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Administration of N-Acetylcysteine (NAC) as Adjunctive Therapy for COVID-19 Patients by Improving Oxidative Stress and Inflammation Through Assessment of MDA, IL-6, IL-1β, TNF-α, and TGF-β

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ABSTRACT

INTRODUCTION: Oxidative stress due to COVID-19 will trigger a widespread inflammatory reaction in the lungs. Malondialdehyde (MDA), Interleukin-6 (IL-6), Interleukin-1 β (IL-1β), Tumor Necrosis Factor-α (TNF-α), and Tumor Growth Factor- β (TGF-β) are pro-inflammatory cytokines which levels may rise in the presence of an inflammatory process. N-Acetylcysteine (NAC) is an antioxidant widely considered as adjunctive therapy in COVID-19. We aim to analyze the effect of NAC administration in improving oxidative stress and inflammation in COVID-19 through assessment of MDA, IL-6, IL-1β, TNF-α, and TGF-β.

METHOD: This is a quasi-experimental study with pre-post design. MDA, IL-6, IL-1β, TNF-α, and TGF-β levels were measured in admission and day 8 of administration of 5000 mg/72 hours of NAC.

RESULT: From 74 samples, between admission and day 8 of NAC therapy, There is a decrease of IL-6 (185.31 ± 181.30 to 97.60 ± 161.86 (p=0.003)), TNF-α (5.83 ± 3.51 to 4.37 ± 3.33 (p=0.019)), IL-1 β (4.65 ± 4.13 to 1.87 ± 0.96 (p<0.001)), and MDA levels (3000.70 ± 2017.98 to 2116.54 ± 1109.58 (p<0.001). However, TGF-β levels were increased from 5.47 ± 2.76 to 7.97 ± 3.24 (p<0.001).

DISCUSSION: From our patients we get a significant decrease of pro-inflammatory cytokines (MDA, IL-6, IL-1β, and TNF-α) after administration of NAC. However, we acquired a significant increased level of TGF-β in our patients despite NAC administration which implies the chronic immune response in COVID-19 patients.

CONCLUSION: There is an improvement of oxidative stress and inflammation by administration of NAC as adjunctive therapy in COVID-19 patients assessed by decrease of MDA, IL-6, IL-1β, and TNF-α levels. There is no effect of NAC as adjunctive therapy in TGF-β levels in COVID-19 patients.

KEYWORDS: NAC, COVID-19, MDA, IL-6, IL-1β, TNF-α, TGF-β

INTRODUCTION

On December 31, 2019, China reported a mysterious case of pneumonia of unknown cause. In 3 days, the number of patients with these cases amounted to 44 patients and continues to grow until now there are thousands of cases (WHO, 2020). Initially, epidemiological data showed that 66% of patients were associated or exposed to a seafood market or live market in Wuhan, Hubei Province of China (Huang et al,

2020). Samples of isolates from patients were studied with the results showing the presence of a coronavirus infection, a new type of betacoronavirus, named 2019 novel Coronavirus (2019-nCoV). On February 11, 2020, the World Health Organization named the new virus Severa acute respiratory syndrome coronavirus-2 (SARS-CoV-2) and the name of the disease as Coronavirus disease 2019 (COVID-19) (WHO, 2020). At first the transmission of this virus could not be determined whether it could pass between humans. The number of cases continues to grow over time. In addition, there were cases of 15 medical workers being infected by one of the patients. One of these patients was suspected of being a "super spreader" case. It was finally confirmed that this pneumonia transmission can be transmitted from human to human. Until now, this virus is rapidly spreading, it is still a mystery and research is still ongoing (Wang *et al*., 2020)

The lungs are the preferred target of Covid-19, where the lungs are also one of the most oxygenated organs of the body. Multiple lung diseases contribute to the increased production of the characteristic reactive oxygen species (ROS) from oxidative stress conditions. Oxidative stress is an important factor that causes metabolic and physiological changes and various diseases in the body. Covid-19 infection triggers an inflammatory reaction that releases the proinflammatory cytokines characteristic of acute lung damage. Good association between pro-inflammatory elements and reactive oxygen species (ROS) in different lung diseases including Coronavirus infection is associated with inflammation and oxidative stress. (Derouiche, 2020)

The production of ROS leads to lipid peroxidation, a continuous process that affects unsaturated fatty acids, which are mainly located in cell membranes, resulting in malondialdehyde (MDA). Fat peroxidation products spread from the area of inflammation to the circulation so that their levels can be measured in the blood which will be an indicator of biomarkers. (Fatani, 2014; Mishra et al.,2018).

