species (ROS), and cell death-induced by this cell events is a cause which can result in significant endothelial and multi-organ damage, so the regulation of oxidative stress is essential.[3,4,7] Thus, modulation of oxidative stress may be able to prevent the cellular and tissue injury and development severe disease symptoms in coronavirus patients by reduce the immuno-pathology of coronavirus infection on patients' health after the active phase of the infection is over, so that, if we could reduce or modulate cellular toxic effects, it would make COVID-19 disease more controllable.[9,11]

Therefore, this study aimed to evaluate the association between different biomarkers of oxidative stress with parameters of mitochondrial and cellular function to evaluate the relationship between oxidative stress and leukocyte function in patients with COVID-19. This process contributes to the understanding of the possible link between oxidative stress and the risk of pathogenesis, severity and mortality in patients affected by SARS-CoV-2 infection.

2 MATERIALS AND METHODS

2.1 Study and subjects . Patients with laboratory confirmed COVID-19 infection, diagnosed using quantitative reverse transcriptase–polymerase chain reaction (qRT-PCR), and clinical symptomatology of COVID-19 (history of fever, any respiratory symptom and chest radiographs confirmed pulmonary lesions (for moderate and severe cases), were included. The baseline characteristics of the patients for their classification are shown in Table 1.

The patients were classified into two groups according to disease severity considering the following parameters. I) Viremia: Patients with fever (>38 °C), d-dimer (0.6-0.9 mg/ml), lactate (¡2mmol/L), leukopenia, anosmia, myalgia, arthralgia, fatigue, cough, sore throat, runny nose, and sneezing. II) Severe sepsis with multi-organ dysfunction syndrome (MODS): Patients with d-dimer (¿10mg/ml), creatinine (>1.5 mg/dl), procalcitonin (+), lactate (¿2mmol/L), hypotension, electrolyte imbalance and who had any of the following were considered in critical condition: acute respiratory distress syndrome (ARDS), respiratory failure requiring mechanical ventilation, may have shock, encephalopathy, myocardial injury, heart failure, coagulation dysfunction and acute kidney injury or failure of other organs requiring admission to the intensive care unit.

In addition, patients with respiratory problems similar to COVID-19 disease but with a negative confirmatory test, were included as damage control.

2.2 Biochemistry analysis

2.2.1 Peroxide assay. The peroxide content was determined in plasma by means of a quantitative colorimetric assay, according to the kit indications "QuantiChrom^{TM} Peroxide Assay Kit (DIOX-250) - BioAssay Systems", at the end of the process the optical density at 570 nm was quantified in ELISA plate reading equipment (Chromate), reporting values of ng/ml.

2.2.2 Nitric oxide assay. The nitric oxide content was determined in plasma by means of a quantitative colorimetric assay, according to the kit indications "QuantiChrom^{TM} Nitric Oxide Assay Kit (D2NO-100) - BioAssay Systems", at the end of the process the optical density was quantified at 570 nm, in an ELISA plate reading equipment (Chromate), reporting in µM values.

2.2.3 Oxidized proteins assay. Protein carbonyl content in plasma was determined as previously described.[12] The plasma was incubated with DNPH (2,4-Dinitrophenylhydrazine; 10 mM) and HCl (2N) and finally with guanidine hydrochloride (6M). Assessment of carbonyl formation was done on the basis of formation of protein hydrazone by reaction with DNPH. The absorbance was measured at 370 nm. Result was expressed as nmol carbonyl per mg protein.

2.3 Mitochondrial function, cell death and morphology by microscopy

2.3.1 Fluorescence assessment for Mitochondrial activity .

Staining against Tetramethylrhodamine methyl-ester (TMRM - was used to assess mitochondrial membrane potential, $\Delta\Psi$m) and Hoechst were assessed as a reliable marker of mitochondrial function and chromatin condensation respectively. Buffy coat was first rinsed in PBS (pH=7.4) for 5 min and then incubated with TMRM (1:200) in PBS for 30 min at 37°C. Thereafter, the mixture was washed and incubated with Hoechst (1:500) in PBS for 5 min at room temperature, and rinsed in PBS (pH 7.4) for 5 min per step. The mixture was then mounted with Vectashield and the image acquisition and processing were made with a microscope (Iroscope) fitted with a digital camera and imaging system using top view acquisition software. Fluorescent photomicrographs were obtained and the number of reactive cells to TMRM and Hoechst per field was determined in ten fields per sample, the results are shown by measurement of the densitometry (relative units) or the number of cells.

