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Exploring the Relationship Between Sod1, 2 And 3 Gene Polymorphisms With Post-Covid19 Symptoms

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KEYWORDS
Covid19; Long Covid19; SOD; Genomic vairation

ABSTRACT
The coronavirus disease 19 (COVID-19) has become a challenge for the media world. Even though they have been declared cured, some Covid-19 survivors still have health complaints. Abnormal symptoms, signs, or clinical parameters that persist two weeks or more after the onset of COVID-19 and do not return to their initial healthy state are potentially considered long-term effects of the disease. Although such changes are primarily reported in people with severe and critical illness, lasting effects also occur in individuals with mild infections that do not require hospitalization. This study aims to explore the role of antioxidants on the pathogenesis of Covid19 and its relationship with SOD1, 2 and 3 genomic variations. Getting alternative biomarkers for long covid19 detection. Research Method: The research sample is blood and questionnaires that will be taken from respondents affected by Covid19 a maximum of 6 months before data collection is held. Furthermore, DNA isolation, DNA amplification, cutting with restriction enzymes, and DNA band documentation with gel electrophoresis will be carried out. Symptoms related to systemic are the most common symptoms found in respondents with Long Covid19. A total of 8 respondents had systemic related symptoms, namely weakness, lethargy and sweating, while skin-related complaints were found in three respondents and one respondent had lung-related complaints. The average SOD activity value of respondents was 2.19 U/ml. In this study, more symptoms of long COVID-19 were found associated with systemic and cardiovascular symptoms. The complaints were not differentiated by the sex of the respondents. The output draft will be processed immediately after getting other results.

1. Introduction

Coronavirus disease 19 (COVID-19) has become a challenge for the media world. Although related to the respiratory system, the pathogenesis of this disease is still not fully understood. COVID-19 was designated a pandemic by the World Health Organization (WHO) in March 2020 because it has spread worldwide and is considered a disease with a high transmission ability from person to person. Symptoms of Covid19 include early symptoms of low-grade fever and dry cough, followed by high fever, fatigue and difficulty breathing with dyspnea, and in more severe cases, hypoxia with oxygen saturation of less than 85%, multi-organ damage and even death (Xu et al., 2020). Even though they have been declared cured, some COVID-19 survivors still have health complaints. Abnormal symptoms, signs, or clinical parameters that persist two weeks or more after the onset of COVID-19 and do not return to their initial healthy condition are potentially considered long-term effects of the disease (Tenforde et al., 2020). Although such changes are primarily reported in people with severe and critical illnesses, lasting effects also occur in individuals with mild infections that do not require hospitalization (Townsend et al., 2021).

Once the virus enters the respiratory tract, the viral replication process begins, and the natural immune response begins to activate macrophages and dendritic cells via toll-like receptors and NODs against the production of inflammatory cytokines and reactive oxygen species (ROS) (Uehara et al., 2015). The spread of the virus in the blood will cause damage to erythrocytes by ROS and other inflammatory mechanisms (Li et al., 2021), leading to the formation of free hemp and Fe. In addition, activated macrophages and neutrophils will produce respiratory bursts that will form superoxide radicals and H2O2, which lead to oxidative stress. Oxidative stress, along with free Fe, will convert soluble plasma fibrinogen into abnormal fibrin clumps in the form of dense matted deposits (DMD) that are resistant to degradation by enzymes. This will cause microthrombosis in the vascular system and pulmonary microcirculation. Cytokine storms arise through the upregulation of cytokine expression via NF-κB. Furthermore, cytokine storms will induce oxidative stress through macrophages and neutrophil respiratory bursts and vice versa. This cycle will provoke serious tissue damage that is not related to viruses. In addition, mitochondria produce ROS, which increases iNOS expression through NF-κB so that NO is formed which will induce mitochondrial dysfunction and cause cytopathic hypoxia. The virus will also inhibit Nrf2, which is responsible for increased antioxidant enzymes, causing oxidative stress. In conclusion, low haemoglobin levels and high lung exudate protein will cause pulmonary hypoxia, cytopathic hypoxia and endothelial damage and disseminated coagulation, which will cause multi-organ collapse (Cecchini & Cecchini, 2020).