MDA is an indicator of lipid peroxidation that is often used. MDA is widely used as a biomarker for oxidative stress for several reasons, including MDA formation increases with oxidative stress, is a specific product of lipid peroxidation and is stable in isolated body fluid samples (Singh et al.,2014).

Increased plasma concentrations of inflammatory markers such as CRP and ferritin, and proinflammatory cytokines such as TNF-alpha, IL-1B, IL-6 and IL-8, and chemokines such as MCP1, along with an increased neutrophil/lymphocyte ratio, have been associated with an increased incidence of infection and inflammation. death from SARS-CoV-2. Although it is possible that lung and other tissue damage in SARS-CoV-2 infection is the result of a multifactorial mechanism, recent studies suggest that ROS may play a major role in the initiation and progression of this inflammatory process. ROS trigger the NLRP3 inflammasome through NF-kB and thioredoxin which interact/inhibit protein activation. In addition, NF-kB inversely regulates the expression of IL-18 and IL-1B, thereby further enhancing the NLRP3 inflammasome (Pasini et al., 2021).

Another study suggested the presence of activated receptor proteinases (PARs) on epithelial cells, monocytes, macrophages, and vascular endothelial cells, and their activation initiated the release of proinflammatory mediators including the cytokines TNF, IL-1β, IL-2, and IL-6, and the chemokine CXCL8 (Fig. IL-8) and CCL2, all of which are associated with the pathogenesis of ARDS (Chang et al., 2020). Neutrophils express interleukin (IL)-6 and IL-!B through a TLR8-mediated mechanism that initiates cytokine storm and further lung damage (Mohamed et al., 2020). IL-1β and NETs form a feedback loop, which contributes to the pathogenesis of ARDS in

COVID-19 pasien patients (Yaqinuddin dan Kashir, 2020).

There is one study that analyze the effect of 15 mM NAC in acute inflammation was evaluated at 2, 4, 6 and 24 h. And mRNA expression of tumor necrosis factor (TNF)-α, IL-1β, IL-6, IL-8 and IL-10 was assessed by real time PCR. It shows that NAC inhibits the inflammatory cytokines TNFα, IL-1β and IL-6 in LPS-activated macrophages under mild oxidative conditions (Palacio *et al.*, 2011). NAC has been demonstrated to improve the redox status especially under oxidative stress, a key phenomenon in SARS-Cov-2. 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. NAC may increase the proliferative response of T cells, inhibit NLRP3 inflammatory pathway (IL1B and IL18) and decrease plasma TNF (Lana *et al.*, 2021).

The evident role of IL-21 in the training of COVID-19 plasmablasts suggests to cognate B cell activation by follicular helper T (Tfh)/Th17 cells, the main makers of IL-21 (Parrish-Novak *et al*., 2000). Furthermore, increased TGF- β expression has been defined as a marker of continuing Th17 cell activation (Gutcher *et al*., 2011, Lee *et al*., 2012). Other sources of TGF- β include Peyer's patches (Rebeldi *et al.*, 2016), subepithelial dendritic cells, regulatory T cells (Tregs), and neutrophils from the peripheral blood and airway tissue. TGF- β1 levels were shown to be higher in mucosal tissues following SARS-CoV infection, which was the cause of the 2003 SARS outbreak (He *et al*., 2006). Furthermore, SARS-CoV nucleoprotein (NP), which is 90% identical to SARS-CoV-2 NP (Grifoni *et al*., 2021), can activate SMAD3 directly, boosting TGF- β mediated gene expression, which could include TGF-β itself (Zhao *et al*., 2008).