2.3.2 Fluorescence assessment for Apoptosis . Staining against annexin-V and propidium iodide were assessed as a reliable marker of apoptosis and necrosis respectively. Buffy coat was first rinsed in annexin-buffer for 5 min and then incubated with annexin-V/FITC (1:200) in annexin-buffer for 20 min at RT. Thereafter, the mixture was washed and incubated with propidium iodide (1:400) for 5 min at RT, and rinsed in PBS (pH 7.4) for 5 min per step. The mixture was then mounted with Vectashield and the image acquisition and processing were made with a microscope (Iroscope) fitted with a digital camera and imaging system using top view acquisition software. Fluorescent photomicrographs were obtained and the number of reactive cells to annexin-V, PI or annexin-V/PI per field was determined in ten fields per sample, the results are shown by counting the number of positive cells.

2.3.3 White blood cells morphology

A peripheral blood film (made from a drop of blood from an EDTA anticoagulated tube or skin puncture) is stained with Wright stain and microscopically examined using scanning (10x), and oil (100x) objectives. The WBC morphology was carefully evaluated using a 100x microscope objective.

2.4 Statistic analyses. Results were expressed as mean values ± SEM. All data were statistically analyzed using one-way analysis of variance (ANOVA) for repeated measures, followed by post hoc Bonferroni's test. All analytical procedures were performed using the scientific statistic software GraphPad Prism 5 (GraphPad Scientific, San Diego, CA, USA). Differences of P¡0.05 were considered as statistically significant.

3 RESULTS

3.1 SARS-CoV-2 induces changes in oxidative stress markers in blood samples of COVID-19 patients.

Different markers of oxidative damage, including hydrogen peroxide and nitric oxide formation and also some major toxic side effect were analyzed such as protein carbonyl levels, were determined in the plasma of COVID-19-patients. The amount of $H_2O_2$was assessed as an index of oxidative stress total, are shown in Fig. 1A. In control patients, baseline H_2O_2levels was 712.7 ± 186.2 ng/mL, while in plasma from COVID-19-patients there were less increases in H_2O_2formation of 28.9% in the viremia group and significant increases of 46.4% in the MODS group ($p<0.05$), above control (919.3+-151.6 and 1044+-125.4; respectively). On the other hand, the peroxide levels in the non-positive MODS COVID-19 group were similar to the control group (11% above control).

In regard to NO production are shown in Fig. 1B, baseline levels in control group were found around 34.91+- 4.463 µmol of NO_2/mg protein. The COVID-19-induced increases in nitrites accumulation were found around 370 % in viremia ($p<0.01$) and 216 % in sepsis whit MODS ($p<0.05$), above control (164.2± 12.60 and 110.4± 13.39; respectively). In addition, non-positive MODS COVID-19 group showing higher levels of nitric oxide ($p<0.01$).

The levels of protein oxidation (expressed as the formation of protein carbonyl as a marker of proteostasis-loss) from all experimental groups are shown in Fig. 1C. Basal levels were 3.238±0.2746 nmol/mg protein. COVID-19 produced changes in protein carbonyl when compared with control group values at any of all times tested in 61.8% in the viremia group and 102% in the MODS group; statistical significance was set at $p<0.05$ (5.240±0.4537) and $p<0.01$ (6.542±0.3925) respectively. The MODS not COVID-19 group, showed the highest protein carbonyl levels ($p<0.01$). Whereas Fig. 1D presents the correlation between peroxide levels and proteins carbonyl content found in blood of COVID-19-patients. Noteworthy a correlation between parameters was found of $r = 0.88$

3.2 SARS-CoV-2 induces inhibition of mitochondrial function and nuclear condensation in leukocytes of COVID-19 patients.

Mitochondrial function is assessed by staining with TMRM and were contrasted with nuclei stained with Hoechst in leukocytes. TMRM fluorescence was diminished in COVID-19 patients (viremia and severe sepsis with MODS), the former indicating a reduction in $\Delta\Psi$m, are show in Fig. 2. The basal levels of mitochondrial function were 70780±5132 in relative units. Regarding mitochondrial function in the viremia group a 44% function was observed (31171±4449), while the MODS group presented a percentage of 67.5% (47785±4005) compared to the control, representing an inhibition of 66% ($p<0.01$) and 32.4% ($p<0.05$) respectively. Regarding the non-COVID MODS group, a significant inhibition of mitochondrial function of 23.2% was observed compared to the control (Figure 2A).