Reactive Oxygen Species (ROS) can be characterized by several markers, including the enzyme Superoxide Dismutase (SOD). SOD functions to accelerate the reaction of the superoxide anion (O2) with itself to form hydrogen peroxide (H2O2) and oxygen ($2O2 + 2H \rightarrow H2O2 + O2$) (Wang et al., 2018). This reaction is important for maintaining redox equilibrium (Sies, 2015). However, excessive ROS production can exceed the capacity of the antioxidant defence system, causing oxidative stress and causing cellular damage by oxidized proteins, lipids, DNA/RNA and other macromolecules. There are three types of SOD; two of them are often found in mitochondria, namely SOD1 and SOD2. SOD 1 can be found in the intermembrane space, while SOD2 can be found in the mitochondrial matrix (Kim et al., 2017). SOD3 is often found extracellularly (Kim et al., 2017).

A relationship between the variation of the third SOD genotype and the risk of occurrence of several diseases has been found, for example, the CC rs4880 genotype is associated with increased hepatotoxicity after asparaginase therapy, the CC genotype rs2234694 increases the risk of T2DM, and the rs1041740 and rs17880487 variants in the SOD1 gene are associated with cardiovascular mortality and rs7655372 the SOD3 gene is associated with ischemic stroke (Alachkar et al., 2017; Ghattas & Abo-Elmatty, 2012; Otaki et al., 2016; Yang et al., 2021). However, the relationship between the third genotypic variant of SOD and post-Covid19 symptoms is still unknown.

The aim of this study was to explore the role of antioxidants in the pathogenesis of COVID-19. The other objective is to Analyze the SOD1, SOD2 and SOD3 mRNA expression levels of COVID-19 survivors. Analyzing SOD1, SOD2 and SOD3 levels in the plasma of Covid19 survivors, Analyzing the genotype variation of SOD1, SOD2 and SOD3 of Covid19 survivors, Analyzing the demographic picture and clinical symptoms of Covid19 survivors, Analyzing the relationship between expression levels and plasma SOD1, SOD2 and SOD3 levels with the duration of recovery and clinical symptoms of long covid19, Analyzing the relationship between SOD1, SOD2 and SOD3 genotype variations with the length of recovery and clinical symptoms of long covid19.

2. Materials and Methods

Research Design

The study will be designed as a case-control study that will test two groups of COVID-19 survivors with and without long-term COVID-19 symptoms.

Research Subjects

This study's research population is composed of COVID-19 survivors in Jakarta. Research subjects who meet the inclusion and exclusion criteria will be asked to join the study and sign an informed consent form.

Research Sample

Research samples are divided into 2 types, namely blood and tears. Blood is taken from a 5cc cubital vein and inserted into an EDTA vacutainer. Cheek mucosal smears will be performed using sterile cotton swabs that are daubed into the cheeks of research subjects and stored in buffers to prevent DNA and RNA damage. All blood samples and cheek mucosal smears were transferred to the laboratory in a styrofoam box filled with ice and stored in a refrigerator at - 80C for further analysis. Inclusion and exclusion criteria. The inclusion criteria are:

- Willing to sign informed consent.
- Willing to follow research procedures.

The exclusion criteria are:

- Individuals confirmed with COVID-19.

Sample Size

The five groups' gene expression differences were determined using G*Power 3.1.9.4 software. Based on the calculation of G*Power, which takes into account the effect size of 0.5, the total number of samples needed is 128 (Figure 1), with a total sample of 64 for each group.

