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Review Paper

Nebulization of glutathione and N-Acetylcysteine as an adjuvant therapy for COVID-19 onset



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ABSTRACT

Ever since its emergence, the highly transmissible and debilitating coronavirus disease spread at an incredibly fast rate, causing global devastation in a matter of months. SARS-CoV-2, the novel coronavirus responsible for COVID-19, infects hosts after binding to ACE2 receptors present on cells from many structures pertaining to the respiratory, cardiac, hematological, neurological, renal and gastrointestinal systems. COVID-19, however, appears to trigger a severe cytokine storm syndrome in pulmonary structures, resulting in oxidative stress, exacerbated inflammation and alveolar injury. Due to the recent nature of this disease no treatments have shown complete efficacy and safety. More recently, however, researchers have begun to direct some attention towards GSH and NAC. These natural antioxidants play an essential role in several biological processes in the body, especially the maintenance of the redox equilibrium. In fact, many diseases appear to be strongly related to severe oxidative stress and deficiency of endogenous GSH. The high ratios of ROS over GSH, in particular, appear to reflect severity of symptoms and prolonged hospitalization of COVID-19 patients. This imbalance interferes with the body's ability to detoxify the cellular microenvironment, fold proteins, replenish antioxidant levels, maintain healthy immune responses and even modulate apoptotic events. Oral administration of GSH and NAC is convenient and safe, but they are susceptible to degradation in the digestive tract. Considering this drawback, nebulization of GSH and NAC as an adjuvant therapy may therefore be a viable alternative for the management of the early stages of COVID-19.

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Introduction

Since the emergence of SARS-Cov-2 and the novel coronavirus disease in December 2019, there have been approximately 16.2 million confirmed cases and 3,364,178 deaths worldwide [1]. COVID-19 has been characterized as a multi-organ disease that affects vital systems like pulmonary, cardiac, gastrointestinal, hematological, neurological, and renal structures. However, the most common manifestations are seen in the lungs ranging from mild pneumonia to more severe presentation associated with acute respiratory distress syndrome, shock, respiratory failure [2,3]. The basic mechanism of tissue injury is attributed to viral binding to angiotensin-converting enzyme 2 (ACE2) receptors located on several different human cells. The ACE2 receptor is expressed on the type II alveolar epithelial cells where the viral binding and replication results in alveolar injury and interstitial inflammation. The respiratory complications are attributed to a "cytokine storm syndrome" [4,5] an exaggerated systemic immune inflammatory response with oxidative stress (Fig. 1) caused by increased levels of interleukin (IL)-6 and tumor necrosis factor α (TNF- α) along with decreased levels of interferon α and interferon β (IFN- α , IFN- β) [6]. The elevated levels of pro-inflammatory cytokines and chemokines such as tumour necrosis factor (TNF)- α , interleukin 1 β (IL-1 β), IL-6, IL-8 [2,7] and others indicate the cytokine storm plays a central role in the immunopathology of COVID-19, but the primary source or the exact virological mechanisms behind it have not been identified yet [8]. Dendritic cells (DCs) and alveolar macrophages phagocytose the virus-infected epithelial cells, followed by an immunosuppressive response characterized by lymphopenia, low CD4 and CD8 T cell counts and therefore an increased risk of bacterial infection [9–11]. At the tissue level, formation of hyaline membranes and alveolar wall oedema and thickening in addition to microvasculature involvement with pulmonary vessel (intra and extra) hyaline thrombosis, hemorrhage, vessel wall oedema, intravascular neutrophil trapping and immune cell infiltration is appreciated [12,13]. Hypoxemia and microthrombi have been speculated to contribute to pulmonary infarction, hemorrhage and pulmonary hypertension [14] leading further to diffuse pulmonary intravascular coagulopathy (PIC) coupled with ARDS in advanced cases [15].

Recently, administration of glutathione (GSH) and even its precursor molecule N-acetylcysteine (NAC) have been receiving some attention and are worthy of consideration as potential adjuvant therapeutics for

the management of COVID-19-related oxidative stress. These antioxidant agents may prove to be a viable solution since they are known to display multiple biological properties, some of which are well associated with antiviral effects and immune responses with the ability to balance the unbridled oxidative stress (Fraternale et al., 2006; Kwon et al., 2019).

To the best of our knowledge, not many studies have been published in this regard due to the recent nature of this infection, which is why no treatment has been able to completely and effectively assure consistent control of the inflammation or the disease process itself. Although in most cases the disease can be resolved on its own, in severe cases patients may not resist the diffuse and massive alveolar damage associated with disease progression and, ultimately, pass away. There is still a lack of effective antiviral agents as well as fully elucidated or validated therapeutic alternatives that can halt disease progression. This has urgently prompted researchers and clinicians alike to propose new treatments in attempts to delay the exacerbated inflammatory response and accelerate the repair of functional lung tissue in the initial phases of COVID-19.

The objective of the present study is to review and discuss nebulization of glutathione and its precursor N-acetylcysteine in attenuating the exacerbated inflammatory and oxidative stress associated with the "cytokine storm syndrome" in the onset of COVID-19, preventing aggravation of disease progression and ICU hospitalization.

The respiratory tract and acute respiratory syndrome

The respiratory tract is known to be a complex structure containing a wide variety of cell populations distributed in different regional microenvironments along its path. The trachea is composed of ducts and submucosal glands, where different stem and progenitor cells are found. In the bronchi, basal cells predominate in the intercartilaginous zones due to their high proliferative rate. In this same structure, there is the presence of clear cells, which are responsible for secretion and absorption of glycoproteins as well as the degradation of toxic substances. Type 1 and 2 alveolar cells are located in the alveoli and become responsible for the production of surfactant substances, which reduce the surface tension at the interface between fluids present in the alveolar cavity and the air [4].

The acute respiratory syndrome is a progressive inflammatory process in the lungs, typically characterized by an alveolar lesion with dif-

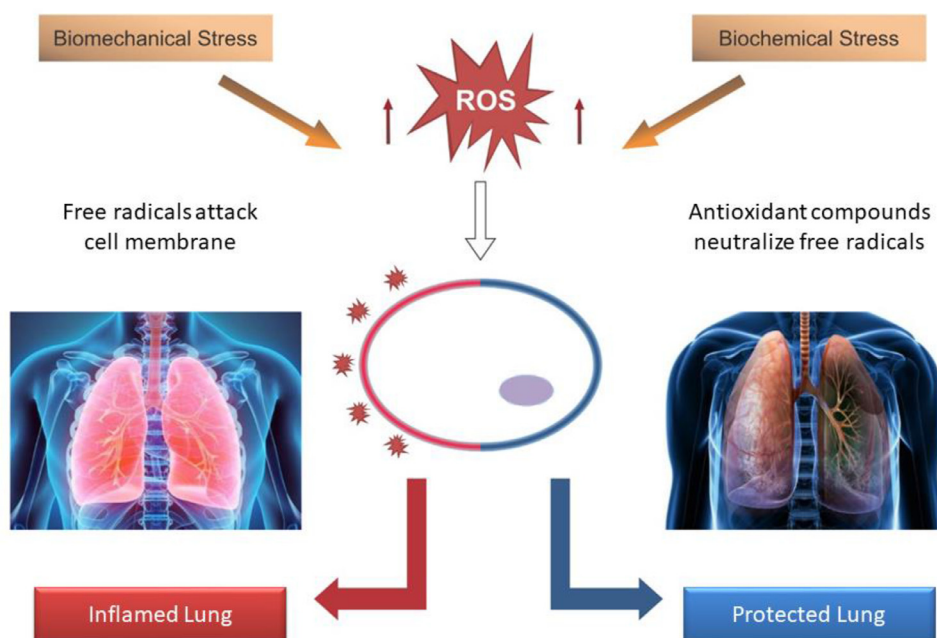


Fig. 1. COVID-19 pathophysiology and lung damage.

fuse alveolar damage. The first cases of severe acute respiratory syndrome (SARS) (as seen in covid-19) were described long ago in patients with symptoms of tachypnea, refractory hypoxemia and diffuse opacity in the lung on radiographs [5].

