Anti-Inflammatory and Antioxidant Response in COVID-19 Infection: Nrf2/HO-1 Pathway

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Abstract

SARS-CoV-2 virus infection starts with the internalization of the viral particle into the host cells, mainly the upper respiratory system epithelial cells which have the highest expression of the ACE2 receptor which is essential for the internalization process. The pathophysiology of severe forms of COVID-19 disease results not only from direct, cytopathic viral effect but also from immune response dysregulation of the host resulting in hyperinflammatory state and oxidative stress. The nuclear factor erythroid 2-related factor 2 (Nrf2) ability to protect cells and induce a rapid anti-inflammatory and antioxidant response primarily depends on its constitutive cellular expression, which can be affected by numerous endogenous and exogenous factors. The binding of Nrf2 to cellular receptors leads to the transcription of a large number of genes encoding various antioxidant enzymes and other cytoprotective molecules, including heme oxygenase-1(HO-1). Activation of HO-1 results in antioxidant, anti-inflammatory and anti-apoptotic effects. Based on previous studies, the Nrf2/HO-1 pathway provides protection against oxidative stress and inflammatory and immune response which is significant in COVID-19 infection, which is characterized by a strong hyperinflammatory response. This narrative review aims to describe the role of the hyperinflammatory response in the development of COVID-19 infection, with a focus on the NrF2/HO-1 pathway.

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Introduction

Coronaviruses belong to the RNA viruses group with the largest genome among the rest of the RNA viruses (27–37 kb). Virus particles have a characteristic membrane with spikey structured proteins on the outer surface, which is where the name coronavirus (lat. corona, crown) comes from. Three groups of coronaviruses differ genotypically, of which viruses from group III are found only in birds, while viruses from groups I and II are also found in mammals. Coronaviruses mainly cause respiratory system diseases in humans, and at least four variants continuously circulate within the population, especially among young children. Two variants were identified in the mid-1960s (hCoV-OC43 and hCoV-229E), and the other two in the early 2000s (hCoV-NL-63 and hCoV-HKU1). The first coronavirus linked with a more serious illness was identified at the end of 2002. Due to its tendency to cause severe acute respiratory syndrome (SARS), it was named SARS-CoV. It's believed to be of wild animals origin, most likely bats, and was transmitted to humans via infected bats that were prepared and sold as food in China [1]. The SARS-CoV epidemic has affected 29 countries, 8.096 cases were registered, and 774 deaths were confirmed, according to which the estimated mortality was 9.6%. Ten years later, in April 2012., another coronavirus associated with severe forms of respiratory infections emerged, and due to its Middle Eastern origin, this strain has been named MERS-CoV (Middle East Respiratory Syndrome). Concurrently with the sporadic isolation of MERS-CoV, in December 2019., a new coronavirus named SARS-CoV-2 was isolated in the city of Wuhan, China. and rapidly spread, not only in China but also in other continents, therefore becoming the first coronavirus that caused a global pandemic[2,3].

Disease Background

Despite numerous research, the SARS-CoV-2 origin remains unclear. The most common theory of this virus origin is associated with bats, which are natural reservoirs of the virus, especially considering the high homology of the genome of viruses isolated from bats with the human SARS-CoV-2 virus, which amounts to 96.2%. Initially, infections of humans by the SARS-CoV-2 virus appeared as a classic zoonosis since most of the first confirmed coronavirus cases had direct contact with animals at the market in Wuhan. The first patients were believed to be infected by consuming infected animals that were prepared as food or sold alive, with bats being the most frequently involved [4]. The rapid spread and appearance of the disease among healthcare workers and people who did not have direct contact with infected animals indicated human-to-human transmission. Since it's mainly a respiratory disease and viruses are isolated from the epithelial cells of the respiratory tract, it can be assumed that the main transmission route of the virus between people is droplet transmission, i. e. through aerosols created when speaking, sneezing or coughing [6,5]. Although SARS-CoV-2 has been detected in urine, stool, blood/serum, saliva, and tears, there is no strong evidence that it can be transmitted in such a manner.

Structure and pathogenesis of SARS-CoV-2 infection

The SARS-CoV-2 genome size is 29.9 kb, and two-thirds of the genome encodes proteins necessary for virus replication. One-third encodes structural proteins: protein E (envelope protein), protein S (spike protein), protein M (membrane protein), and protein N (nucleocapsid protein). Proteins S, M, and E form the virus particle envelope, and protein N, which binds the RNA molecule to itself, is found inside the virus particle [7,8]. The SARS-CoV-2 genome is highly variable, resulting from errors that occur during RNA molecule replication, whereby they accumulate and create new mutations, resulting in the emergence of new viral subvariants or strains. To date, more than 20 strains of this virus have been described. As some mutations cause pathogenicity or virulence modifications (either positive or negative ones), the World Health Organization identifies five variants of concern (VOCs), given their public health impact: alpha (B.1.1.7) which was originally isolated in the UK in late December 2020., beta (B.1.351), which was