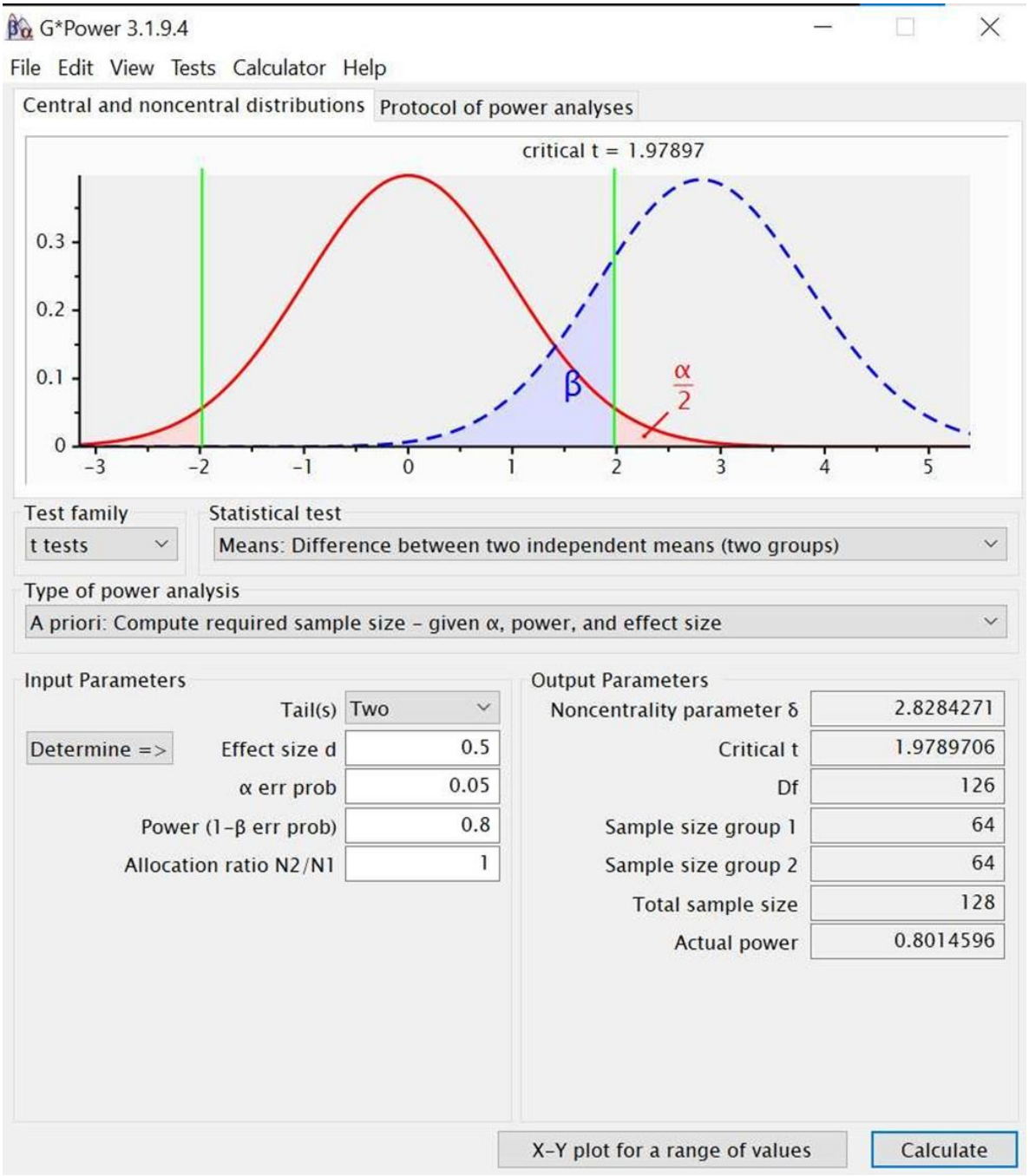

Figure 1 Calculation of total sample size on G*Power

Research materials and methods

The materials needed in this study are:

1) RNA isolation kit
2) Kit synthesis cDNA
3) The DNA is the.
4) Enzyme restrictions