Population estimates of SARS range from 10 to 86 cases per 100,000, with the highest rates reported in Australia and the United States. Since the diagnosis is based on imaging exams, SARS is likely to be under-reported in low-income countries, where the resources to obtain chest radiographs and measure arterial blood gases are limited [6].

SARS is known to develop within seven days after clinical recognition of the known risk factor, most commonly pneumonia or sepsis. These risk factors can be classified as direct ones, which include aspiration of gastric contents, pulmonary contusion, inhalation injury or drowning; and indirect ones, such as trauma or non-thoracic shock, pancreatitis, burns, overdose by illicit drugs, and the presence of edema after lung transplantation [16].

"SARS mimics" should not be disregarded account because they comprise a large number of diseases or syndromes that may require specific treatments. Examples include interstitial lung disease, polymyositis, diffuse alveolar hemorrhage, cancer and lung diseases caused by drug abuse [17]. Previous definitions excluded volume overload or heart failure, but recent evidence suggests that these problems can coexist in up to a third of patients with SARS.

Histologically, SARS can be interpreted as "diffuse alveolar lesion", a term described by Katzenstein et al. The authors described the rapid development of capillary congestion, atelectasis, hemorrhage and alveolar edema followed by formation of hyaline membrane, epithelial cell hyperplasia and interstitial edema [18]. In fact, animal models of SARS have been developed to recapitulate these findings; however, the Berlin definition as well as the definition of the 1994 American-European Consensus Conference [19] has little specificity for diffuse alveolar damage. In cadaver exams, for instance, 40% to 58% of patients with a moderate to severe clinical diagnosis of SARS have diffuse alveolar damage. Pulmonary edema and pneumonia without hyaline membranes are other common findings, although 14% of patients do not have lung injuries, which may be attributed to atelectasis disguised as SARS [20,21].

Additionally, over 40 genes have been associated with the development of SARS, including genes encoding the angiotensin converting enzyme (ACE), interleukin 10 (IL-10), tumor necrosis factor (TNF) and vascular endothelial growth factor (VEGF), as well as SOD3, MYLK, NFE2L2, NAMPT and SFTPB. In the only genome-wide association study in SARS-associated trauma, no polymorphism was found to be significant [22].

Elevated levels of plasma biomarkers, especially markers of systemic inflammation (interleukin-6 and interleukin-8), epithelial injury (receptor for advanced glycation end products and surfactant protein D) and endothelial injury (angiopoietin 2), as well as dysregulated coagulation markers (low levels of protein C and high levels of plasminogen activator inhibitor 1), have all been associated with adverse SARS results. These molecules provide information on the pathogenesis of the disease and can assist in monitoring the response to treatment [23].

Pathogenesis of SARS

Speaking of pathogenesis, SARS begins with the exudative phase, characterized by a response mediated by innate immune cells of the alveolar endothelial and epithelial barriers as well as the accumulation of protein-rich fluids in the interstitium and alveolus. Activation of the resident alveolar macrophages leads to the release of potent pro-inflammatory mediators and chemokines that promote the accumulation of neutrophils and monocytes. Once activated, neutrophils further aggravate injury by releasing toxic mediators. This leads to loss of barrier function and increased interstitial and intra-alveolar fluid build-up. The mediated expression of TNF promotes the aggregation of platelets, formation of microthrombi, intra-alveolar coagulation and the subsequent formation of a hyaline membrane. Endothelial activation and mi-

crovascular injury also contribute to the rupture of the barrier in SARS pathology and can be significantly aggravated by mechanical stretching [23,24].

In the second phase, also known as the proliferative phase, there is the initiation of repair processes which are essential in dictating survival outcomes. It is during this phase where the objective is to restore tissue homeostasis via the transient expansion of fibroblasts and the formation of a temporary matrix, as well as the proliferation of airway progenitor cells and type II alveolar epithelial cells (AECII), specifically envisaging the differentiation of biologic precursors into type I alveolar epithelial (AECI) cells [25].

The final phase is commonly referred to as the fibrotic phase. It is strongly associated with mechanical ventilation and extensive damage to the basement membrane, where inadequate or delayed re-epithelialization can lead to the development of changes such as interstitial fibrosis. Once the epithelial integrity is restored, resorption of the alveolar edema and the provisional matrix can then progressively lead to restoration of the alveolar architecture and function [23].

In the case of severe coronavirus-related respiratory syndrome (COVID-19), the reported consequences are severe pneumonia and kidney failure with an even higher mortality rate, reaching approximately 55% in documented cases [26]. The severity of the disease is strongly associated with advanced age and other existing comorbidities in patients, such as metabolic syndrome, resulting in mortality rates above 50% in individuals over 65 years of age. Regarding the pathophysiological mechanism of SARS-CoV there is an infection of type 2 pneumocytes. These cells are responsible for the production of pulmonary surfactants and also act as progenitor cells for type 1 pneumocytes [27]. In the acute phase of SARS-CoV infection, there is erosion of the epithelial cells that line the airways and accumulation of debris and particles that obstruct the regular breathing pattern [28,29]. There is also a risk of progression to more severe complications such as acute lung injury (ALI) and the subsequent Acute Respiratory Distress Syndrome (ARDS), which cover alveolar damage [27].

Exacerbated inflammatory stress from such an infection can stimulate an excess of secretions in the lung, including fibrin compounds and proteinaceous materials, impeding the essential gas exchange for the maintenance of vital organs [30]. If this secretion is not removed, the pathophysiology progresses to the state of pulmonary fibrosis, established by misplaced collagen deposition as well as the conversion of mucus into fibrous tissue [30]. In this sense, acute injury and alveolar damage can be linked to an exacerbated reaction in response to microbiological agent exposure, in this case, the SARS-CoV-2.

Genome sequencing and phylogenetic analysis suggest that the coronavirus strain responsible for COVID-19 is a beta-coronavirus in the same subgenus as the severe acute respiratory syndrome virus (SARS), but now in a different clade. The structure of the receptor-binding genetic region is very similar to that of the SARS coronavirus, and the new virus also utilizes the same ACE2 (angiotensin-converting enzyme 2) receptor to enter and infect host cells [31].

The Coronavirus Study Group of the International Virus Taxonomy Committee has proposed that this virus be called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The degrees of symptomatic infection can vary from mild to critical. Most infections can in fact be less severe. According to a study, no pneumonia was found in 81% of cases; severe disease (with dyspnea, hypoxia or >50% of lung injury in images within 24 to 48 hours) was reported in 14%; and critical illness with respiratory failure, shock or multi-organ dysfunction was reported in 5% [3].

In another study evaluating 201 patients hospitalized due to COVID-19 in Wuhan, 41% of the individuals developed SARS; constraints such as advanced age (65 and above), diabetes mellitus and hypertension were strongly correlated with SARS [32].