As a result, we concentrated our next investigation on SARS-CoV-2-reactive CD4+ T cells. We looked examined the transcriptomes and TCR repertoires of single T cells from three patients who had been in the ICU for 13, 29, and 32 days, respectively, and were seropositive for IgM and IgG, with one of them also being seropositive for IgA. To do this, PBMCs were stimulated for 6 hours with a peptide pool that included the SARS-CoV-2 spike glycoprotein (S), membrane glycoprotein (M), and nucleocapsid phosphoprotein (NP) (NP). Antigen-experienced regulatory and effector cells were isolated magnetically from reactive CD4+ T lymphocytes expressing CD137 or CD154 (Bacher *et al.*, 2016)

While we were only able to separate a few of these cells from a healthy donor, antigen-reactive CD4+ T cells made up 3.5 percent, 1.5 percent, and 4.9 percent of total CD4+ T cells in the three patients, respectively. UMAP clustering revealed two significant CD3E-expressing populations: Tregs that express FOXP3 and IKZF2 (Helios) and Tfh cells that express ICOS and PDCD1. Tfh cells expressed CD40LG, IFNg, IL2, and TNF after 6 hours of antigenic stimulation, but not IL17, IL10, or any type 2 T helper cell-related cytokines. Th cells expressing IL21 (1–10%), TGF- β1 (2.9–9.5%), and IL21 plus TGFB1 (0.3–3.5%) were equally common in SARS-CoV-2-reactive Th cells as they were in healthy controls.

From a healthy control, no substantial numbers of IL21 and/or TGF- β1 expressing SARS-CoV-2 reacting Th cells could be identified. It's worth noting that the TCR repertoires of SARS-CoV-2-specific Tregs and Tfh cells are distinct and do not overlap, with only 13 of the 1473 TCR clones found in both subsets, a phenomena that has also been reported for aero-antigen-specific Tregs and conventional T cells. These findings show that seriously affected COVID-19 patients have large populations of circulating, SARS-CoV-2-infected CD4+ T cells that are imprinted to express IL-21

and TGF-β, and therefore qualify as instructors of B cell activation in ongoing SARS-CoV-2-triggered immunological reactions. (Bacher *et al.,* 2016)

Even though datas regarding the effect of NAC for inhibiting TGF- β1 mediated immune response is not yet available, NAC blocks TGF-β-mediated epithelial-mesenchymal transition (EMT) of rat type II cells in vitro. Mechanistically, it was shown that TGF-β decreased glutathione levels and significantly increased reactive oxygen species production. NAC restored both to levels similar to those found in TGF-β-untreated cells. These findings are consistent with the idea that NAC acts as an antioxidant and cellular redox stabilizer, and prevents EMT of type II cells by maintaining intracellular glutathione stores.

Interleukin-6 (IL-6) is a cytokine that plays a central role in acute inflammation. IL-6, discovered by Weissenbach et al. in 1980. IL-6 is a multifunctional cytokine that plays an important role in human metabolism, autoimmune cell differentiation, disease treatment, etc. Interleukin-6 (IL-6) has fairly strict regulation and low levels in healthy individuals. During infection, trauma, or other stress, IL-6 is expressed in much higher concentrations and has been implicated in the pathogenesis of several chronic disease conditions including cardiovascular disease, atherosclerosis, and obesity.. (Abeywardena et al., 2009; Zhang et al, 2020)

IL-6 is a small polypeptide consisting of four main α-helix, has a molecular weight of 19-28 kDa, and consists of 184 amino acid residues, in monomer form, with an isoelectric point of 5.0, a glycosylation site, and two disulfide bonds. The coding of the IL-6 gene is located on chromosomes 7p 15-21, including 4 introns and 5 exons. Helix A and B run in the same direction, while helixes C and D travel in opposite directions, and their representations are in the form of ribbons.. (Zhang et al., 2020; Kaur et al., 2020)