5) Conventional PCR mix
6) SYBR Green PCR Master Mix
7) Primer
8) Agarose Powder
9) Buffer TAE 1x
10) DNA Fluorosafe

Research Methods

1) RNA Isolation Research Methods
   RNA will be extracted from blood and cervical smear samples in tubes containing RNA later. RNA extraction and DNase cleaning using RNAqueous-Micro® Kit (Cat# AM1931, Thermo Fisher Scientific, Waltham, MA, USA) in accordance with the manufacturer's protocol. The RNA concentration was determined using a nanodrop spectrophotometer based on a wavelength of 260. All samples with an absorption ratio of 2.0 to 260:280 will be used for further analysis.
2) Synthesis Cdna
   Complement DNA (cDNA) will be synthesized from 200 ng of DNase-treated RNA. This method uses 250 ng/ul random hexamers (Sigma, Adelaide, SA, Australia) and 200 U Superscript Reverse Transcriptase III (Thermo Fisher Scientific, Waltham, MA, USA). To ensure the absence of genomic contamination, negative controls will be analyzed by adding water that DEPC has added in place of Superscript Reverse Transcriptase III.
3) Quantitative real-time PCR
   For Quantitative real-time PCR (qPCR) analysis, primers will be designed based on publicly published RNA sequences using Primer3 plus (Rozen and Skaletsky, 2000) and primary Net software (PREMIERE Biosof). Quantitative real-time PCR analysis will be performed using the Rotor-Gene 6000 series 1.7 thermal cyclers (Qiagen GmbH) by duplication at 95C for 15 seconds, then 60C for 60 seconds for 40 cycles. CDNA amplification will be prepared in a 10 ml reaction containing 2 ml 1:20 cDNA, 5 ml Power SYBR Green PCR Master Mix (Applied Biosystems), 0.2 ml forward and reserve primers for target genes and 2.6 ml water mixed with DEPC. The Ct value is determined using Rotor-Gene 6000 software (Q series; Qiagen GmbH) at a threshold of 0.05 normalized with fluorescence units. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is used as a "housekeeping" gene due to its stable expression in human tissues.
4) DNA Isolation
   According to the manufacturer's protocol, DNA will be isolated from venous blood and cheek mucosal smears using Quick-DNA™ Miniprep Plus Kit (Zymo Research, Irvine, CA, USA).
   The concentration of DNA was determined using nanophotometers based on 260 wavelengths. All samples with a ratio of 1.8 to absorbance of 260:280 will be used for further analysis.
5) Target gene amplification
   The target gene will be amplified using conventional PCR machines. According to the manufacturer's protocol, DNA will be mixed with MyTagTM HS Red Mix (Bioline-Meridian Bioscience Memphis, Tennessee, USA).
6) Incubation with restriction enzymes
   The amplification results are incubated at a certain time and temperature with restriction enzymes (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's protocol.
7) Gel Elektroforesis
   Electrophoresis using 2% agarose gel to see the size of the incubation restriction fragment band

Analysis Methods

Data In this study, the data obtained on quantitative PCR will be entered into the following formula:

$$2 - ave\Delta Ct$$

Where:

Average ΔCt = mean of the results of reducing the cycle threshold of the GAPDH gene and the target gene studied.

The formula compares the expression of the target gene with the expression of the GAPDH (housekeeping gene) gene, which is constantly expressed in each type of body cell. The final result will be numerical data describing the target expression. The higher number describes the higher gene expression, while the lower number also describes the lower gene expression.

All statistical analysis will be performed using Microsoft Office Excel 2010 and GraphPad Prism Version 6.00 (GraphPad Software Inc.). Results will be presented in mean ± SEM if the data distribution is normal, and results will be presented in median if the data distribution is abnormal. All bivariate data will be analyzed using the t-test in the normal distribution; for normal distribution, data will be analyzed in non-Mann-Whitney statistical tests. The result will be considered significant at $p < 0.05$ with a 95% confidence interval.