Some patients experiencing severe COVID-19 symptoms have laboratory evidence of an exuberant inflammatory response, similar to the cytokine release syndrome. Typical observations include persistent fever,

elevated inflammatory markers (eg, D-dimer, ferritin) and elevated pro-inflammatory cytokines, which have all been associated with critical and fatal diseases. Initial plasma concentrations of IL-1B, IL-1RA, IL-7, IL-8, IL-9, IL-10, basic FGF, GCSF, GM-CSF, IFN γ , IP10, MCP1, MIP1A, MIP1B, PDGF, TNF α and VEGF were higher in ICU and non-ICU patients than in healthy adults. Plasma levels of IL-5, IL12p70, IL-15, Eotaxin and RANTES were similar between healthy adults and patients infected with the novel 2019 coronavirus. The comparison between ICU and non-ICU patients showed that plasma concentrations of IL-2, IL-7, IL-10, GCSF, IP10, MCP1, MIP1A and TNF α were higher in ICU patients than in non-ICU patients [33].

Other authors propose that the development of ALI and alveolar damage can also occur in other types of respiratory infections, such as the influenza virus H1N1 and the respiratory syncytial virus (RSV), which also put the elderly population at greater risks [34–36]

Oxidative stress in COVID-19

Oxidative stress has been implicated in the manifestation of critical illnesses, including ischemia and reperfusion injury and systemic inflammatory states. The state of oxidative stress is defined as an imbalance between the presence of antioxidants and free radicals/pro-oxidants in a biological system. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are the two most common residual products of various cellular processes, including aerobic metabolism [37]. When the redox balance is disrupted, excessive production of free-radicals start to cause significant damage as the body is left unable to counteract the over-production of these oxidant agents. The unstable atoms in free-radicals take away electrons from other biological structures, causing instability and damage to cells and essential components such as DNA, proteins and lipids from the membrane, which ultimately lead to inflammation tissue injury [38].

The ACE2 is a protease that participates in the renin-angiotensin system (RAS). Both ACE and ACE2 are present on cell membranes and compete for the same substrates, angiotensin I and II. ACE2, however, can neutralize ACE activity, influencing the amount of angiotensin-II (ANGII) and increased ANG 1-7 peptide [39,40].

The downstream effects of the two enzymes are opposite as ACE activity leads to vasoconstriction, oxidative stress, inflammation and apoptosis. ACE2 on the other hand causes vasodilation and angiogenesis, and promotes anti-inflammatory, antioxidant and anti-apoptotic effects [40]. The oxidative stress generated by ACE activity is linked to the effects of ANGII produced, which increases the production of ROS through the activation of NADPH oxidase and the generation of peroxynitrite anions. In contrast, the ANG 1-7 peptide synthesized by ACE2 activity affect pro-oxidant pathways, preventing or attenuating cell damage induced by oxidative stress.

Every individual has a unique ACE/ACE2 balance and may therefore be more susceptible to inflammation if ACE prevails. In coronavirus-related cases, SARS-CoV-2 infection negatively regulates the abundance of ACE2 on cell surfaces, as suggested by evidence [41]. The result is a toxic over-accumulation of ANGII, exacerbated inflammation and, finally, acute respiratory distress syndrome and fulminant myocarditis.

Suboptimal ratio of ACE to ACE2 may explain the heterogeneous responses to the viral infection. The link between deregulation of the RAS cascade and probability and severity of SARS-CoV-2 infection has been discussed in recent investigations [42].

Studies suggest that a delicate disulfide-thiol balance is crucial for viral entry and fusion in the host cell. It turns out that oxidative stress generated by free radicals can negatively affect this balance [37]. Particular focus is shifted towards the impact of antioxidants, such as NADPH and glutathione, and redox proteins, such as thioredoxin and protein disulfide isomerase, which maintain the disulfide-thiol balance in the cell [37]. The possible influence of these biomolecules on the binding of the viral protein to the host cell's ACE2 receptor protein is discussed, as well as on the severity of COVID-19 infection [37]. Exacerbated ROS

production and a disproportionate cellular antioxidant/oxidant balance may play an important role in the pathogenesis of respiratory infections (Fig. 1), especially SARS-CoV infections. More recently, it has been suggested that individuals with pre-existing comorbidities such as metabolic syndrome and lung, heart and kidney disease are at a higher risk of developing a serious infection. Additionally, pathologies like cancer, diabetes mellitus, cardiovascular diseases, and chronic kidney disease can cause an increase in oxidative stress.

Recent studies have also highlighted the importance of the disulfide-thiol balance in the viral entry of the SARS-CoV and SARS-CoV-2 coronaviruses. Similar to the HIV gp120 protein, experimental data showed that as the thiol content in SARS-CoV S1 (the receptor-binding subunit) increased, its ability to bind to target cells decreased, suggesting that both viruses require a specific thiol content for fusion and entry to occur [37].

Managing SARS-CoV infection

The accurate identification and immediate treatment of the underlying cause in patients with SARS is imperative. Supportive therapy for SARS is focused on limiting more pulmonary actions through a combination of pulmonary protection to prevent ventilator-associated lung injuries and conservative fluid therapy to not only prevent but stimulate resorption of pulmonary edema. Currently, there are no ideal or “gold standard” parameters for pulmonary protection. There may be no safe level of tidal volume or airway pressure in patients with acute injury. As the volume of aerated lung is reduced in patients with SARS, even the normal volumes delivered with airway pressures surveyed for the uninjured lung can cause volumetric trauma, further damaging the epithelium and stimulating inflammation. The repetitive opening and closing of the lung structures amplifies the regional pulmonary tension and denatures the surfactant. Epithelial and endothelial damage, in turn, causes translocation of pro-inflammatory mediators and bacterial products, worsening systemic inflammation [23,43].

At the moment, there are no well-known pharmacological therapies for SARS proving to reduce mortality in the short or long term. Inhaled nitric oxide may improve oxygenation and lung function in the long-term among patients who survive. However it does not reduce mortality and is often associated with acute kidney injury [44]. Surfactant substitution, neutrophil elastase inhibition and anticoagulation have not yielded very optimistic results in clinical trials. The same is true for NSAIDs (ketoconazole and lysophylline), statins and albuterol, for example [45].

More recently, however, researchers have considered the potential applications of specific antioxidants, more specifically NAC and GSH, to assist in the management of these respiratory complications.

Biological effects of glutathione and N-acetylcysteine

As previously introduced, the immunoinflammatory mechanisms implicated in the pathophysiology of SARS-Cov-2 have led scientists to believe that disulfide-thiol balance is critical for viral entry into the host cell. Oxidative stress remains a key factor in this disease process, playing a crucial role in the balance of homeostasis [37,46–48]. Therefore, antioxidants like Glutathione (GSH) and its precursor, N-acetylcysteine (NAC) have been considered in treatment of this condition given their influence on binding of the viral protein on to the host cell ACE2 receptor protein that is specifically implicated in the SARS-Cov-2 infection [37,41,48].