In regard to nuclear condensation by count of cells with the highest staining for Hoechst are show in Fig. 3. In the control group, an average number of 11.6 cells with the highest fluorescents was observed, while the COVID groups (viremia and MODS) showed an increase in nuclear condensation of 31 (165.6%) and 63 (442.7%) cells respectively ($p<0.01$). While the MODS group not positive for COVID also showed an increase in the number of cells with nuclear condensation (26.3 cells; 126.7% above of control) (Fig. 3A).

On the other hand, the graphs of correlation between peroxide levels and mitochondrial function are presented (Fig. 2B) and the correlation of peroxide levels and nuclear condensation (Fig. 3B), determined in the leukocytes of patients with COVID -19. It should be noted that a correlation was found between the parameters of $r = 0.84$ (Fig. 2B) and $r = 0.90$ (Fig. 3B).

3.3 SARS-CoV-2 induces cell death and morphological changes in leukocytes of COVID-19 patients.

We analyzed cell death levels by annexin-V and propidium iodide reactivity as a phenotypic marker for apoptosis and necrosis. Single annexin-V expression shows early apoptosis, while the presence of annexin-V and propidium iodide results in late apoptosis, and only staining with propidium iodide establishes necrosis.

According to cell death (Fig. 4), an increase in both early apoptosis (243.1%, Fig. 4A) and late apoptosis (466.7%, Fig. 4B) was observed in the viremia-COVID-19 group, respect to control; in contrast, the MODS-COVID-19 group, showed a greater increase in late apoptosis (1363%, $p<0.001$) compared to the early apoptosis (147.6%, $p<0.01$), was presented reflecting rapid changes in cellular damage processes. Also, the MODS-COVID-19 negative group showed an increase in early and late apoptosis processes (130% and 496%, respectively) compared to the control group.

Finally, in the necrotic death process (Fig. 4C) it is observed that the viremia-COVID-19 group presents higher levels (3.8 folds more, $p<0.01$) than the control; while the MODS-COVID-19 group presented lower levels compared to the viremia group, but higher than the control (1.6 folds higher, $p<0.05$). Regarding the negative MODS COVID group, there was also a slight increase in necrosis (0.5 folds), although it was not significant compared to the control.

In regard to morphological changes in leukocytes are show in Fig. 5. It is observed that infection by SARS-CoV-2 induces modifications in the morphology of leukocytes, generating the presence of cellular structures that indicate changes in their functions. In Figure 5A, Band neutrophils with coarsely clumped chromatin and cytotoxic granulation, observed in greater quantity in the MODS stage. 5B and 5C, Hypersegmented neutrophils with vacuoles, in addition, loss of membrane integrity is observed, these cells were found in the sepsis stage. 5D, Band neutrophils with cytotoxic granulation and vacuoles, observed at all stages. 5E, monocytes with both cytoplasmic and nuclear digestive vacuoles are observed, indicating increased phagocytic activity. These monocytes were observed in greater quantity in COVID-19 patients in MODS stage and in smaller quantity in the sepsis stage; 5F, vacuolated neutrophils, these neutrophils present vacuoles in the cytoplasm and were observed in all stages of the disease. 5G: Leukocytes with membrane rupture. In the MODS-COVID-19 stage, leukocytes with a completely ruptured membrane were observed, indicating death from cell lysis. in 5H: Hypersegmented neutrophils with cytotoxic granulation are observed, granulations indicate a systemic inflammatory process in addition to a viral or bacterial infectious process, granulations were observed in all stages of the disease, however hypersegmentation was observed at the stage of MODS. 5I, reactive lymphocytes (hyperbasophilic) are observed, which presents a scarce cytoplasm in addition to a marked basophilia, smaller in size as a mature lymphocyte and observed at all stages of SARS-CoV-2 infection.

4 DISCUSSION

The present study highlights an impairment of mitochondrial function in leukocytes from COVID-19 patients, expressed as a decrease in mitochondria membrane potential, associated by increase in ROS production that induces morphological changes and cell death in leukocytes. Our results confirm the pro-oxidant and cytotoxic profile of SARS-CoV-2 in leukocytes and reveal a modulatory action of cellular and organic damage events as part of an integral lesion response. Summarizing these findings showed that SARS-CoV-2 infection increases the levels of oxidative stress markers such as peroxide and nitric oxide, as well as markers of organic damage such as protein carbonylation. These results were associated with an increase in the inhibition of mitochondrial function that leads to both morphological and functional cell injury and therefore culminate in cell death of leukocytes involving an immunosuppression event which contributes to generalized tissue injury in COVID-19 patients.