IL-6 plays a central role in cytokine storms and is a multi-effective cytokine with anti-inflammatory and proinflammatory effects. There are three main lines of IL-6 signal transduction: classical signal transduction, trans-signal transduction, and trans-presentation. In the classical signal transduction pathway, IL-6 binds to the IL-6 receptor (IL-6R) to form a complex and then binds to gp130 to initiate intracellular signal transduction. IL-6R exists not only in transmembrane form but also in dissolved form (sIL-6R). IL-6 binds to both of these forms and then interacts with gp130 to trigger downstream signal transduction and gene expression. Both mIL-6R and sIL-6R can also transmit IL-6 signals via classical lines and trans-signal lines. (Zhang et al, 2020; Kaur et al., 2020; Abbasifard dan Khorramdelazad, 2020)

Increased expression of IL-6 in some viral infections, such as COVID-19, causes damage to lung tissue and affects the progressiveness and severity of the disease. Studies on patients infected with CoV (i.e., SARS-CoV, MERS-CoV, and SARS-CoV-2) have shown that lymphopenia and cytokine storms are two significant immunopathological findings. Cytokine storms also occur due to impaired expression and production of inflammatory cytokines such as IL-1β, IL-4, IL-6, IL-10, IL-18 IL-33, IFN-γ, and TNF-a which can result in uncontrolled and potentially damaging increases and inflammation and potentially damage various tissues and organs. (Abbasifard dan Khorramdelazad, 2020)

Increased levels of IL-6 can interfere with epithelial fluid transport and surfactant production by type II alveolar cells by producing platelet-activating factor (PAF), leukotrienes, reactive oxygen species (ROS), and other proteases. Recent studies show a positive and significant correlation between serum viral load and IL-6 levels in patients with severe and critical COVID-19 degrees. Viral load is also associated with the severity of ARDS and damage to lung

tissue. All evidence suggests the possible destructive role of IL-6 in patients with SARS-COV-2 infection. (Abbasifard dan Khorramdelazad, 2020)

METHOD

This is a quasi-experimental study with pre-post design to assess the effects of NAC administration as adjunctive therapy in COVID-19 confirmed patients. Study population is confirmed COVID-19 patients by RT-PCR nasopharyngeal swab result admitted in our hospital. Inclusion criteria includes confirmed COVID-19 patients admitted both in non-intensive and intensive wards and exclusion criteria are those who passed away before RT PCR swab results can be obtained (probable cases), pregnant woman, and asymptomatic cases. The study was commenced in Saiful Anwar general Hospital, Malang Regency, East Java, Indonesia between May 2020 to July 2021. Samples were checked for full laboratory workup including serum cytokines (MDA, IL-6, IL-1β, TNF-α, AND TGF-β) using ELISA kits in admission and in day 8 after administration of 5000 mg/72 hours of NAC. The data was collected and analyzed using appropriate statistical analysis.

RESULT

We screened 74 patients in the analyses which fulfilled the included criteria. Men had a higher number for infection with COVID-19 than women (44 (59.5%) vs 30 (40.5%)). With a higher number for severe and critically ill of COVID-19's severity (40 (54%) vs 34 (46%)). The analyses also showed that patients admit to hospital with many complaint, such as fever (53 (71.6) dyspnea (61 (82.4%)), cough (60 (81.1%)), and GIT disturbances (42 (56.8%)). Recent smoker or active smoker predominantly admit to hospital with 43 (58.1%) vs 31 (41%). Only 33 (44.59%) have a comorbid on this analyses which higher number of recovery outcome (64 (86.5%)) of all subject compared with death outcome (10 (13.5%)).