first reported in South Africa in December 2020., gamma (P.1), which was first reported in early January 2021. In Brazil, delta (B.1.617.2), first reported in December 2020. In India and omicron (B.1.1.529), first reported in South Africa in November 2021. [9]. Both pathogenicity and virulence of the SARS-CoV-2 result from its ability of cellular internalization, i.e., the ability of the virus to enter cells. The entry of the SARS-CoV-2 virus into the cells is mediated by the S protein of the virus particle and the ACE2 receptor (Angiotensin-Converting Enzyme 2) of the host cells. ACE2 receptor was identified in 2003. and the SARS-CoV-2 and other coronaviruses (hCoV-229E, hCoV-OC43 and hCoV-HKU1) are uptaken via aforementioned receptor. Protein S contains two subunits: the S1 subunit enables viral particle binding to the ACE2 receptor via the receptor-binding domain (RBD), while the S2 subunit enables the fusion of the viral particle and the host cell membranes [10]. In addition to the ACE2 receptor, integrins and CD174-SP are also mentioned as potential receptors for the internalization of SARS-CoV-2 into host cells. There is no strong experimental evidence for these assumptions [11,12]. Since ACE2 has a wide biodistribution in the organism, including the respiratory system, digestive system (small and large intestine), myocardium, kidneys, thyroid gland, liver, brain, and olfactory neuroepithelium, SARS-CoV-2 has a broad tissue and cell tropism, resulting in wide clinical presentation and a clinically diverse manifestation [13]. Despite the broad distribution of these receptors, the respiratory system is predominantly and most severely affected by this viral infection. This can be explained by the fact that the respiratory system epithelial cells have the largest expression of ACE2 receptors, as well as the fact that the main transmission route of the disease is droplet, and the respiratory system mucous membrane is the initial site for the virus entrance into the body. Epithelial cells of nasal mucosa and bronchial epithelial cells are the site of the largest ACE2 receptor expression, while in the alveoli the ACE2 receptor is found only in type II pneumocytes [14]. Upregulated expression of the ACE2 receptor in the respiratory system epithelial cells is affected by proinflammatory cytokines that are produced during infection, especially IL-1β and type I and III interferons, which can accelerate its spread and replication [15].

Clinical characteristics COVID-19 infection

The respiratory system is the main organ system affected by this disease, but due to broad SARS-CoV-2 virus tropism, other clinical manifestations are possible as the result of other tissues and organs involvement. It's a highly contagious illness that has a wide clinical spectrum, that varies from asymptomatic to mild, severe, and critical (29). Clinically, COVID-19 infection is most often presented as a common cold or a flu-like illness, which in certain individuals can progress to more severe forms, leading to pneumonia and acute respiratory distress syndrome, and also to manifestations that are caused by other tissues and organs involvement [16]. Acute respiratory distress syndrome (ARDS) represents the most severe form of the respiratory system involvement by the SARS-CoV-2 virus and is accompanied by a high rate of severe complications such as sepsis, septic shock, and multiorgan failure, all of which increase the mortality rate. ARDS is characterized by new-onset severe respiratory insufficiency and bilateral pulmonary infiltrates, which occurs as a result of still insufficiently elaborated pathophysiological mechanisms that include the direct viral cytopathic effect, immune response dysregulation (hyperinflammatory state or cytokine storm) as well as increased oxidative stress. The final result is extensive lung parenchyma damage and consequently severely impaired oxygen and carbon dioxide exchange (hypoxemia, hypercapnia) [17]. As previously discussed, SARS-CoV-2 predominantly affects the respiratory system, but other organs and organ systems can also be affected, either as a single organ disease, but most common with the respiratory system involvement. The most common extrapulmonary COVID-19 manifestations involve gastrointestinal and hepatobiliary, hematological, cardiac, renal, and neurological systems [16].

Immune response in COVID-19 infection

SARS-CoV-2 virus infection starts by the internalization of the viral particle into the host cells, mainly the upper respiratory system epithelial cells which have the highest expression of the ACE2 receptor which is essential for the internalization process. The viral entry into the cells triggers an immune response whose role is to neutralize the virus and bring the infection under the control. Today, it's considered that the pathophysiology of severe forms of COVID-19 infection results not only from direct, cytopathic viral effect but also from immune response dysregulation of the host resulting in hyperinflammatory state and oxidative stress [18]. By viral particle entrance and the viral RNA release in the host cell cytoplasm begins a cellular response, in addition to the process of virus replication. The cellular response aims to activate the immune response as a protective mechanism of the host against the virus [19]. In this process PRR receptors (Pattern recognition receptors) are essential. These are found in epithelial cells cytoplasm and have role of recognizing PAMP molecules (Pathogen-associated molecular patterns) [20]. These PRR receptors recognize long chains of viral RNA that are formed as intermediates during replication, causing their activation and triggering signaling pathways that include interferon regulatory factor (IRF) and nuclear factor-κB (NF-κB) [21]. IRF promotes the transcription of interferons I and III, while NF-κB promotes the transcription of pro-inflammatory cytokines and chemokines [22]. Interferons, pro-inflammatory cytokines, and chemokines are responsible for chemotaxis and mutual interaction of innate immunity cells: neutrophils, monocytes that differentiate into macrophages, dendritic cells and NK cells. They form the first line of defense of the organism against the SARS-CoV-2 virus. In addition to innate immunity cells, T and B lymphocytes also participate in the immune response to the SARS-CoV-2 virus. After phagocytosis of infected epithelial cells, antigen presenting cells (APC), among which respiratory dendritic cells dominate move to regional lymph nodes and present there the viral antigen, which is expressed on their surface

trough molecules of the MHC complex (Major histocompatibility complex), to the naïve circulating T lymphocytes. Viral antigens are recognized by T-cell receptors (TCR) of naïve T lymphocytes, and as a result, their interaction leads to their sensitization, whereby they are activated, proliferate, and migrate to the infection localization. At the infection localization activated cytotoxic T lymphocytes (CD8+) produce antiviral cytokines (INF-γ, TNF-α, IL-2) that inhibit viral replication, chemokines (CXCL-9, 10 and 11) that enhance the chemotaxis of other effector T lymphocytes and cytotoxic molecules (perforin, granzyme B) that kill infected cells, thus trying to eliminate the virus. In addition to classic CD4+ and CD8+ lymphocytes, the so-called unconventional lymphocytes T (uT) that accumulate in the mucous membranes, especially of the respiratory system, are also a part of immune reaction against the SARS-CoV-2 virus. They represent a heterogeneous T lymphocytes group that can recognize non-peptide viral antigens and are not limited to the presentation of antigens via the MHC system like the classic lymphocytes [23]. The humoral immune response is the most effective and long-lasting immune mechanism in terms of host defense against viruses, including the SARS-CoV-2 virus. It's based on the interaction of naïve B lymphocytes with antigen and CD4+ T lymphocytes, the ultimate consequence of which is maturation into plasma cells and the production of specific antibodies [24]. In the host's battle with the SARS-CoV-2 virus antibodies targeted at the RBD domain of the S protein are the most effective because they most successfully block the binding of the virus particle to the ACE2 receptor on the cell surface [25].