Table 1 Research Achievement Indicators

Aspects		CHECKLIST
Featured Scale	International Scale	
	National Scale	✓
	Local Scale	
Research Topics/Themes	Top Down	
	Semi Top Down	
	Bottom Up	✓
Funding Scheme	Block Grant	
	Competitive	
RESEARCH IMPLEMENTER	Research Center	
	Individual	✓
	Riset Group	
Source Of Funds	Decentralized Fund	

3. Result and Discussion

This study analyzes the demographic characteristics of patients and their relationship with symptoms of long-term COVID-19 that occur a maximum of 6 months after being declared recovered from COVID-19. Symptoms will be described based on the organ systems of the human body. There have been 55 respondents with the same portion for gender. Most respondents had never had Covid19 before but 10 respondents had had Covid19 before twice. Generally, respondents are self-isolating at home, and most respondents have no comorbidities for COVID-19 disease and no previous history of smoking.

Table 2 Characteristics of research respondents (n = 55)

No.	Information	Frequency (n)	Percentage (%)
1.	Gender		
	Man	27	49.09
	Woman	28	50.91
2.	History of Covid19		
	Never	24	43.64
	Once	21	38.18
	Twice	10	18.18
3.	Hospital history		
	Self-isolation	54	98.18
	Treated	1	1.82
4.	Comorbid		
	None	49	89.09
	Diabetes Mellitus	2	3.64
	Hypertensive	3	5.45
	Heart Disease	0	0
	Lung Disease	1	1.82
	Other	1	1.82
5.	Habit history		
	Smoke	16	29.09
	Drinking alcohol	0	0
	Drugs	0	0
	Other	39	70.91
6.	Symptoms of Long COVID-19		
	Yes	28	51
	No	27	49

Of the 55 respondents, there were 27 respondents without long-term covid symptoms and 28 respondents with long-term covid symptoms

Based on the data, it is stated that most patients who experience symptoms of long-term COVID-19 are women, while male patients have a tendency not to experience symptoms of long-term COVID-19. Based on the analysis, it was found that around 60% of patients suffering from ling covid were women. This is in line with a publication from ISOS related to Long Covid which states that people who have weak immunity to infection, women, and people with severe disease are more likely to experience Long COVID.

As for the history of COVID-19, patients who experience symptoms of long-term COVID-19 are mostly patients who have previously experienced positive COVID-19 once. All patients who experience symptoms are patients undergoing self-isolation. Some patients do not have cormobids. As many as 3 patients suffered from comorbidity, namely diabetes militias, as many as 1 people and 2 people with hypertension. Most patients do not have the habit of smoking, drinking alcohol or using drugs (Proskurnina et al., 2020; Uehara et al., 2015). Only 5 patients had a smoking habit. As for

patients who smoke, both regular cigarettes and vapes. Their smoking habits are intense sometimes, and they spend as much as 6 cigarettes to 1 pack of cigarettes in one day.

The virus that causes Covid19 is SARS-CoV-2 which has mutated several times. To date, there have been thousands of variants WHO has recorded in three categories; the dangerous one is the Variant of Concern (VOC). At the beginning of the COVID-19 pandemic, the alpha and beta variants had spread throughout the world, but it was indeed the delta variant that had a high morbidity and mortality rate compared to other variants.

The virus must be able to infect host cells and evade the immune system in order to survive. Therefore, the virus will continue to mutate. Similar to SARS-CoV2, this virus also continues to mutate, resulting in many variants, including the omicron variant currently detected in many countries worldwide.

Symptoms related to systemic are the most common symptoms found in respondents with Long Covid-19. A total of 8 respondents had systemic-related symptoms, namely weakness, lethargy and sweating, while skin-related complaints were found in three respondents, and one respondent had lung-related complaints.