Table 1. Patients' Demographic

Variable and Cathegory	Frequency	Percentage
Age	53.67 yo ± 10.81 (min. 24 yo with max. 75 yo)	N sample = 74 (100%)
Gender		
Male	44	59.5%
Female	30	40.5%
Severity		
Moderate	34	45.9%
Severe	32	43.2%
Critically Ill	8	10.8%
Admission's Complaint		
Fever	53	71.6%
Shortness of Breath	61	82.4%
Cough	60	81.1%
GIT disturbances	42	56.8%
Recent Smoker or History of Smoking		
Yes	43	58.1%
No	31	41.9%
Comorbidities		
Yes	33	44.59%
No	41	55.40%
Corticosteroid Used		
Yes	40	54.1%
No	34	45.9%
Outcome		
Recovery	64	86.5%
Death	10	13.5%

The concentration of IL-6 in the group which we give NAC decreased markedly from day 1 to day 8 (185.31 ± 181.30 to 97.60 ± 161.86, p=0.003). TNF-a concentrations also show a decreased pattern at day 8 after NAC treatment (5.83 ± 3.51 to 4.37 ± 3.33, p=0.019). Also, IL-1B concentrations were reduced by NAC

treatment on days 1–8 (4.65 ± 4.13 to 1.87 ± 0.96, p<0.001). Similar to the IL-6, IL-1B, and TNF-a, NAC treatment result in a decrease in the MDA concentrations on days 1–8(3000.70 ± 2017.98 to 2116.54 ± 1109.58, p<0.001). However, only TGF-B concentrations were increased by NAC treatment at day 8 (5.47 ± 2.76 to 7.97 ± 3.24, p<0.001).

Table 2. Pre- and Post-NAC Results of Test Markers

Parameter	Pre (Mean ± SD)	Post (Mean ± SD)	P value
IL-6	185.31 ± 181.30	97.60 ± 161.86	0.003
IL-1β	4.65 ± 4.13	1.87 ± 0.96	< 0.001
TNF-α	5.83 ± 3.51	4.37 ± 3.33	0.019
MDA	3000.70 ± 2017.98	2116.54 ± 1109.58	< 0.001
TGF-β1	5.47 ± 2.76	7.97 ± 3.24	< 0.001

DISCUSSION

In our study, it was found that MDA levels in covid 19 patients increased (3000.70 ± 2017,98), this is in line with the study of Mehri et al, where MDA as a marker of oxidative stress increased significantly in Covid-19 patients. (Mehri et al., 2021) Elevated levels of MDA in COVID-19 patients compared to the control group showed an overproduction of free radicals that destroy membrane lipids with the formation of MDA as a by-product. (Muhammad et al., 2021) After administration of NAC, MDA decreased significantly after administration of NAC for 7 days (p<0.001), this is in accordance with the study of Cazzola et al which stated that NAC significantly reduced the pro-oxidant response caused by LPS. by reducing the level of peroxidase activity by 30% and levels of H2O2, malondialdehyde (MDA), and nitric oxide (NO) (Cazzola et al., 2021).

From our samples we got an increase of TGF-β levels from 5.47 ± 2.76 before NAC administration to 7.97 ± 3.24 in day 8. This increase in an expected phenomenon in patients with COVID-19. However, there is no effect of NAC administration to TGF-β levels. More study is needed to explore further effects of NAC to TGF-β mediated immune response in COVID-19.

Tumor necrosis factor-α (TNF-α) is a pro-inflammatory cytokine that is overproduced during the course of COVID-19. Tumor necrosis factor-α has been widely associated with poor prognosis in patients with Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS). In addition, TNF- levels are also related to the degree of disease in COVID-19 patients, therefore TNF- antibodies have the potential to be one approach to COVID-19 therapy (Coperchini et al., 2020)

Based on the data, it can be seen that from 75 samples of patients with NAC, on the first day they had TNF- with an average of 5.83 pg/ml, and on the 8th day the average TNF- became 4.37 pg/ml. Based on the results of statistical tests obtained p-value of 0.019 (p <0.05), so it can be concluded that there is a significant difference in TNF- group with NAC between H1 and H8, where on H8 after administration of adjuvant therapy, TNF- NAC decreased by 1.46 pg/ml