Hyperinflammatory immune response and cytokine storm in COVID-19 infection

Immune system dysregulation in response to the presence of the SARS-CoV-2 virus, and not only the cytopathic effect of the virus, is responsible for development of more severe clinical forms of the COVID-19 infection [18]. Hyperinflammatory response is a condition

resulting from uncontrolled activation of the immune system, which is primarily manifested by increased production and release of pro-inflammatory cytokines, which is known in clinical practice as a cytokine storm or cytokine release syndrome. The current focus in the development of hyperinflammatory syndrome pertains to dysregulation of the host's innate immunity response cells in the presence of the SARS-CoV-2 virus [26]. The basis of this theory is pyroptosis, the programmed cell death of infected cells, which is activated during viral RNA replication. This is why pyroptosis is also known as an inflammatory form of programmed cell death. During pyroptosis, various molecules that can function as stimulators of innate immunity cells are released [27,28]. Among these is IL-1β, whose high levels are detected in the serum and bronchoalveolar lavage of patients suffering from severe forms of COVID-19 disease [29]. By disruption of infected cells during pyroptosis parts of viral RNA that act as PAMP molecules (Pathogen-associated molecular patterns) and other viral proteins are released. These are recognized by previously described PRR receptors of the surrounding cells and trough the IL-1R signaling pathway stimulate transcription and production of proinflammatory cytokines such as IL-6, IL-8, IL-10, TNF-α, INF-γ, MCP, MIP and others. They lead to chemotaxis of macrophages and other effector cells of innate immunity as well as T lymphocytes which further enhance the immune response, cause local damage of cells and tissues and additionally produce cytokines resulting in constant amplification and stimulation of the immune response. Finally, produced cytokines also enter the circulation and can cause damage in distant tissues and organs, which explains the occurrence of multiorgan failure in critical patients with COVID-19 [30,31]. The hyperinflammatory state or cytokine storm is considered one of the main factors in the development of the most severe forms of COVID-19 infection, which is acute respiratory distress syndrome (ARDS) and associated multiorgan failure [32]. Multiorgan failure is promoted by activation of endothelial cells mediated by TNF-α and IL-1β, which brings to increased expression of selectin P,

fibrinogen and von Willebrand factor, as well as induced tissue factor release, which initiates the coagulation cascade and the formation of clots, especially at microcirculatory level [33].

Oxidative stress in COVID-19 infection

The production of reactive oxygen species and oxidative stress is one type of the host response to the presence of viruses in its cells and is part of the overall defense against infection, due to active participation of reactive oxygen species in elimination of pathogens. Also, several reactive oxygen species are involved in intercellular and intracellular signaling pathways of the immune system cells [34]. There are several assumed mechanisms of increased reactive oxygen species concentration in COVID-19 infection. The general mechanism is based on the renin-angiotensin-aldosterone system, that is, the interaction of angiotensin II and NADPH oxidase. Angiotensin II is known as a stimulator of NADPH oxidase, which generates reactive oxygen species and oxidative stress. Under physiological conditions, ACE2 catalyzes the conversion of angiotensin II to angiotensin, which doesn't affect NADPH oxidase, but moreover, has an antioxidative effect. During the SARS-CoV-2 virus infection, the availability of "free" ACE2 decreases due to its binding to the virus and/or entry into cells. The result is an increased concentration of angiotensin II and NADPH oxidase stimulation, which causes an increased production of reactive oxygen species and oxidative stress [35,36]. Therefore, the inflammatory reaction intensity and the degree of innate immune response dysregulation (excessive activation of monocyte-macrophage system cells) are proportional to oxidative stress level, and reactive oxygen species together with proinflammatory cytokines lead to the cell-tissue damage present in patients suffering from severe forms of COVID-19.

Nrf2/HO-1 signaling pathway in COVID-19 infection

The relation between the hyperinflammatory state and oxidative stress represents a great challenge in patients suffering from COVID-19

infection, especially in terms of discovering molecular pathways that could potentially be therapeutic targets and whose modulation could reduce the inflammatory response and oxidative stress, essential elements in the development of severe forms of COVID-19 disease (Figure 1).
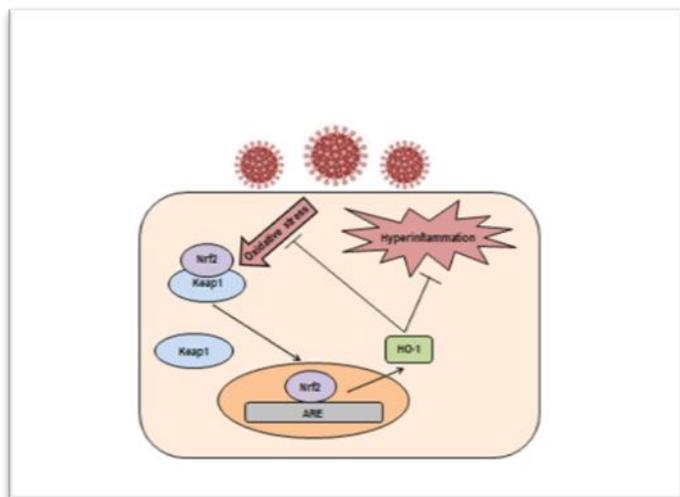