Table 3 Long COVID-19 Complaints Related to Systemic Symptoms

No.	Information	Frequency (n)
1	Systemic Symptoms	
	Weak and lethargic	6
	Perspire	2
2	Lungs	
	Shortness of breath Skin	1
3	Hair loss	1
	Dry scaly skin	1
	Red stain	1
4	Cardiovascular	
	Low blood pressure	2
	Heart palpitations	1
	Chest pain	1

Low blood pressure is the most common cardiovascular-related symptom, followed by heart palpitations and chest pain.

From the results of SOD activity, it was found that the average SOD activity was 2.19 U/ml, with the highest SOD activity value being 3,164 U/ml while the lowest was 1,062 U/ml.

Table 4 The Value of SOD Activity in Respondents

No. Respond	SOD activity (U/ml)
1	2.570
2	2.088
3	2.317
4	2.852
5	3.164
6	2.852
7	2.570
8	2.341
9	1.786
10	2.570
11	2.088
12	2.317
13	1.882
14	2.088
15	2.317
16	2.317
17	2.707
18	2.852
19	2.440
20	2.852
21	1.529
22	2.199
23	1.451
24	2.199
25	2.088
26	2.570
27	1.982
28	1.882
29	1.882
30	2.852
31	2.088
32	1.529
33	2.088
34	1.882
35	1.378

36	2.570
37	1.786
38	1.882
39	1.062
40	1.696

Lopez-Leon et al. (2022) showed that there are five most common manifestations: fatigue, headache, attention disorder, hair loss, and shortness of breath. Other symptoms are related to breathing, cardiovascular, and neurological organs. Some studies report that complaints are more common in women, especially fatigue and panting (Townsend et al., 2021; Xiong et al., 2021).

4. Conclusion

In this study, more symptoms of long COVID-19 were found associated with systemic and cardiovascular symptoms. The complaints were not differentiated by the sex of the respondent.

5. References

Alachkar, H., Fulton, N., Sanford, B., Malnassy, G., Mutonga, M., Larson, R. A., Bloomfield, C. D., Marcucci, G., Nakamura, Y., & Stock, W. (2017). Expression and polymorphism (rs4880) of mitochondrial superoxide dismutase (SOD2) and asparaginase induced hepatotoxicity in adult patients with acute lymphoblastic leukemia. *The Pharmacogenomics Journal*, *17*(3), 274–279. https://doi.org/10.1038/tpj.2016.7

Cecchini, R., & Cecchini, A. L. (2020). SARS-CoV-2 infection pathogenesis is related to oxidative stress as a response to aggression. *Medical Hypotheses*, *143*, 110102. https://doi.org/10.1016/j.mehy.2020.110102

Ghattas, M. H., & Abo-Elmatty, D. M. (2012). Association of Polymorphic Markers of the Catalase and Superoxide Dismutase Genes with Type 2 Diabetes Mellitus. *DNA and Cell Biology*, *31*(11), 1598–1603. https://doi.org/10.1089/dna.2012.1739

Kim, Y., Gupta Vallur, P., Phaëton, R., Mythreye, K., & Hempel, N. (2017). Insights into the Dichotomous Regulation of SOD2 in Cancer. *Antioxidants*, *6*(4), 86. https://doi.org/10.3390/antiox6040086

Li, S., Li, S., Disoma, C., Zheng, R., Zhou, M., Razzaq, A., Liu, P., Zhou, Y., Dong, Z., Du, A., Peng, J., Hu, L., Huang, J., Feng, P., Jiang, T., & Xia, Z. (2021). SARS-CoV-2: Mechanism of infection and emerging technologies for future prospects. *Reviews in Medical Virology*, *31*(2). https://doi.org/10.1002/rmv.2168