N-acetylcysteine (NAC) works through various mechanisms that are mediated in cells by GSH. It has long been known that NAC is a ROS elimination agent, especially hypochlorous acid (HOCL) and –OH. NAC inhibition of ROS that produces Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase can prevent hypertension and various pathological conditions associated with inflammation such as atherosclerosis (Griendling et al., 2000). In addition, NAC has also been shown to have a protective effect against ARDS. It is known that ROS plays an important role in the pathogenesis of lung injury and that the alveolar epithelial lining of ARDS patients is deficient in GSH. N-acetylcysteine is also able to inhibit viral replication and the expression of pro-inflammatory molecules. N-acetylcysteine

(NAC) has been shown to be able to inhibit pulmonary inflammation, myeloperoxidase (MPO) activity, neutrofilm macrophages, IL-6, IL-1β, CXCL-10, and TNF- (De Flora et al., 2020)

Various studies have explained the benefits and effects of NAC on IL-6 levels, but for COVID-19 cases more data is needed. The effects of NAC on IL-6 are through resistance to NFKB and ROS, as well as glutathione metabolism. Guo et al. in studies using osteoblastic cells (MC3T3-E1) proved that IL-6 levels and NFKB expression decreased after administration of NAC. The study conducted by Paterson et al., proved that IL-6 levels and NFKB expression decreased in the group of sepsis patients given NAC for 72 hours, compared to the group of patients given a placebo. Similarly, a study conducted by Gosset et al., in which there was a significant decrease in IL-6 levels in BAL fluid samples after 48 hours of NAC administration.

Of the 75 samples of group patients with NAC, on the first day had IL-6 levels with an average of 196.95 pg/ml, and on day 8 the average IL-6 became 102.47 pg/ml. It can be concluded that there was a significant difference in il-6 groups with NAC between H1 and H8, wherein H8 after the administration of NAC IL-6 adjuvant therapy decreased by 94.49 pg/ml ($p = 0.001$).

Based on these results it can be concluded that through the administration of NAC adjuvant therapy 5 grams for 72 hours in COVID-19 patients significantly lowered IL-6 levels. Of course, this directly affects the severity of the patient. So that the provision of NAC as an adjuvant therapy can be recommended as a fairly qualified procedure in COVID-19 patients.

CONCLUSION

There is an improvement of oxidative stress and inflammation by administration of NAC as adjunctive therapy in COVID-19 patients assessed by decrease of MDA, IL-6, IL-1β, and TNF-α levels. There is no effect of NAC as adjunctive therapy in TGF-β levels in COVID-19 patients.

Declaration by Authors

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REFERENCES

1. Avdeev S.N., Gaynitdinova V.V. and Merzhoeva Z.M. N-acetylcysteine for the treatment of COVID-19 among hospitalized patients. m5G, 2021, 18:55.
2. Bacher, P. et al. Regulatory T cell specificity directs tolerance versus allergy against aeroantigens in humans. *Cell* 167, 1067–1078 (2016). e1016.
3. Bartziokas, K. and Kostikas, K., 2021. Lactate dehydrogenase, COVID-19, and mortality. Medicina Clinica (English Ed.), 156(1), p.37.
4. Cazzola, M., Rogliani, P., Salvi, S.S., Ora, J. and Matera, M.G., 2021. Use of Thiols in the Treatment of COVID-19: Current Evidence. Lung, pp.1-9.
5. Chu, H. W., Trudeau, J. B., Balzar, S. & Wenzel, S. E. Peripheral blood and airway tissue expression of transforming growth factor beta by neutrophils in asthmatic subjects and normal control subjects. *J. Allergy Clin. Immunol.* 106, 1115–1123 (2000).
6. Connelly K.G., Moss M., Parsons P.E., Moore E.E., Moore F.A., Giclas P.C., et al. Serum ferritin as a predictor of the acute respiratory distress syndrome. *Am J Respir Crit Care Med* 1997;155:21–5.
7. Derouiche, S. (2020). Oxidative stress associated with SARS-Cov-2 (COVID-19) increases the severity of the lung disease-a systematic review. J. Infect. Dis. Epidemiol, 6, 121.
8. Dominari A, Hathaway Iii D, Kapasi A, et al. Bottom-up analysis of emergent properties of N-acetylcysteine as an adjuvant therapy for COVID-19. *World J Virol*. 2021;10(2):34-52. doi:10.5501/wjv.v10.i2.34
9. Fatani S. H. 2014. Biomarkers of oxidative stress in acute and chronic bronchial asthma. J Asthma, Early Online, pp. 1–7.