Figure 1. Nrf2/HO-1 signaling pathway in COVID-19 infection

The transcriptional factor Nrf2 (Nuclear factor erythroid 2-related factor 2, Nrf2) is one of the most frequently researched molecules in terms of the cellular anti-inflammatory and antioxidant response in viral infections. The ability of Nrf2 to protect cells and induce a rapid anti-inflammatory and antioxidant response primarily depends on its constitutive cellular expression, which can be affected by numerous endogenous and exogenous factors [37]. The basic binding site of Nrf2 is the promoter region of DNA, ARE region (antioxidant response element), responsible for the transcription of genes whose products are part of the antioxidant response [38]. The binding of Nrf2 to the ARE region leads to the transcription of a large number of genes encoding various antioxidant enzymes and other cytoprotective molecules. These are enzymes that participate in the metabolism of iron and heme (heme oxygenase-1), enzymes that participate in the direct neutralization of reactive oxygen species (NADPH quinone reductase, superoxide dismutase, catalase, glutathione peroxidase, glutathione transferase), as well as enzymes that participate in the synthesis and regeneration of glutathione and NADPH (glutamate cysteine ligase, glutathione reductase, glucose-6-phosphate dehydrogenase) [39]. In addition to the previously discussed effect of Nrf2 by binding to the ARE region, there are also findings of its direct anti-inflammatory effect, independent of gene expression. Kobayashi et al. described in their research that Nrf2 can reduce the expression of pro-inflammatory cytokines (IL-1, IL-6) in activated macrophages by directly inhibiting their transcription in the cell nucleus [40]. Heme oxygenase-1 (HO-1) is commonly researched enzyme that is produced as a result of the interaction of Nrf2 and the ARE region. It is involved in the catabolism of pro-oxidant heme, which results in numerous effects, including antioxidant, anti-inflammatory and anti-apoptotic effects. HO-1 catalyzes the reaction that breaks down heme and produces iron (Fe^{2+}), which is stored in the cell in the form of ferritin, carbon monoxide (CO) and biliverdin. Biliverdin is quickly converted by biliverdin reductase into bilirubin, which has higher electrophilicity than biliverdin and a stronger antioxidant effect. In addition to heme degradation products, HO-1 also has direct beneficial effects that may have certain implications for the development of the COVID-19 infection. The antiviral effect of HO-1 is based on several mechanisms. The basic mechanism involves an increase in the interferon I level, which has been proven to be suppressed in cells infected with the SARS-CoV-2 virus. Interferon I, in addition to interfering with viral RNA replication, also enhances the cytolytic effect of innate immunity cells against cells infected with the SARS-CoV-2 virus [41]. Studies on animal models also indicate a direct anti-inflammatory effect of HO-1 mediated by a reduction in the production of pro-inflammatory cytokines [42]. Considering the antioxidant, anti-inflammatory and antiviral effects of Nrf2, its reduced concentration can result in increased oxidative stress, enhanced immune response, as well as faster and uncontrolled virus replication [40,43].

Clinical implications of NrF2/HO-1 pathway in COVID-19 infection, possible therapeutic approach

Research shows that dysregulation of the immune system and increased expression of oxidative stress are essential in the development of the most severe forms of the COVID-19 infection [26,44], therefore reduced Nrf2 values result in a worse clinical course and severe clinical forms of the disease. The key question research has addressed is the mechanisms of Nrf2 activation. A possible explanation is that the Nrf2 expression is mainly affected by endogenous or exogenous stimulus, and not by its spontaneous transcription because its expression is inhibited in basal conditions, which is why its activation is also one of the cellular defense mechanisms [45]. Viremia acts as a direct activator of Nrf2, and it could be expected that as a result, the serum concentrations of Nrf2 are higher in viral infections [46]. The activation of Nrf2 results in antiviral activity, some authors assume that its lower values in viral infections, especially in severe ones, may also be the result of a direct viral effect on its reduced expression in order to insure a smoother replication [38]. The presence of complications such as bacteriemia and sepsis can inhibit Nrf2 activation [47-49]. This is in line with research conducted on patients with COVID-19 infection [50-52] in which patients with the most severe form of the disease have lower Nrf2 values. When analyzing other exogenous activators of Nrf2, 1,25-dihydroxy vitamin D should be pointed out in COVID-19 infection. In line with numerous studies lower values of 1,25-dihydroxy vitamin D are associated with a worse outcome, a more severe clinical picture, considering that a lower value of the vitamin is correlated with reduced Nrf2 activation. As proven in numerous studies, in patients with COVID-19 infection, Nrf2 is affected by the oxygen therapy, which leads to increased oxidative stress and inflammatory response and has an activating effect on Nrf2 [53-56]. Oxygen administration is required in severe forms of the disease, which results in additional oxidative stress that increases Nrf2 expression. However, patients with the most severe forms of the