GSH (γ -L-Glutamyl-L-cysteine) is found in the cytosolic compartment of most cells in the body [49]. This tripeptide is made up of three amino acids: glycine, cysteine and glutamate. GSH acts on several enzymatic systems in the body that help eliminate free-radicals and detoxify fat-soluble components [50]. It also exerts a metabolic role in various biochemical processes, including amino acid transport, DNA synthesis and immune system improvement [51]. In the epithelial lining fluid of the

lower respiratory tract, GSH is the first line of defense against oxidative stress [51]. The concentrations are 140 times higher than in serum, and alterations in GSH metabolism in the lungs are considered central in the context of inflammatory lung diseases [52–54]. NAC has been demonstrated to improve the redox status especially under oxidative stress, a key phenomenon in SARS-Cov-2 [6]. In fact, it has been previously suggested that the deficiency of endogenous GSH as a whole is reflective of a significant factor for the pathogenesis of various diseases through mechanisms involving oxidative stress and inflammation. Also, the main risk factors for the more aggressive forms and lethal manifestations of COVID-19 appear exactly in the population that naturally presents a natural or pathological depletion of GSH [48]. The glutathione precursor also activates additional mechanisms that are beneficial in ameliorating the pathophysiological pathways of the viral attack and subsequent immunomodulatory inflammatory response. It may increase the proliferative response of T cells, inhibit NLRP3 inflammatory pathway (IL1B and IL18) and decrease plasma TNF [55] According to Zhou et al [56] NAC decreases IBF-alpha, IL-1b, IL-6, IL-8, IL-10, IL-17 serum levels in patients with sepsis, severe burns, acute liver failure, and may also diminish the potency of the cytokine storm, thereby attenuating the detrimental effects of SARS-Cov-2 on multi-system organ failure.

In addition to being a potent antioxidant and an anti-inflammatory agent, NAC has demonstrated the potential to serve as a mucolytic agent, further strengthening its role in pulmonary diseases [57]. Although acknowledged in the guidelines of the American Thoracic Society and the European Respiratory Society, more robust studies need to be conducted to further elucidate its applicability [58]. Grandjean et al concluded through their meta-analysis of eight trials that oral delivery of NAC at doses ranging from 400 to 1200 mg per day to as little as 600 mg three times per week proved to be superior to placebo in the treatment of chronic bronchitis. They also noted decrease in the number of acute exacerbations by 23% [59].

Repine et al reviewed the beneficial effects of NAC in chronic obstructive pulmonary disease via and learned that it was directly associated with improvement of symptoms, decreasing the number of viral infections and airway bacterial colonization [60].

Both NAC and GSH have attracted attention for their therapeutic efficacy in a variety of conditions, given their multimodal mechanism of action. NAC, best known in the past as an antidote for Acetaminophen poisoning has since been appreciated as a potential therapeutic agent for psychiatric, neurological, infectious, addictive and pulmonary diseases [57].

In terms of mechanisms of action, studies have shown that there is no change in the plasma concentration of GSH, except locally, with more pronounced effects on the upper and lower respiratory tract. The predominant mechanism is attributed to its antioxidant properties that confer protection against oxidative damage, in an attempt to reestablish equilibrium in the respiratory tract. Most studies were unable to demonstrate changes in oxidation markers with the use of GSH. Additional explanations for the effects of GSH may include improved host defenses such as increased cytotoxic lymphocytes and better oxygenation. Interestingly, GSH inhalation produces clinically significant results for most of the diseases studied. More specifically, GSH inhalation has shown improvement in respiratory function markers that impact quality of life and disease progression [61].

Administration routes

In inflammatory lung diseases, supplementation with exogenous sources of GSH may help to reduce the oxidant content. Few clinical studies have concluded that oral administration of GSH was ineffective in increasing plasma levels of healthy controls [62], whereas the intravenous route increases its levels in the pulmonary epithelial lining fluid, albeit for a short period of time [63]. Due to its versatility, NAC dosing and route of administration has been varied. In the case of acetaminophen poisoning, for example, which is by far the most traditional

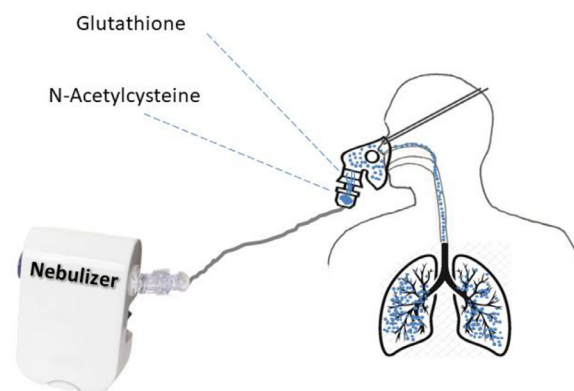


Fig. 2. Nebulization of N-acetylcysteine and Glutathione for the management of initial COVID-19-related respiratory stress.

and time-tested application of NAC, it is often administered as an intravenous infusion of 100-150 mg/kg along in glucose or saline solutions [64].

When used for pulmonary conditions, oral delivery [65] or nebulization [66] with water or saline or in form of tablet or capsule may be more suitable. Topical preparations are generally available as a 200 mg/ml preparation and are absorbed into the systemic circulation from the site administered. The oral bioavailability of NAC is approximately 6-10%, due to extensive first-pass metabolism, with T max at 1-2 hours after dosing [67]. He et al identified an amide derivative of NAC, N-acetylcysteine amide (NACA) in murines as a promising precursor of NAC that increases bioavailability to 67% and increases GSH replenishing capacity by 3-4 fold [68]. Although the oral dosing seems to be fairly well tolerated, mild gastrointestinal symptoms such as nausea, vomiting and diarrhea have been reported in a dose-dependent fashion. For instance, Mardikian et al [69] reported the occurrence of some mild side-effects between 1200, 2400 and 3600 mg/day oral doses of NAC, lying in parallel with the findings of Berk et al [70,71] who evaluated a daily dose of 2 g of NAC (1000 mg twice daily) and also perceived some mild adverse effects.

Moreover, oral administration of GSH can be more expensive than supplementation with cysteine and glycine, and its systemic bioavailability may be poor due to its natural degradation in the digestive tract. Therefore, its suitability for use in a large population may be limited. Interestingly, a case study showed that the repeated use of 2000 mg of oral administration and intravenous delivery of glutathione was effective in alleviating the severe respiratory symptoms of COVID-19, illustrating for the first time the effectiveness of this antioxidant therapy for COVID-19 [72].

In regards to NAC, there is still some limitation in regards to the comprehension of the exact pharmacokinetic profile due to limited studies and early research using low doses and primitive analytical technologies. Most prior studies demonstrate extensive inter-subject variation in plasma concentrations following oral administration of NAC [14,67] which would put forward the inhalation route as a preferred method for treatment of acute respiratory conditions.

We propose that nebulization of 3000 mg per day of NAC or GSH may suffice in controlling the aggravation of oxidative stress and inflammation arising from the severe cytokine storm in pulmonary structures (Fig. 2). This alternative is quite convenient since it is relatively easy and quick for patients to do at any time they wish to, without requiring them to leave the comfort of their own home. Also, portable nebulizers may be even more convenient in this regard. Moreover, at least in comparison to oral administration, for instance, nebulization may offer more viability. This alternative route does not appear to cause any discomfort and does not render the therapeutic agents susceptible to rapid biodegradation as seen in oral ingestion [67], for example, where

molecules may prematurely react with gastric juice. This would reduce their efficacy and, in some cases, even generate mildly discomforting sensations such as nausea, vomiting and diarrhea [69–71].

To elaborate, nebulized NAC, either alone or in combination with other agents has demonstrated to be effective and well tolerated in a variety of conditions. Han et al [73] studied the effect of NAC in lung cancer patients diagnosed with post radiation pneumonitis. The researchers revealed that the mean patient-rated severity score associated with sputum production was decreased in the NAC group between visits 1 and 4 ($P=0.08$) in comparison to the controls, and none of the patients had to use any additional expectorant agents after receiving nebulization. Similarly, Volgers et al [74] studied high dose nebulized NAC in COPD patients and demonstrated that NAC exerts a strong bacteriostatic effect in patients with airway conditions.