10. Ferreira-Gomes, M., Kruglov, A., Durek, P. *et al.* SARS-CoV-2 in severe COVID-19 induces a TGF-β-dominated chronic immune response that does not target itself. *Nat Commun* 12, 1961 (2021). https://doi.org/10.1038/s41467-021-22210-3
11. Garcia P.C., Longhi F., Branco R.G., Piva J.P., Lacks D., Tasker R.C. Ferritin levels in children with severe sepsis and septic shock. *ACTA Paediatr* 2007;96:1829–31.
12. Grifoni, A. et al. A sequence homology and bioinformatic approach can predict candidate targets for immune responses to SARS-CoV-2. *Cell Host Microbe* 27, 671–680 e672 (2020).
13. Gutcher, I. et al. Autocrine transforming growth factor-beta1 promotes in vivo Th17 cell differentiation. *Immunity* 34, 396–408 (2011).
14. He, L. et al. Expression of elevated levels of pro-inflammatory cytokines in SARS-CoV-infected ACE2+ cells in SARS patients: relation to the acute lung injury and pathogenesis of SARS. *J. Pathol.* 210, 288–297 (2006).
15. Henry B.M., de Oliveira M., Benoit S., Plebani M., Lippi G. Hematologic, biochem- ical and immune biomarker abnormalities associated with severe illness and mortality in coronavirus disease 2019 (COVID-19): a meta-analysis. *Clin Chem Lab Med* 2020.
16. Ibrahim H, Perl A, Smith D, et al. Therapeutic blockade of inflammation in severe COVID-19 infection with intravenous N-acetylcysteine. *Clin Immunol*. 2020;219:108544. doi:10.1016/j.clim.2020.108544
17. Kell D.B., Pretorius E.. Serum ferritin is an important inflammatory disease marker, as it is mainly a leakage product from damaged cells. *Metallomics* 2014;6:748–73.
18. Kim Y.S., Son A., Kim J., Kwon S.B., Kim M.H., Kim P., et al. Chaperna-mediated assembly of ferritin-based middle east respiratory syndrome-coronavirus nanoparticles. *Front Immunol* 2018;9:1093.
19. Kobune M., Kohgo Y., Kato J., Miyazaki E., Niitsu Y. Interleukin-6 en- hances hepatic transferrin uptake and ferritin expression in rats. *Hepatology* 1994;19:1468–75.
20. Lagan A.L., Quinlan G.J., Mumby S., Melley D.D., Goldstraw P., Bellingan G.J., et al. Variation in iron homeostasis genes between patients with ARDS and healthy control subjects. *Chest* 2008;133:1302–11.
21. Lana J, Lana A, Rodrigues Q, Santos G, Navanie R, Navani A., et al. Nebulization of glutathione and N-Acetylcysteine as an adjuvant therapy for COVID-19 onset. Advances in Redox Research, 2021, 3, 100015. https://doi.org/10.1016/j.arres.2021.100015) .
22. Lee, Y. et al. Induction and molecular signature of pathogenic TH17 cells. *Nat. Immunol.* 13, 991–999 (2012).
23. Li H., Xiang X., Ren H., Xu L., Zhao L., Chen X., et al. Serum amyloid A is a biomarker of severe coronavirus disease and poor prognosis. *J Infect* 2020;80:646–55.
24. Mehri, F., Rahbar, A.H., Ghane, E.T., Souri, B. and Esfahani, M., 2021. The comparison of oxidative markers between Covid-19 patients and healthy subjects: oxidative stress and Covid-19. Archives of Medical Research.
25. Mishra V., Banga J., Silveyra P. 2018. Oxidative Stress and Cellular Pathways of Asthma and Inflammation: Therapeutic Strategies and Pharmacological Targets. Pharmacology and Therapeutics, vol, 181, pp. 169- 182.
26. Muhammad, Y., Kani, Y.A., Iliya, S., Muhammad, J.B., Binji, A., El-Fulaty Ahmad, A., Kabir, M.B., Umar Bindawa, K. and Ahmed, A.U., 2021. Deficiency of antioxidants and increased oxidative stress in COVID-19 patients: A cross-sectional comparative study in Jigawa, Northwestern Nigeria. SAGE open medicine, 9, p.2050312121991246.
27. Palacio J.R, Market U and Martinez P. Anti-inflammatory properties of N-acetylcysteine on lipopolysaccharide-activated macrophages. Inflammation Research, 2011, 60(7):695-704).
28. Parrish-Novak, J. et al. Interleukin 21 and its receptor are involved in NK cell expansion and regulation of lymphocyte function. *Nature* 408, 57–63 (2000).
29. Pasini F, Stranieri C, Cominacini L and Mozzini C. Potential Role of Antioxidant and Anti-Inflammatory Therapies to Prevent Severe SARS-Cov-2 Complications.