disease have lower Nrf2 values. One of the explanations is that the application of mechanical ventilation i.e. longer exposure to FiO_2 results in decreasing in antioxidant and anti-inflammatory effects, and the period and expression of Nrf2 [52,57]. Although for now there is not much clinical research, these have indicated the correlation of serum HO-1 level with worse clinical outcomes and clinical findings [58]. A large amount of evidence, primarily based on animal models, support the idea that Nrf2 and HO-1 can provide protection against oxidative stress as well as inflammatory and immune responses [59]. Many studies indicate the importance of Nrf2 and its equivalents in embryonic development, stress signaling and aging [60]. Moreover, Nrf2-dependent genes, such as HO-1 provide a cytoprotective effect and play a crucial role in the development of oxidative and aging-related disorders. A more detailed analysis of microRNAs involvement in Nrf2/HO-1 regulation could provide new ideas for the treatment of many diseases (malignant, autoimmune). The involvement of Nrf2 in reductive stress in an animal model of protein aggregation cardiomyopathy indicates the contribution of this transcriptional regulator of antioxidant genes in other pathological situations related to unbalanced redox homeostasis. Due to animal models, our knowledge of the Nrf2/HO-1 pathway in controlling physiology and disease progression advanced significantly, but additional clinical research concerning individual pathological conditions is needed. In this review, we systematically presented the immune response as part of the COVID-19 infection. We focused on the development of anti-inflammatory and antioxidant responses, with an emphasis on the Nrf2/HO-1 pathway. It should be emphasized that clinical studies have been described, the connection with the severity of the disease, using mechanical ventilation. The above is intended to encourage future research to consider a therapeutic approach through Nrf2 activation.

Conclusion

One of the important features of COVID-19 infection is hyperinflammatory response and due to it, the role of the Nrf2/HO-1 pathway is extremely important. Previous research proposed many mechanisms that positively or negatively affect the activation of Nrf2 and HO-1, as well as their correlation with inflammatory response markers. All further studies concern the factors that affect its activation, including the exogenous factors, as well as the consequences of the adverse effects of the patient's comorbidities on the activation of the Nrf2/HO-1 pathway.

References

1. Ksiazek TG, Erdman D, Goldsmith CS, Zaki SR, Peret T, Emery S, Tong S, Urbani C, Comer JA, Lim W, Rollin PE, Dowell SF, Ling AE, Humphrey CD, Shieh WJ, Guarner J, Paddock CD, Rota P, Fields B, DeRisi J, Yang JY, Cox N, Hughes JM, LeDuc JW, Bellini WJ, Anderson LJ; SARS Working Group. A novel coronavirus associated with severe acute respiratory syndrome. N Engl J Med. 2003;348(20):1953-66. doi: 10.1056/NEJMoa030781.

2. Azhar EI, Hui DSC, Memish ZA, Drosten C, Zumla A. The Middle East Respiratory Syndrome (MERS). Infect Dis Clin North Am. 2019 Dec;33(4):891-905. doi: 10.1016/j.idc.2019.08.001.

3. Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, Si HR, Zhu Y, Li B, Huang CL, Chen HD, Chen J, Luo Y, Guo H, Jiang RD, Liu MQ, Chen Y, Shen XR, Wang X, Zheng XS, Zhao K, Chen QJ, Deng F, Liu LL, Yan B, Zhan FX, Wang YY, Xiao GF, Shi ZL. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020;579(7798):270-273. doi: 10.1038/s41586-020-2012-7.

4. Li H, Tao Z, Yang Y, Deng Z, Liu B, Ma Z, Zhang Y, Shi G, Lam TTY, Wu JT, Gao GF, Cowling BJ, Yang B, Leung GM, Feng Z. Early Transmission Dynamics in Wuhan, China, of Novel Coronavirus-Infected Pneumonia. N Engl J Med. 2020;382(13):1199-1207. doi: 10.1056/NEJMoa2001316.

5. Ralp R, Lew J, Zeng T. 2019-nCoV (Wuhan virus), a novel Coronavirus: human-to-human transmission, travel-related cases, and vaccine readiness. J Infect Dev Ctries. 2020;14:3–17. doi: 10.3855/jidc.12425.

6. Wong SCY, Kwong RT, Wu TC, Chan JWM, Chu MY, Lee SY, Wong HY, Lung DC. Risk of nosocomial transmission of coronavirus disease 2019: an experience in a general ward setting in Hong Kong. J Hosp Infect. 2020;105:119-27. doi: 10.1016/j.jhin.2020.03.036.

7. Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, Wang W, Song H, Huang B, Zhu N, Bi Y, Ma X, Zhan F, Wang L, Hu T, Zhou H, Hu Z, Zhou W, Zhao L, Chen J, Meng Y, Wang J, Lin Y, Yuan J, Xie Z, Ma J, Liu WJ, Wang D, Xu W, Holmes EC, Gao GF, Wu G, Chen W, Shi W, Tan W. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. Lancet. 2020;395(10224):565-574. doi: 10.1016/S0140-6736(20)30251-8.

8. Wu A, Peng Y, Huang B, Ding X, Wang X, Niu P, Meng J, Zhu Z, Zhang Z, Wang J, Sheng J, Quan L, Xia Z, Tan W, Cheng G, Jiang T. Genome Composition and Divergence of the Novel Coronavirus (2019-nCoV) Originating in China. Cell Host Microbe. 2020;27(3):325-328. doi: 10.1016/j.chom.2020.02.001.

9. Aleem A, Akbar Samad AB, Slenker AK. Emerging Variants of SARS-CoV-2 And Novel Therapeutics Against Coronavirus (COVID-19). In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022. PMID: 34033342.

10. Ou X, Liu Y, Lei X, Li P, Mi D, Ren L, Guo L, Guo R, Chen T, Hu J, Xiang Z, Mu Z, Chen X, Chen J, Hu K, Jin Q, Wang J, Qian Z. Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV. Nat Commun. 2020;11(1):1620. doi: 10.1038/s41467-020-15562-9.

11. Sigrist CJ, Bridge A, Le Mercier P. A potential role for integrins in host cell entry by SARS-CoV-2. Antivir Res. 2020;177:104759. doi: 10.1016/j.antiviral.2020.104759.