In contrast, Masoompour et al [75] conducted a randomized clinical trial of 40 mechanically ventilated patients ages 15-90 years who received NAC vs. normal saline via their nebulizers three times a day for one day. It was noted that although nebulized NAC did not show superior results than normal saline in reducing density of mucus plugs in mechanically ventilated patients, mean secretion density was significantly lower in the NAC group ($P=0.006$) and NAC increased O_2 saturation significantly ($P=0.014$).

Based on the aforementioned information regarding pulmonary conditions, it would appear that inhalation (Fig. 2) is one of the methods that can significantly increase the levels of GSH in the pulmonary epithelial lining fluid [61]. Previous studies on GSH inhalation have also revealed positive results for an array of additional conditions such as cystic fibrosis, chronic otitis, HIV positive patients, idiopathic pulmonary fibrosis and chronic rhinitis [61]. Medical experts should, however, beware of the dispersion of particles in patients undergoing nebulization, which is why this route of administration is strongly recommended for patients who do not respond well to other interventional strategies and administration routes.

The respiratory tract of patients affected by COVID-19 ends up presenting acute inflammation due to escalated immune responses and cytokine production, which are responsible for injury, multiple organ failure and death. The early nebulization of GSH in suspected cases of acute respiratory syndrome due to COVID-19 can help control pulmonary epithelial oxidants. Improvement in lung function is expected, with increased oxygen saturation as well as control of pulmonary inflammation. Lower glutathione levels have been associated with increased cellular oxidative stress as well as several disease states and immune disorders that lead to greater susceptibility to viral infections, including SARS-CoV-2 infection [72]. Uncontrolled viral replication leads to prolonged oxidative damage to the lungs, thus increasing the severity of the infection [76]. Conversely, high levels of GSH may very well assist in preventing the virus from replicating efficiently, producing lower viral loads and, therefore, attenuating symptoms if accurately diagnosed and treated during the initial stages. For instance, an investigation conducted at Kursk State Medical University evaluated the effects of GSH levels on an individual's ability to recover from COVID-19 infection and concluded that high ratios of ROS over GSH appeared to be strongly related to symptom severity and extended recovery times [76].

GSH deficiency can interfere with the body's ability to detoxify the cellular microenvironment, fold proteins, replenish antioxidants, maintain healthy immune responses and even modulate apoptotic events, promoting suboptimal cellular function and development of diseases [53].

Conclusion

COVID-19 has caused a major global impact ever since its emergence in December 2019. This highly transmissible and debilitating disease spread at an incredibly fast rate in a matter of months, causing the infection and unfortunate death of millions of people. SARS-CoV-2 is able to invade host cells in humans by binding to ACE2 receptors lo-

cated in several cells throughout the body. It affects multiple organ systems including the respiratory, cardiac, hematological, neurological, renal and gastrointestinal structures. The most significant manifestations expressed by this disease can be attributed to the predominant cytokine storm syndrome in the lungs, resulting in severe oxidative stress, exacerbated inflammation and alveolar injury. This disease is still quite recent and not fully understood, which may also explain why no treatments have shown complete efficacy and safety. Therefore, individuals still need to rely on other forms of protection and prevention, including vaccination, but safety is still not fully ascertained. GSH and its precursor, NAC, are two natural antioxidant compounds with demonstrated ability to participate in several biological processes in the body, especially in the management of the redox equilibrium. Recent studies have suggested that COVID-19 and even other diseases are strongly related to oxidative stress and deficiency of endogenous GSH. The high ratios of ROS over GSH, in particular, appear to reflect severity of symptoms and prolonged hospitalization of patients. This imbalance interferes with the body's ability to detoxify the cellular microenvironment, fold proteins, replenish antioxidants, maintain healthy immune responses and even modulate apoptotic events, promoting suboptimal cellular function and development of diseases. Although nebulization of GSH and NAC as an adjuvant therapy could be beneficial in managing the early stages of COVID-19, more studies are required.

Declaration of competing interest

The authors declare that they have no conflict of interest.

References

- [1] JPA. Ioannidis, Global perspective of COVID-19 epidemiology for a full-cycle pandemic, *Eur. J. Clin. Invest.* (2020) [PMID: 33026101 DOI: 10.1111/eci.13423].
- [2] M Gavriatopoulou, E Korompoki, D Fotiou, I Ntanasis-Stathopoulos, T Psaltopoulou, E Kastritis, E Terpos, MA. Dimopoulos, Organ-specific manifestations of COVID-19 infection, *Clin. Exp. Med.* (2020) [PMID: 32720223 DOI: 10.1007/s10238-020-00648-x].
- [3] Z Wu, JM. McGoogan, Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China, *JAMA* (2020) [DOI: 10.1001/jama.2020.2648].
- [4] CJ Blaisdell, DB Gail, EG. Nabel, National heart, lung, and blood institute perspective: Lung progenitor and stem cells - Gaps in knowledge and future opportunities, *Stem Cells* (2009) [DOI: 10.1002/stem.148].
- [5] DG Ashbaugh, DB Bigelow, TL Petty, BE. Levine, Acute respiratory distress in adults, *Lancet* (1967) [PMID: 4143721 DOI: 10.1016/s0140-6736(67)90168-7].
- [6] J Villar, J Blanco, RM. Kacmarek, Current incidence and outcome of the acute respiratory distress syndrome, *Curr. Opin. Crit. Care* (2016) [DOI: 10.1097/MCC.000000000000266].
- [7] C Bryce, Z Grimes, E Pujadas, S Ahuja, MB Beasley, R Albrecht, T Hernandez, A Stock, Z Zhao, MR AlRasheed, J Chen, L Li, D Wang, A Corben, GK Haines, WH Westra, M Umphlett, RE Gordon, J Reidy, B Petersen, F Salem, MI Fiel, SM El Jamal, NM Tsankova, J Houldsworth, Z Mussa, B Veremis, E Sordillo, MR Gitman, M Nowak, R Brody, N Harpaz, M Merad, S Gnjatic, WC Liu, M Schotsaert, L Miorin, TA Aydilillo Gomez, I Ramos-Lopez, A Garcia-Sastre, R Donnelly, P Seigler, C Keys, J Cameron, I Moultrie, KL Washington, J Treatman, R Sebra, J Jhang, A Firpo, J Lednicky, A Paniz-Mondolfi, C Cordon-Cardo, ME Fowkes, Pathophysiology of SARS-CoV-2: the Mount Sinai COVID-19 autopsy experience, *Mod Pathol* (2021) [PMID: 33795830 DOI: 10.1038/s41379-021-00793-y].
- [8] SH. Huang, What we know so far (As of March 26, 2020) about COVID-19—an MRT point of view, *J. Med. Imaging Radiat. Sci.* (2020) [PMID: 32279977 DOI: 10.1016/j.jmir.2020.03.004].
- [9] R Channappanavar, J Zhao, S. Perlman, T cell-mediated immune response to respiratory coronaviruses, *Immunol. Res.* (2014) [PMID: 24845462 DOI: 10.1007/s12026-014-8534-z].
- [10] B XU, FAN C yu, WANG A lu, ZOU Y long, YU Y han, C HE, XIA W guang, ZHANG J xian, Q MIAO, Suppressed T cell-mediated immunity in patients with COVID-19: a clinical retrospective study in Wuhan, China, *J Infect* (2020) [PMID: 32315725 DOI: 10.1016/j.jinf.2020.04.012].
- [11] G Zhou, S Chen, Z. Chen, Advances in COVID-19: the virus, the pathogenesis, and evidence-based control and therapeutic strategies, *Front. Med.* (2020) [PMID: 32318975 DOI: 10.1007/s11684-020-0773-x].
- [12] LM Buja, D Wolf, B Zhao, B Akkanti, M McDonald, L Lelenwa, N Reilly, G Ottaviani, MT Elghetany, DO Trujillo, GM Aisenberg, M Madjid, B. Kar, The emerging spectrum of cardiopulmonary pathology of the coronavirus disease 2019 (COVID-19): Report of 3 autopsies from Houston, Texas, and review of autopsy findings from other United States cities, *Cardiovasc Pathol* (2020) [PMID: 32434133 DOI: 10.1016/j.carpath.2020.107233].