The relevance of these findings is considerable since it represents, to our knowledge, one of the first reports available in the literature that describes the ability of mitochondrial inhibition by SARS-CoV-2 infection in response to inflammation-modulation to evoke an integral oxidative response in leukocytes, thus contrasting the cytotoxic profile of the virus with respect to endothelial and alveolar cell in COVID-19 disease.[13] It has been determined that viral stimulation in COVID-19 is prone to elicit intensive immunological reactions, cytokine storm and immune-cell infiltration.[4] However, some immunocytes can produce numerous ROS including peroxide, nitric oxide and hydroxyl radical, as reported in other virus studies.[14] ROS is important for regulating immunological responses, but excessive ROS will induce the oxidize proteins, lipids, DNA leading to destruction not only virus-infected cells but also normal cells in lung, endothelial tissue and even the immune cells themselves.[15] Therefore, it could be established that modulation of the cellular redox state

in early stages is very important to mitigate the cytotoxic events caused by COVID-19 infection.

Most studies have tried to show physio-pathological mechanisms, where inflammation and oxidative stress as a result of inflammation have been implicated in the pathogenesis of COVID-19.[1,3,5] Thus, a high number of leukocytes are involved in the inflammatory process and an elevated level of interleukins has been detected in the plasma of COVID-19 patients,[16] that promote degranulation of polymorphonuclear cells and the production of ROS which promotes oxidative stress inducing cellular and tissue damage.[17] This oxidative stress is also active epithelial and endothelial cells to generate chemotactic molecules that recruit neutrophils, monocytes and lymphocytes which potentiate inflammation and oxidative stress and therefore tissue injury.[7,17,18] During viral infection, circulating neutrophils increases free radical release, lipid peroxidation and reduce nitric oxide, which is an endothelial vasodilator. Moreover, oxidative stress affect repair mechanisms and the immune system function, which is one of the main events of the inflammatory response, increasing cytotoxic processes such as mitochondrial inhibition and accelerated apoptosis, that can be related to the severity and progression of COVID-19 disease.[19] Studies indicate that COVID-19 infection is capable of producing an excessive immune reaction in the host, generating an leukocytes activation where monocytes larger than normal can be seen.[20] Therefore, COVID-19 infection leads to excessive activation of monocytes / macrophages with the development of a cytokine storm and, consequently, leads to the appearance of acute respiratory distress syndrome (ARDS).[21] From these reported studies and the found findings, it is suggested that the intervention in modulating the immune response be in early stages, to prevent leukocyte over-activation and cellular toxic effects.

On the other hand, mitochondria play pivotal roles in cell homeostasis of leukocytes as well as other cells. Accordingly, the increased energy expenditure secondary to a cytokine storm can lead to a non-adaptive state, overwhelming the metabolic reserve capacity of mitochondria both from cells infected with COVID-19 and those that respond to infection. As a normal body function against pathogens, mitochondria also produce ROS, however, excessive ROS production can be damaging in a similar way to the infection generated by coronavirus, thus inducing a mitochondrial dysfunction which potentiates cellular damage.[22]Together, the combination of impaired respiration, diminished ATP production, increased ROS, and reduced detoxification capacity with dysregulated immune functions seems likely to play a pivotal role in the increased inflammation and severity of COVID-19.[22,23]

It has been reported that the depletion of cellular adenosine triphosphate (ATP) can lead to cellular dysfunction induced by COVID-19[24] and the immune cells are not an exception. A hypothesis has been proposed, stating that ATP depletion can lead to induce the cytotoxic mechanisms of COVID-19 and promote suppression of the immune system. Adequate levels of ATP have been established to be essential for maintaining active JAK/STAT cell signaling pathways that are involved in INF-1 function, as well as preventing cytokine storm by modulating the function of lysosomal TLR7. These events can potentially make recruited immune cells more prone to early exhaustion against COVID-19 if ATP levels decrease.[24,25]

In the study reported by Varga et al.,[8] they observed the presence of viral elements within endothelial cells and an accumulation of inflammatory cells, with evidence of endothelial and inflammatory cell death. In addition, they report that induction of apoptosis and pyroptosis might have an important role in endothelial cell injury in patients with COVID-19. And that damage is most likely induced by over-activation of the phenotype changes of the immune system. Several current reports emphasize the occurrence of lymphopenia with drastically reduced numbers of both CD4 and CD8 T-cells in moderate and severe COVID-19 cases. The extent of lymphopenia-seemingly correlates with COVID-19-associated disease severity and mortality.[19,26] Furthermore, damage accumulation and a poor DNA repair system in immune cells have been reported. The overexpression of oxidative stress seen with a viral infection, along with attenuated DNA repair capacity, could accelerate genome instability and apoptosis in infected and non-infected cells.[23]

During the SARS-CoV-2 pandemic, quantitative hematologic abnormalities have been reported in COVID-19 patients. Most of these common hematological findings include lymphocytopenia, neutrophilia, eosinopenia, mild thrombocytopenia (35%) or, less frequently, thrombocytosis.[27] However, similarly, morphological changes in circulating cell lines have been reported like those observed in this work.