12. Wang K, Chen W, Zhang Z, Deng Y, Lian JQ, Du P, Wei D, Zhang Y, Sun XX, Gong L, Yang X, He L, Zhang L, Yang Z, Geng JJ, Chen R, Zhang H, Wang B, Zhu YM, Nan G, Jiang JL, Li L, Wu J, Lin P, Huang W, Xie L, Zheng ZH, Zhang K, Miao JL, Cui HY, Huang M, Zhang J, Fu L, Yang XM, Zhao Z, Sun S, Gu H, Wang Z, Wang CF, Lu Y, Liu YY, Wang QY, Bian H, Zhu P, Chen ZN. CD147-spike protein is a novel route for SARS-CoV-2 infection to host cells. Signal Transduct Target Ther. 2020;5(1):283. doi: 10.1038/s41392-020-00426-x.

13. Wang Y, Wang Y, Luo W, Huang L, Xiao J, Li F, Qin S, Song X, Wu Y, Zeng Q, Jin F, Wang Y. A comprehensive investigation of the mRNA and protein level of ACE2, the putative receptor of SARS-CoV-2, in human tissues and blood cells. Int J Med Sci. 2020;17(11):1522-1531. doi: 10.7150/ijms.46695.

14. Lee IT, Nakayama T, Wu CT, Goltsev Y, Jiang S, Gall PA, Liao CK, Shih LC, Schürch CM, McIlwain DR, Chu P, Borchard NA, Zarabanda D, Dholakia SS, Yang A, Kim D, Chen H, Kanie T, Lin CD, Tsai MH, Phillips KM, Kim R, Overdevest JB, Tyler MA, Yan CH, Lin CF, Lin YT, Bau DT, Tsay GJ, Patel ZM, Tsou YA, Tzankov A, Matter MS, Tai CJ, Yeh TH, Hwang PH, Nolan GP, Nayak JV, Jackson PK. ACE2 localizes to the respiratory cilia and is not increased by ACE inhibitors or ARBs. Nat Commun. 2020;11(1):5453. doi: 10.7150/ijms.46695.

15. Zhuang MW, Cheng Y, Zhang J, Jiang XM, Wang L, Deng J, Wang PH. Increasing host cellular receptor-angiotensin-converting enzyme 2 expression by coronavirus may facilitate 2019-nCoV (or SARS-CoV-2) infection. J Med Virol. 2020;92(11):2693-2701. doi: 10.1002/jmv.26139.

16. Cascella M, Rajnik M, Aleem A, Dulebohn SC, Di Napoli R. Features, Evaluation, and Treatment of Coronavirus (COVID-19). In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022. PMID: 32150360

17. Aslan A, Aslan C, Zolbanin NM, Jafari R. Acute respiratory distress syndrome in COVID-19: possible mechanisms and therapeutic management. Pneumonia (Nathan). 2021;13:14. doi: 10.1186/s41479-021-00092-9.

18. Borczuk AC, Yantiss RK. The pathogenesis of coronavirus-19 disease. J Biomed Sci. 2022;29:87. doi: 10.1186/s12929-022-00872-5.

19. Lamers MM, Haagmans BL. SARS-CoV-2 pathogenesis. Nat Rev Microbiol. 2022;20:270–84. doi: 10.1038/s41579-022-00713-0.

20. Kikkert M. Innate immune evasion by human respiratory RNA viruses. J Innate Immun. 2020;12:4–20. doi: 10.1159/000503030.

21. Wilkins C, Gale M. Recognition of viruses by cytoplasmic sensors. Curr Opin Immunol. 2010;22:41-7. doi: 10.1016/j.coi.2009.12.003.

22. Blanco-Melo D, Nilsson-Payant BE, Liu WC, Uhl S, Hoagland D, Møller R, Jordan TX, Oishi K, Panis M, Sachs D, Wang TT, Schwartz RE, Lim JK, Albrecht RA, tenOever BR. Imbalanced Host Response to SARS-CoV-2 Drives Development of COVID-19. Cell. 2020;181(5):1036-1045.e9. doi: 10.1016/j.cell.2020.04.026.

23. Jouan Y, Guillon A, Gonzalez L, Perez Y, Boisseau C, Ehrmann S, Ferreira M, Daix T, Jeannet R, François B, Dequin PF, Si-Tahar M, Baranek T, Paget C. Phenotypical and functional alteration of unconventional T cells in severe COVID-19 patients. J Exp Med. 2020;217(12):e20200872. doi: 10.1084/jem.20200872.

24. Chen S, Guan F, Candotti F, Benlagha K, Camara NOS, Herrada AA, James LK, Lei J, Miller H, Kubo M, Ning Q, Liu C. The role of B cells in COVID-19 infection and vaccination. Front Immunol. 2022;13:988536. doi: 10.3389/fimmu.2022.988536.

25. Ju B, Zhang Q, Ge J, Wang R, Sun J, Ge X, Yu J, Shan S, Zhou B, Song S, Tang X, Yu J, Lan J, Yuan J, Wang H, Zhao J, Zhang S, Wang Y, Shi X, Liu L, Zhao J, Wang X, Zhang Z, Zhang L. Human neutralizing antibodies elicited by SARS-CoV-2 infection. Nature. 2020;584(7819):115-119. doi: 10.1038/s41586-020-2380-z.

26. Gustine JN, Jones D. Immunopathology of Hyperinflammation in COVID-19. Am J Pathol. 2021;191:4-17. doi: 10.1016/j.ajpath.2020.08.009.