- [13] LM Barton, EJ Duval, E Stroberg, S Ghosh, S. Mukhopadhyay, COVID-19 autopsies, Oklahoma, USA, *Am J Clin Pathol* (2020) [PMID: 32275742 DOI: 10.1093/AJCP/AQAA062].
- [14] R Wölfel, VM Corman, W Guggemos, M Seilmaier, S Zange, MA Müller, D Niemeyer, TC Jones, P Vollmar, C Rothe, M Hoelscher, T Bleicker, S Brünink, J Schneider, R Ehmann, K Zwirgmaier, C Drosten, C. Wendtner, Virological assessment of hospitalized patients with COVID-2019, *Nature* (2020) [PMID: 32235945 DOI: 10.1038/s41586-020-2196-x].
- [15] D McGonagle, JS O'Donnell, K Sharif, P Emery, C Bridgewood, Immune mechanisms of pulmonary intravascular coagulopathy in COVID-19 pneumonia, *Lancet Rheumatol* (2020) [DOI: 10.1016/S2665-9913(20)30121-1].
- [16] ND Ferguson, E Fan, L Camporota, M Antonelli, A Anzueto, R Beale, L Brochard, R Brower, A Esteban, L Gattinoni, A Rhodes, AS Slutsky, JL Vincent, GD Rubenfeld, B Taylor Thompson, V Marco Lanieri, The Berlin definition of ARDS: An expanded rationale, justification, and supplementary material, *Intensive Care Med* (2012) [PMID: 22926653 DOI: 10.1007/s00134-012-2682-1].
- [17] M Aublanc, S Perinel, C. Guérin, Acute respiratory distress syndrome mimics: The role of lung biopsy, *Curr. Opin. Crit. Care* (2017) [DOI: 10.1097/MCC.0000000000000373].
- [18] ALA Katzenstein, CM Bloor, AA. Leibow, Diffuse alveolar damage: the role of oxygen, shock, and related factors. A review, *Am J Pathol* (1976) [PMID: 788524].
- [19] GR Bernard, A Artigas, KL Brigham, J Carlet, K Falke, L Hudson, M Lamy, JR Legall, A Morris, R Spragg, B Cochlin, PN Lanken, KV. Leeper, J Marini, JF Murray, L Oppenheimer, A Pesenti, L Reid, J Rinaldo, The American-European Consensus Conference on ARDS: definitions, mechanisms, relevant outcomes, and clinical trial coordination, *Am. J. Respir. Crit. Care Med.* (1994).
- [20] A Esteban, P Fernández-Segoviano, F Frutos-Vivar, JA Aramburu, L Nájera, ND Ferguson, I Alía, F Gordo, F. Ríos, Comparison of clinical criteria for the acute respiratory distress syndrome with autopsy findings, *Ann. Intern. Med* (2004) [PMID: 15381517 DOI: 10.7326/0003-4819-141-6-200409210-00009].
- [21] AW Thille, A Esteban, P Fernández-Segoviano, JM Rodriguez, JA Aramburu, O Peñuelas, I Cortés-Puch, P Cardinal-Fernández, JA Lorente, Frutos-Vivar F. Comparison of the berlin definition for acute respiratory distress syndrome with autopsy, *Am. J. Respir. Crit. Care Med.* (2013) [PMID: 23370917 DOI: 10.1164/rccm.201211-1981OC].
- [22] C J.D., W M.M., F R., O G.E., B J., W L.B., C D.C., C C.S., C M.J., M M., M N.J., K C., L M., A J., B K.C., S J., L P.N., M A.K., A R., M J.P., H H., Genome wide association identifies PPF1A1 as a candidate gene for acute lung injury risk following major trauma, *PLoS One* (2012) [DOI: 10.1371/journal.pone.0028268 LK - <http://limo.libis.be/resolver?sid=EMBASE&issn=19326203&id=doi:10.1371%2Fjournal.pone.0028268&title=Genome+wide+association+identifies+PPF1A1+as+a+candidate+gene+for+acute+lung+injury+risk+following+major+trauma> &title=PLoS+ONE&title=PLoS+ONE&volume=7&issue=1&spage=&epage=&aulast=Christie&aufirst=Jason+D.&auinit=J.D.&aufull=Christie+J.D.&coden=&isbn=&pages=&date=2012&auinit1=J&auinitm=D].
- [23] A Rhodes, LE Evans, W Alhazzani, MM Levy, M Antonelli, R Ferrer, A Kumar, JE Sevransky, CL Sprung, ME Nunnally, B Rochweg, GD Rubenfeld, DC Angus, D Annane, RJ Beale, GJ Bellingham, GR Bernard, JD Chiche, C Coopersmith, DP De Backer, CJ French, S Fujishima, H Gerlach, JL Hidalgo, SM Hollenberg, AE Jones, Karnad DiR, RM Kleinpelt, Y Koh, TC Lisboa, FR MacHado, JJ Marini, JC Marshall, JE Mazuski, LA McIntyre, AS McLean, S Mehta, RP Moreno, J Myburgh, P Navalesi, O Nishida, TM Osborn, A Perner, CM Plunkett, M Ranieri, CA Schorr, MA Seckel, CW Seymour, L Shieh, KA Shukri, SQ Simpson, M Singer, BT Thompson, SR Townsend, T Van Der Poll, JL Vincent, WJ Wiersinga, JL Zimmerman, RP Dellinger, Surviving sepsis campaign: international guidelines for management of sepsis and septic shock: 2016, *Crit. Care Med.* (2017) [PMID: 28098591 DOI: 10.1097/CCM.0000000000002255].
- [24] NR Aggarwal, LS King, FR D'Alessio, Diverse macrophage populations mediate acute lung inflammation and resolution, *Am. J. Physiol. - Lung Cell. Mol. Physiol.* (2014) [DOI: 10.1152/ajplung.00341.2013].
- [25] AE Vaughan, AN Brumwell, Y Xi, JE Gotts, DG Brownfield, B Treutlein, K Tan, Y Tan, FC Liu, MR Looney, MA Matthay, JR Rock, HA. Chapman, Lineage-negative progenitors mobilize to regenerate lung epithelium after major injury, *Nature* (2015) [PMID: 25533958 DOI: 10.1038/nature14112].
- [26] AM Zaki, S Van Boheemen, TM Bestebroer, ADME Osterhaus, RAM. Fouchier, Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia, *N Engl J Med* (2012) [DOI: 10.1056/NEJMoa1211721].
- [27] TJ Franks, PY Chong, P Chui, JR Galvin, RM Lourens, AH Reid, E Selbs, PL McEvoy, DL Hayden, J Fukuoka, JK Taubenberger, WD. Travis, Lung pathology of severe acute respiratory syndrome (SARS): a study of 8 autopsy cases from Singapore, *Hum Pathol* (2003) [DOI: 10.1016/S0046-8177(03)00367-8].
- [28] JM Nicholls, LLM Poon, KC Lee, WF Ng, ST Lai, CY Leung, CM Chu, PK Hui, KL Mak, W Lim, KW Yan, KH Chan, NC Tsang, Y Guan, KY Yuen, JSM. Peiris, Lung pathology of fatal severe acute respiratory syndrome, *Lancet* (2003) [DOI: 10.1016/S0140-6736(03)13413-7].
- [29] DM Hwang, DW Chamberlain, SM Poutanen, DE Low, SL Asa, J. Butany, Pulmonary pathology of severe acute respiratory syndrome in Toronto, *Mod Pathol* (2005) [DOI: 10.1038/modpathol.3800247].
- [30] EAJ Hoste, CDVK Roossens, S Bracke, JMA Decruyenaere, DD Benoit, KHDK Vandewoude, FA. Colardyn, Acute effects of upright position on gas exchange in patients with acute respiratory distress syndrome, *J Intensive Care Med* (2005) [DOI: 10.1177/0885066604271616].
- [31] P Zhou, Yang X Lou, XG Wang, B Hu, L Zhang, W Zhang, HR Si, Y Zhu, B Li, CL Huang, HD Chen, J Chen, Y Luo, H Guo, Jiang R Di, MQ Liu, Y Chen, XR Shen, X Wang, XS Zheng, K Zhao, QJ Chen, F Deng, LL Liu, B Yan, FX Han, YY Wang, GF Xiao, ZL Shi, A pneumonia outbreak associated with a new coronavirus of probable bat origin, *Nature* (2020) [PMID: 32015507 DOI: 10.1038/s41586-020-2012-7].