Otaki, Y., Watanabe, T., Nishiyama, S., Takahashi, H., Arimoto, T., Shishido, T., Miyamoto, T., Konta, T., Shibata, Y., Sato, H., Kawasaki, R., Daimon, M., Ueno, Y., Kato, T., Kayama, T., & Kubota, I. (2016). The Impact of Superoxide Dismutase-1 Genetic Variation on Cardiovascular and All-Cause Mortality in a Prospective Cohort Study: The Yamagata (Takahata) Study. *PLOS ONE*, *11*(10), e0164732. https://doi.org/10.1371/journal.pone.0164732

Proskurnina, E. V., Izmailov, D. Yu., Sozarukova, M. M., Zhuravleva, T. A., Leneva, I. A., & Poromov, A. A. (2020). Antioxidant Potential of Antiviral Drug Umifenovir. *Molecules*, *25*(7), 1577. https://doi.org/10.3390/molecules25071577

Sies, H. (2015). Oxidative stress: a concept in redox biology and medicine. *Redox Biology*, *4*, 180–183. https://doi.org/10.1016/j.redox.2015.01.002

Tenforde, M. W., Kim, S. S., Lindsell, C. J., Billig Rose, E., Shapiro, N. I., Files, D. C., Gibbs, K. W., Erickson, H. L., Steingrub, J. S., Smithline, H. A., Gong, M. N., Aboodi, M. S., Exline, M. C., Henning, D. J., Wilson, J. G., Khan, A., Qadir, N., Brown, S. M., Peltan, I. D., … Wu, M. J. (2020). Symptom Duration and Risk Factors for Delayed Return to Usual Health Among Outpatients with COVID-19 in a Multistate Health Care Systems Network — United States, March–June 2020. *MMWR. Morbidity and Mortality Weekly Report*, *69*(30), 993–998. https://doi.org/10.15585/mmwr.mm6930e1

Townsend, L., Dowds, J., O'Brien, K., Sheill, G., Dyer, A. H., O'Kelly, B., Hynes, J. P., Mooney, A., Dunne, J., Ni Cheallaigh, C., O'Farrelly, C., Bourke, N. M., Conlon, N., Martin-Loeches, I., Bergin, C., Nadarajan, P., & Bannan, C. (2021). Persistent Poor Health after COVID-19 Is Not Associated with Respiratory Complications or Initial Disease Severity. *Annals of the American Thoracic Society*, *18*(6), 997–1003. https://doi.org/10.1513/AnnalsATS.202009-1175OC

Uehara, E. U., Shida, B. de S., & de Brito, C. A. (2015). Role of nitric oxide in immune responses against viruses: beyond microbicidal activity. *Inflammation Research*, *64*(11), 845–852. https://doi.org/10.1007/s00011-015-0857-2

Wang, Y., Branicky, R., Noë, A., & Hekimi, S. (2018). Superoxide dismutases: Dual roles in controlling ROS damage and regulating ROS signaling. *Journal of Cell Biology*, *217*(6), 1915–1928. https://doi.org/10.1083/jcb.201708007

Xiong, Q., Xu, M., Li, J., Liu, Y., Zhang, J., Xu, Y., & Dong, W. (2021). Clinical sequelae of COVID-19 survivors in Wuhan, China: a single-centre longitudinal study. *Clinical Microbiology and Infection*, *27*(1), 89–95. https://doi.org/10.1016/j.cmi.2020.09.023

Xu, Z., Shi, L., Wang, Y., Zhang, J., Huang, L., Zhang, C., Liu, S., Zhao, P., Liu, H., Zhu, L., Tai, Y., Bai, C., Gao, T., Song, J., Xia, P., Dong, J., Zhao, J., & Wang, F.-S. (2020). Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *The Lancet Respiratory Medicine*, *8*(4), 420–422. https://doi.org/10.1016/S2213-2600(20)30076-X

Yang, X., Yang, S., Xu, H., Liu, D., Zhang, Y., & Wang, G. (2021). Superoxide Dismutase Gene Polymorphism is Associated With Ischemic Stroke Risk in the China Dali Region Han Population. *The Neurologist*, *26*(2), 27–31. https://doi.org/10.1097/NRL.0000000000000301