27. Schifanella L, Anderson JL, Galli M, Corbellino M, Lai A, Wieking G, Gryzwacz B, Klatt N.R, Haase A.T, Schacker T.W. Massive viral replication and cytopathic effects in early COVID-19 pneumonia. https://doi.org/10.48550/arXiv.2005.00004 (30.4.2020.)

28. Zhang H, Zhou P, Wei Y, Yue H, Wang Y, Hu M, Zhang S, Cao T, Yang C, Li M, Guo G, Chen X, Chen Y, Lei M, Liu H, Zhao J, Peng P, Wang CY, Du R. Histopathologic Changes and SARS-CoV-2 Immunostaining in the Lung of a Patient With COVID-19. Ann Intern Med. 2020;172(9):629-632. doi: 10.7326/M20-0533.

29. Liao M, Liu Y, Yuan J, Wen Y, Xu G, Zhao J, Cheng L, Li J, Wang X, Wang F, Liu L, Amit I, Zhang S, Zhang Z. Single-cell landscape of bronchoalveolar immune cells in patients with COVID-19. Nat Med. 2020;26(6):842-844. doi: 10.1038/s41591-020-0901-9.

30. Chen G, Wu D, Guo W, Cao Y, Huang D, Wang H, Wang T, Zhang X, Chen H, Yu H, Zhang X, Zhang M, Wu S, Song J, Chen T, Han M, Li S, Luo X, Zhao J, Ning Q. Clinical and immunological features of severe and moderate coronavirus disease 2019. J Clin Invest. 2020;130(5):2620-2629. doi: 10.1172/JCl137244.

31. Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ COVID-19: consider cytokine storm syndromes and immunosuppression. Lancet. 2020;395:1033–4. doi: 10.1016/S0140-6736(20)30628-0.

32. Hidayat M, Handayani D, Nurwidya F, Andarini SL. Hyperinflammation Syndrome in COVID-19 Disease: Pathogenesis and Potential Immunomodulatory Agents. Turk J Immunol. 2021;9:1-11. doi: 10.5222/TJI.2021.92486

33. Chen W, Pan JY. Anatomical and Pathological Observation and Analysis of SARS and COVID-19: Microthrombosis Is the Main Cause of Death. Biol Proced Online. 2021;23(1):4. doi: 10.1186/s12575-021-00142-y.

34. Yang Y, Bazhin AV, Werner J, Karakhanova S. Reactive oxygen species in the immune system. Int. Rev. Immunol. 2013;32:249–70. doi: 10.3109/08830185.2012.755176.

35. Verdecchia P, Cavallini C, Spanevello A, Angeli F. The pivotal link between ACE2 deficiency and SARS-CoV-2 infection. Eur J Intern Med. 2020;76:14-20. doi: 10.1016/j.ejim.2020.04.037.

36. Liu Y, Hu G, Wang Y, Ren W, Zhao X, Ji F, Zhu Y, Feng F, Gong M, Ju X, Zhu Y, Cai X, Lan J, Guo J, Xie M, Dong L, Zhu Z, Na J, Wu J, Lan X, Xie Y, Wang X, Yuan Z, Zhang R, Ding Q. Functional and genetic analysis of viral receptor ACE2 orthologs reveals a broad potential host range of SARS-CoV-2. Proc Natl Acad Sci U S A. 202;118(12):e2025373118. doi: 10.1073/pnas.2025373118.

37. Robledinos-Antón N, Fernández-Ginés R, Manda G, Cuadrado A. Activators and Inhibitors of NRF2: A Review of Their Potential for Clinical Development. Oxid Med Cell Longev. 2019;2019:9372182. doi: 10.1155/2019/9372182.

38. Herengt A, Thyrsted J, Holm CK. NRF2 in Viral Infection. Antioxidants (Basel). 2021;10(9):1491. doi: 10.3390/antiox10091491.

39. Tonelli C, Chio IIC, Tuveson DA. Transcriptional Regulation by Nrf2. Antioxid Redox Signal. 2018 Dec 10;29(17):1727-1745. doi: 10.1089/ars.2017.7342.

40. Kobayashi EH, Suzuki T, Funayama R, Nagashima T, Hayashi M, Sekine H, Tanaka N, Moriguchi T, Motohashi H, Nakayama K, Yamamoto M. Nrf2 suppresses macrophage inflammatory response by blocking proinflammatory cytokine transcription. Nat Commun. 2016;7:11624. doi: 10.1038/ncomms11624.

41. Sa Ribero M, Jouvenet N, Dreux M, Nisole S. Interplay between SARS-CoV-2 and the type I interferon response. PLoS Pathog. 2020;16(7):e1008737. doi: 10.1371/journal.ppat.1008737.

42. Tsoyi K, Lee TY, Lee YS, Kim HJ, Seo HG, Lee JH, Chang KC. Heme-oxygenase-1 induction and carbon monoxide-releasing molecule inhibit lipopolysaccharide (LPS)-induced high-mobility group box 1 release in vitro and improve survival of mice in LPS- and cecal ligation and puncture-induced sepsis model in vivo. Mol Pharmacol. 2009;76(1):173-82. doi: 10.1124/mol.109.055137.

43. Zinovkin RA, Grebenchikov OA. Transcription Factor Nrf2 as a Potential Therapeutic Target for

Prevention of Cytokine Storm in COVID-19 Patients. Biochemistry (Mosc). 2020;85(7):833-837. doi: 10.1134/S0006297920070111.