- [32] C Wu, X Chen, Y Cai, J Xia, X Zhou, S Xu, H Huang, L Zhang, X Zhou, C Du, Y Zhang, J Song, S Wang, Y Chao, Z Yang, J Xu, X Zhou, D Chen, W Xiong, L Xu, F Zhou, J Jiang, C Bai, J Zheng, Y. Song, Risk factors associated with acute respiratory distress syndrome and death in patients with coronavirus disease 2019 pneumonia in Wuhan, China, *JAMA Intern Med* (2020) [PMID: 32167524 DOI: 10.1001/jamainternmed.2020.0994].
- [33] C Huang, Y Wang, X Li, L Ren, J Zhao, Y Hu, L Zhang, G Fan, J Xu, X Gu, Z Cheng, T Yu, J Xia, Y Wei, W Wu, X Xie, W Yin, H Li, M Liu, Y Xiao, H Gao, L Guo, J Xie, G Wang, R Jiang, Z Gao, Q Jin, J Wang, B Cao, Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China, *Lancet* (2020) [PMID: 31986264 DOI: 10.1016/S0140-6736(20)30183-5].
- [34] JO Ebbert, AH. Limper, Respiratory syncytial virus pneumonitis in immunocompromised adults: clinical features and outcome, *Respiration* (2005) [DOI: 10.1159/000085367].
- [35] PW Harms, LA Schmidt, LB Smith, DW Newton, MA Pletneva, LL Walters, SA Tomlins, A Fisher-Hubbard, LM Napolitano, PK Park, M Blaivas, J Fantone, JL Myers, JM. Jentzen, Autopsy findings in eight patients with fatal H1N1 influenza, *Am J Clin Pathol* (2010) [DOI: 10.1309/AJCP35KOZSAVNQZ].
- [36] NT Liem, N Nakajima, LP Phat, Y Sato, HN Thach, PV Hung, LT San, H Katano, T Kumasaka, T Oka, S Kawachi, T Matsushita, T Sata, K Kudo, K. Suzuki, H5N1-infected cells in lung with diffuse alveolar damage in exudative phase from a fatal case in Vietnam, *Jpn J Infect Dis* (2008).
- [37] S Suhail, J Zajac, C Fossom, H Lowater, C McCracken, N Severson, B Laatsch, A Narkiewicz-Jodko, B Johnson, J Liebau, S Bhattacharyya, S. Hati, Role of oxidative stress on SARS-CoV (SARS) and SARS-CoV-2 (COVID-19) infection: a review, *Protein J* (2020) [PMID: 33106987 DOI: 10.1007/s10930-020-09935-8].
- [38] JD. Crapo, Oxidative stress as an initiator of cytokine release and cell damage, *In: Eur. Respir. J., Suppl.* (2003).
- [39] EM Bulger, RV. Maier, Antioxidants in critical illness, *Arch. Surg.* (2001) [PMID: 11585516 DOI: 10.1001/archsurg.136.10.1201].
- [40] S.A. Capellini, Role of renin-angiotensin system in inflammation, immunity and aging, *Curr. Pharm. Des.* (2012) [PMID: 22283774 DOI: 10.2174/138161212799436593].
- [41] K Kuba, Y Imai, S Rao, H Gao, F Guo, B Guan, Y Huan, P Yang, Y Zhang, W Deng, L Bao, B Zhang, G Liu, Z Wang, M Chappell, Y Liu, D Zheng, A Leibbrandt, T Wada, AS Slutsky, D Liu, C Qin, C Jiang, JM. Penninger, A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus-induced lung injury, *Nat. Med.* (2005) [PMID: 16007097 DOI: 10.1038/nm1267].
- [42] X Ji, W Tan, C Zhang, Y Zhai, Y Hsueh, Z Zhang, C Zhang, Y Lu, B Duan, G Tan, R Na, G Deng, G. Niu, TWIRLS, a knowledge-mining technology, suggests a possible mechanism for the pathological changes in the human host after coronavirus infection via ACE2, *Drug. Dev. Res.* (2020) [PMID: 32657473 DOI: 10.1002/ddr.21717].
- [43] AS Slutsky, VM. Ranieri, Ventilator-induced lung injury, *N. Engl. J. Med.* (2013) [PMID: 24283226 DOI: 10.1056/NEJMra1208707].
- [44] MJD Griffiths, TW. Evans, Inhaled nitric oxide therapy in adults, *N. Engl. J. Med.* (2005) [DOI: 10.1056/NEJMra051884].
- [45] AJ Boyle, RM Sweeney, DF. McAuley, Pharmacological treatments in ARDS: a state-of-the-art update, *BMC Med* (2013) [DOI: 10.1186/1741-7015-11-166].
- [46] F Raghu, M Berk, PA Campochiaro, H Jaeschke, G Marenzi, L Richeldi, F-Q Wen, G Nicoletti, PMA. Calverley, The multifaceted therapeutic role of N-Acetylcysteine (NAC) in disorders characterized by oxidative stress, *Curr. Neuropharmacol.* (2020) [DOI: 10.2174/1570159x19666201230144109].
- [47] MA Darenkaya, LI Kolesnikova, SI. Kolesnikov, COVID-19: oxidative stress and the relevance of antioxidant therapy, *Ann. Russ. Acad. Med. Sci.* (2020) [DOI: 10.15690/vramn1360].
- [48] TM. Setti, The role of Glutathione as an adjunct therapy in the treatment of patients with COVID-19-Related acute respiratory syndrome, *Adv. Res. J. Med. Clin. Sci.* (2021) [DOI: 10.15520/arjms.v7i02.247].
- [49] Gropper SS, Smith JL. *Advanced Nutrition and Human Metabolism.* 2013.
- [50] Kidd PM. Glutathione, Systemic protectant against oxidative and free radical damage, *Altern. Med. Rev.* (1997).
- [51] BM Lomaestro, M. Malone, Glutathione in health and disease: Pharmacotherapeutic issues, *Ann. Pharmacother.* (1995) [DOI: 10.1177/106002809502901213].
- [52] I Rahman, W. MacNee, Oxidative stress and regulation of glutathione in lung inflammation, *Eur. Respir. J.* (2000) [PMID: 11028671 DOI: 10.1034/j.1399-3003.2000.016003534.x].
- [53] JH Routh, Z Borok, NG McElvaney, GJ Grimes, AD Bokser, R Buhl, RG. Crystal, Glutathione aerosol suppresses lung epithelial surface inflammatory cell-derived oxidants in cystic fibrosis, *J Appl Physiol* (1999) [DOI: 10.1152/jappl.1999.87.1.438].
- [54] J Behr, B Degenkolb, T Beinert, F Krombach, C. Vogelmeier, Pulmonary glutathione levels in acute episodes of farmer's lung, *Am. J. Respir. Crit. Care Med.* (2000) [DOI: 10.1164/ajrcm.161.6.9907112].
- [55] FL Poe, Corn J. N-Acetylcysteine, A potential therapeutic agent for SARS-CoV-2, *Med. Hypotheses* (2020) [PMID: 32504923 DOI: 10.1016/j.mehy.2020.109862].
- [56] N Zhou, X Yang, A Huang, Z. Chen, The potential mechanism of N-acetylcysteine in treating COVID-19, *Curr. Pharm. Biotechnol.* (2020) [DOI: 10.2174/1389201021999201228212043].
- [57] S Dodd, O Dean, DL Copolov, GS Malhi, M. Berk, N-acetylcysteine for antioxidant therapy: pharmacology and clinical utility, *Expert. Opin. Biol. Ther.* (2008) [PMID: 18990082 DOI: 10.1517/14728220802517901].
- [58] DJ. Pierson, Clinical practice guidelines for chronic obstructive pulmonary disease: A review and comparison of current resources, *Respir. Care* (2006) [PMID: 16533418].
- [59] EM Grandjean, P Berthet, R Ruffmann, P. Leuenberger, Efficacy of oral long-term N-acetylcysteine in chronic bronchopulmonary disease: a meta-analysis of pub-