An increase in reactive lymphocytes, sometimes called activated lymphocytes or virocytes, have been reported in viral diseases. Showing morphological and functional differences with respect to normal leukocytes, this because they are the result of a polyclonal immune response produced by antigenic stimulation derived from various factors.[28] Reactive lymphocytes are normally found in 2% in a healthy adult, we observed reactive lymphocytes, whose percentage was 10%. These lymphocytes had a low cytoplasm in addition to a marked basophilia. Indicating an over activation to counteract the SARS CoV-2 infection. However, some studies show that the hematological line of lymphocytes is reduced in covid-19 patients, even decreasing in advanced stages.[29,30] Therefore, it is considered a poor prognostic factor. Monocytes such as macrophages have been described to express ACE2 receptors, this characteristic makes them vulnerable to infection with SARS-CoV-2, leading to activation and transcription of proinflammatory genes.[20,31] This activation and fight of monocytes with SARS-CoV-2 shows morphological changes in the monocytes, showing vacuoles in their cytoplasm and in the nucleus, deformity of the membrane. Regarding neutrophils, toxic granulation has been observed to be dense lysosomes with a high content of peroxidases, alkaline phosphatase, and acid phosphatase. These abnormally stained azurophil granules can be lysed, which is morphologically evidenced as cytoplasmic vacuolization. According to recent studies, such quantitative and qualitative abnormalities can be related to cytokine storm and hyperinflammation, which is a fundamental pathogenic factor in the evolution of the COVID-19 disease.[27,32] Moreover, the inflammatory response and viral effects on leukocytes could be responsible for these morphological changes, which can be easily identified in leukocytes and can be monitored with a Wright stain.

Finally, modulation of oxidative stress and inflammation can decrease the rate of progression of cellular and tissue damage, preventing the development of local and systemic complications of the disease. Likewise, proper management of inflammation and mitochondrial dysfunction can decrease the extent of tissue injury and therefore improve recovery conditions and quality of life. Finally, oxidative stress modulation, as well as inflammation and leukocyte mitochondrial dysfunction form an essential part of comprehensive treatment from early stages of SARS-CoV-2 infection. Therefore, studies should be carried out to clarify the processes that lead to the premature death of leukocytes and that therefore generate an effect similar to immunosuppression, and that this entails should be avoided with earlier treatment.

Establishing the processes of cellular dysfunction during the pathophysiological evolution of COVID-19 disease is essential. The results obtained show how the mitochondrial activity decreases throughout the infection caused by the SARS-CoV-2 virus promoted by oxidative stress, and that they modify the mechanisms of cellular adaptation, losing the ability to regulate the immune system, and the endothelial damage, giving a synergistic effect on the sustained inflammatory response (Fig. 6).

Therefore, it is plausible to believe that a mechanistic possibility for our model could imply interaction between the generation of oxidative stress and mitochondrial dysfunction, said mitochondrial inhibition would cause an increase in the production of free radicals and cytokines, which would cause a cellular change, increasing cellular damage processes such as protein carbonylation and oxidation of the macromolecules that lead to cytotoxic damage and culminate in cell death (Fig. 7).

5 CONCLUSIONS

The evidence gathered in this study suggests that SARS-CoV-2 infection induces an oxidative response that potentiates cell damage evidenced by inhibition of mitochondrial function, morphological changes and cell death in leucocytes. The signaling associated with inflammation and oxidative stress in COVID-19 disease establishes a paradigm of comprehensive injury that must be consider in physiopathology. These processes explain the rapid changes in the immune system, and that present an initial over-activation and early massive death due to SARS-CoV-2 infection, promoting endothelial-alveolar damage that would cause multi-organ failure. From this, a relevant approach is shown to develop therapeutic strategies based on this and other early processes related to the pathogenic characteristics of the disease, although more research is needed to detail the mechanisms of cytotoxicity, it is essential to start establishing early actions in the therapy of this disease.

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