44. Chernyak BV, Popova EN, Prikhodko AS, Grebenchikov OA, Zinovkina LA, Zinovkin RA. COVID-19 and Oxidative Stress. Biochemistry (Mosc). 2020 Dec;85(12):1543-1553. doi: 10.1134/S0006297920120068

45. Fratta Pasini AM, Stranieri C, Ferrari M, Garbin U, Cazzoletti L, Mozzini C, Spelta F, Peserico D, Cominacini L. Oxidative stress and Nrf2 expression in peripheral blood mononuclear cells derived from COPD patients: an observational longitudinal study. Respir Res. 2020;21(1):37. doi: 10.1186/s12931-020-1292-7.

46. Hagman K, Hedenstierna M, Rudling J, Gille-Johnson P, Hammas B, Grabbe M, Jakobsson J, Dillner J, Ursing J. Duration of SARS-CoV-2 viremia and its correlation to mortality and inflammatory parameters in patients hospitalized for COVID-19: a cohort study. Diagn Microbiol Infect Dis. 2022;102(3):115595.doi: 10.1016/j.diagmicrobio.2021.

47. Gunne S, Heinicke U, Parnham MJ, Laux V, Zacharowski K, von Knethen A. Nrf2-A Molecular Target for Sepsis Patients in Critical Care. Biomolecules. 2020 Dec 17;10(12):1688. doi: 10.3390/biom10121688.

48. Tomasi ML, Ryoo M, Yang H, Iglesias Ara A, Ko KS, Lu SC. Molecular mechanisms of lipopolysaccharide-mediated inhibition of glutathione synthesis in mice. Free Radic Biol Med. 2014;68:148-58. doi: 10.1016/j.freeradbiomed.2013.11.018.

49. Lamonte G, Tang X, Chen JL, Wu J, Ding CK, Keenan MM, Sangokoya C, Kung HN, Ilkayeva O, Boros LG, Newgard CB, Chi JT. Acidosis induces reprogramming of cellular metabolism to mitigate oxidative stress. Cancer Metab. 2013;1(1):23. doi: 10.1186/2049-3002-1-23.

50. Gümüş H, Erat T, Öztürk İ, Demir A, Koyuncu I. Oxidative stress and decreased Nrf2 level in pediatric patients with COVID-19. J Med Virol. 2022;94(5):2259-2264. doi: 10.1002/jmv.27640.

51. Olagnier D, Farahani E, Thyrsted J, Blay-Cadanet J, Herengt A, Idorn M, Hait A, Hernaez B, Knudsen A, Iversen MB, Schilling M, Jørgensen SE, Thomsen M, Reinert LS, Lappe M, Hoang HD, Gilchrist VH, Hansen AL, Ottosen R, Nielsen CG, Møller C, van der Horst D, Peri S, Balachandran S, Huang J, Jakobsen M, Svenningsen EB, Poulsen TB, Bartsch L, Thielke AL, Luo Y, Alain T, Rehwinkel J, Alcamí A, Hiscott J, Mogensen TH, Paludan SR, Holm CK. SARS-CoV2-mediated suppression of NRF2-signaling reveals potent antiviral and anti-inflammatory activity of 4-octyl-itaconate and dimethyl fumarate. Nat Commun. 2020;11(1):4938. doi: 10.1038/s41467-020-18764-3.

52. Mihić D, Loinjak D, Maričić L, Smolić R, Šahinović I, Steiner K, Viland S, Šerić V, Duvnjak M. The Relationship between Nrf2 and HO-1 with the Severity of COVID-19 Disease. Medicina (Kaunas). 2022;58(11):1658. doi: 10.3390/medicina58111658.

53. Phillips M, Cataneo RN, Greenberg J, Grodman R, Gunawardena R, Naidu A. Effect of oxygen on breath markers of oxidative stress. Eur Respir J. 2003;21(1):48-51. doi: 10.1183/09031936.02.00053402.

54. Oldman AH, Martin DS, Feelisch M, Grocott MPW, Cumpstey AF. Effects of perioperative oxygen concentration on oxidative stress in adult surgical patients: a systematic review. Br J Anaesth. 2021;126(3):622-632. doi:10.1016/j.bja.2020.09.050.

55. Foschino Barbaro MP, Serviddio G, Resta O, Rollo T, Tamborra R, Elisiana Carpagnano G, Vendemiale G, Altomare E. Oxygen therapy at low flow causes oxidative stress in chronic obstructive pulmonary disease: Prevention by N-acetyl cysteine. Free Radic Res. 2005;39(10):1111-8. doi: 10.1080/10715760500250257.

56. Nagatomo F, Fujino H, Kondo H, Ishihara A. Oxygen concentration-dependent oxidative stress levels in rats. Oxid Med Cell Longev. 2012;2012:381763. doi: 10.1155/2012/381763.

57. Koksal GM, Dikmen Y, Erbabacan E, Aydin S, Çakatay U, Sitar ME, Altindas F. Hyperoxic oxidative stress during abdominal surgery: a randomized trial. J Anesth. 2016 Aug;30(4):610-9. doi: 10.1007/s00540-016-2164-7.

58. Hara Y, Tsukiji J, Yabe A, Onishi Y, Hirose H, Yamamoto M, Kudo M, Kaneko T, Ebina T. Heme oxygenase-1 as an important predictor of the severity of COVID-19. PLoS One.

2022;17(8):e0273500. doi: 10.1371/journal.pone.0273500.

59. Alcaraz MJ, Ferrándiz ML. Relevance of Nrf2 and heme oxygenase-1 in articular diseases. Free Radic Biol Med. 2020;157:83-93. doi:10.1016/j.freeradbiomed.2019.12.007.

60. Loboda A, Damulewicz M, Pyza E, Jozkowicz A, Dulak J. Role of Nrf2/HO-1 system in development, oxidative stress response and diseases: an evolutionarily conserved mechanism. Cell Mol Life Sci. 2016;73(17):3221-47. doi: 10.1007/s00018-016-2223-0.

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