Antioxidants 2021, 10, 272. https://doi.org/10.3390/ antiox10020272.
30. Poggiali, E., Zaino, D., Immovilli, P., Rovero, L., Losi, G., Dacrema, A., Nuccetelli, M., Vadacca, G.B., Guidetti, D., Vercelli, A. and Magnacavallo, A., 2020. Lactate dehydrogenase and C-reactive protein as predictors of respiratory failure in CoVID-19 patients. Clinica chimica acta, 509, pp.135-138.
31. Reboldi, A. et al. IgA production requires B cell interaction with subepithelial dendritic cells in Peyer's patches. *Science* 352, aaf4822 (2016).
32. Singh, Z., Karthigesu, I. P., Singh, P., & Rupinder, K. A. U. R. 2014. Use of malondialdehyde as a biomarker for assessing oxidative stress in different disease pathologies: a review. Iranian Journal of Public Health, 43(Supple 3), 7-16.
33. Tenório M.C, Graciliano N.G, Moura F.A, Oliveira A.C and Goulart M.O.F. N-Acetylcysteine (NAC): Impacts on Human Health. Antioxidants, 2021, 10, 967. https://doi.org/10.3390/ antiox10060967.
34. Van Obberghen-Schilling, E., Roche, N. S., Flanders, K. C., Sporn, M. B. & Roberts, A. B. Transforming growth factor beta 1 positively regulates its own expression in normal and transformed cells. *J. Biol. Chem.* 263, 7741–7746 (1988).
35. Wong K, Lee S and Kua K. N-Acetylcysteine as Adjuvant Therapy for COVID-19 – A Perspective on the Current State of the Evidence. Journal of Inflammation Research, 2021, 14 2993–3013.
36. Wong KK, Lee SWH, Kua KP. N-Acetylcysteine as Adjuvant Therapy for COVID-19 - A Perspective on the Current State of the Evidence. *J Inflamm Res*. 2021;14:2993-3013. Published 2021 Jul 6. doi:10.2147/JIR.S306849
37. Wu C., Chen X., Cai Y., Xia J., Zhou X., Xu S., et al. Risk Factors associated with acute respiratory distress syndrome and death in patients with coronavirus disease 2019 pneumonia in Wuhan, China. *JAMA Intern Med* 2020.
38. Yaqinuddin A and Kashir J. Novel therapeutic targets for SARS-CoV-2-induced acute lung injury: targeting a potential IL-1beta/neutrophil extracellular traps feedback loop. Med. Hypotheses, 2020, 143, 109906. doi: 10.1016/j.mehy.2020.109906.
39. Zhao, X., Nicholls, J. M. & Chen, Y. G. Severe acute respiratory syndrome-associated coronavirus nucleocapsid protein interacts with Smad3 and modulates transforming growth factor-beta signaling. *J. Biol. Chem.* 283, 3272–3280 (2008).

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