- lished double-blind, placebo-controlled clinical trials, *Clin. Ther.* (2000) [DOI: 10.1016/S0149-2918(00)88479-9].
- [60] JE Repine, A Bast, I. Lankhorst, Oxidative stress in chronic obstructive pulmonary disease, *Am. J. Respir. Crit. Care Med.* (1997) [PMID: 9279209 DOI: 10.1164/ajrccm.156.2.9611013].
- [61] J. Prousky, The treatment of pulmonary diseases and respiratory-related conditions with inhaled (nebulized or aerosolized) glutathione. Evidence-based Complement, *Altern. Med.* (2008) [DOI: 10.1093/ecam/nem040].
- [62] A Witschi, S Reddy, B Stofer, BH. Lauterburg, The systemic availability of oral glutathione, *Eur. J. Clin. Pharmacol.* (1992) [PMID: 1362956 DOI: 10.1007/BF02284971].
- [63] R Buhl, C Vogelmeier, M Critenden, RC Hubbard, RF Hoyt, EM Wilson, AM Cantin, RG. Crystal, Augmentation of glutathione in the fluid lining the epithelium of the lower respiratory tract by directly administering glutathione aerosol, *Proc. Natl. Acad. Sci. U S A* (1990) [DOI: 10.1073/pnas.87.11.4063].
- [64] VA Selvan, SHS Calvert, G Cavell, E Glucksman, M Kerins, J. Gonzales, Weight-based N-acetylcysteine dosing chart to minimise the risk of calculation errors in prescribing and preparing N-acetylcysteine infusions for adults presenting with paracetamol overdose in the emergency department, *Emerg. Med. J.* (2007) [PMID: 17582039 DOI: 10.1136/emj.2006.043141].
- [65] DF. Rogers, Mucoactive agents for airway mucus hypersecretory diseases, *Respiratory Care*, 2007.
- [66] R Tirouvanziam, CK Conrad, T Bottiglieri, LA Herzenberg, RB Moss, LA. Herzenberg, High-dose oral N-acetylcysteine, a glutathione prodrug, modulates inflammation in cystic fibrosis, *Proc. Natl. Acad. Sci. USA* (2006) [PMID: 16537378 DOI: 10.1073/pnas.0511304103].
- [67] MR. Holdiness, Clinical Pharmacokinetics of N-Acetylcysteine, *Clin Pharmacokinet* (1991) [PMID: 2029805 DOI: 10.2165/00003088-199120020-00004].
- [68] R He, W Zheng, T Ginman, H Ottosson, S Norgren, Y Zhao, M. Hassan, Pharmacokinetic profile of N-acetylcysteine amide and its main metabolite in mice using new analytical method, *Eur. J. Pharm. Sci.* (2020) [PMID: 31740394 DOI: 10.1016/j.ejps.2019.105158].
- [69] PN Mardikian, SD LaRowe, S Hedden, PW Kalivas, RJ. Malcolm, An open-label trial of N-acetylcysteine for the treatment of cocaine dependence: a pilot study, *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* (2007) [PMID: 17113207 DOI: 10.1016/j.pnpbp.2006.10.001].
- [70] M Berk, DL Copolov, O Dean, K Lu, S Jeavons, I Schapkaitz, M Anderson-Hunt, AI Bush, N-Acetyl cysteine for depressive symptoms in bipolar disorder-a double-blind randomized placebo-controlled trial, *Biol. Psychiatry* (2008) [PMID: 18534556 DOI: 10.1016/j.biopsych.2008.04.022].
- [71] M Berk, D Copolov, O Dean, K Lu, S Jeavons, I Schapkaitz, M Anderson-Hunt, F Judd, F Katz, P Katz, S Ording-Jespersen, J Little, P Conus, M Cuenod, KQ Do, AI Bush, N-Acetyl cysteine as a glutathione precursor for Schizophrenia-A double-blind, randomized, placebo-controlled trial, *Biol. Psychiatry* (2008) [PMID: 18436195 DOI: 10.1016/j.biopsych.2008.03.004].
- [72] RI Horowitz, PR Freeman, J. Bruzzese, Efficacy of glutathione therapy in relieving dyspnea associated with COVID-19 pneumonia: a report of 2 cases, *Respir. Med. Case Rep.* (2020) [DOI: 10.1016/j.rmcr.2020.101063].
- [73] DW Han, W Ji, JC Lee, SY Song, CM Choi, Efficacy of nebulized acetylcysteine for relieving symptoms and reducing usage of expectorants in patients with radiation pneumonitis, *Thorac Cancer* (2019) [PMID: 30585684 DOI: 10.1111/1759-7714.12938].
- [74] C Volgers, BJ Benedikter, GE Grauls, PHM Hellebrand, PHM Savelkoul, FRM. Stassen, Effects of N-acetyl-L-cysteine on the membrane vesicle release and growth of respiratory pathogens, *FEMS Microbiol. Lett.* (2017) [PMID: 28444395 DOI: 10.1093/femsle/fnx087].
- [75] SM Masoompour, A Anushiravani, AT. Norouz, Evaluation of the effect of nebulized n-acetylcysteine on respiratory secretions in mechanically ventilated patients: randomized clinical trial, *Iran J. Med. Sci.* (2015) [PMID: 26170516].
- [76] A. Polonikov, Endogenous deficiency of glutathione as the most likely cause of serious manifestations and death in COVID-19 patients, *ACS Infect. Dis.* (2020) [PMID: 32463221 DOI: 10.1021/acsinfectdis.0c00288].