Oxidative stress and viral Infections: rationale, experiences, and perspectives on N-acetylcysteine

P. SANTUS[1,2], F. DANZO[1,2], A. ZUFFI[1,2], S. PINI[1,2], M. SAAD[1], A. VISCONTI[3], D. RADOVANOVIC[1]

[1]Division of Respiratory Diseases, Ospedale Luigi Sacco, Polo Universitario, ASST Fatebenefratelli-Sacco, Milan, Italy
[2]Department of Biomedical and Clinical Sciences, Università degli Studi di Milano, Milan, Italy
[3]General Management Office, ASST Fatebenefratelli-Sacco, Milan, Italy

Abstract. – This article explores current evidence on the role of oxidative stress in viral infections, and on the use of antioxidant drugs as adjunctive treatment.

MEDLINE/PubMed was searched for appropriate keywords, and preclinical and clinical studies with reviews were retrieved and examined by authors.

Old and current evidence shows that GSH content reduction is the main mechanism of redox imbalance in viral-infected cells. Clinical studies found that GSH levels are depleted in patients with viral infections such as HIV and SARS-CoV. Viral infections activate inflammation through different pathways, and several of these mechanisms are related to oxidative stress. NAC is a precursor of GSH, and many of its intracellular effects are mediated by GSH replenishment, but it also activates some anti-inflammatory mechanisms. NAC has an excellent safety profile and better oral and topical bioavailability than GSH. These characteristics make NAC a suitable option as a repurposed drug.

Adjunctive antioxidant treatment may improve the outcomes of antiviral therapies. Current evidence supports the rationale for this practice and some clinical experience showed encouraging results.

Key Words:
Oxidative stress, Cytokines, Glutathione, N-acetylcysteine, Viral infection.

Introduction

The recent COVID-19 pandemic has prompted research on treatments for managing viral respiratory infections and has drawn attention to drugs suitable for repurposing and likely viable for rapid introduction as adjunctive therapy[1]. This line of research has drawn information from recent preclinical and clinical experience on SARS-CoV-2 and previous observations on other viruses.

Oxidative stress and increased release of cytokines have been among the main mechanisms responsible for infection and disease[2]. They have been suggested as effective therapeutic targets for respiratory viral infections[1]. The effects of antioxidants were investigated, and N-acetylcysteine (NAC), which was found to inhibit viral replication several years before, was presented as a candidate for repurposing, being a safe and experienced drug with antioxidant and anti-inflammatory activities[1-6].

Methodology

This review explores current evidence on the role of oxidative stress in viral infections. It discusses the antioxidant mechanisms involved, with the clinical perspectives for the therapeutic use of antioxidant drugs in the adjunctive treatment of viral infections.

To address the objective of this article, a review of the literature has been carried out. MEDLINE/PubMed was scoured for appropriate keywords: "oxidative stress", "inflammation", "viral infection", "SARS-CoV-2", "HIV", "influenza", "NAC", "glutathione (GSH)", and "anti-oxidant". *In vitro* and *in vivo* preclinical studies, clinical studies, reviews, and meta-analyses were retrieved; articles in English or English abstracts were considered. All retrieved articles were read and examined by authors and were selected based on relevance. This selection was based on the authors' clinical and scientific expertise. A narrative review article was written, reporting published evidence and the expert opinion of the authors.

Corresponding Author: Pierachille Santus, MD, Ph.D; e-mail: pierachille.santus@unimi.it

Oxidative Stress Role in Viral Infections

When respiratory viral infection occurs, blood and tissue redox markers are imbalanced in infected cells. In contrast, lung infection is associated with cytokine production, inflammation, cell death, and tissue damage, triggered by enhanced reactive oxygen species (ROS) production[1,7].

Many years ago, HIV-positive patients were found to have reduced GSH and increased malondialdehyde and total hydroperoxide plasma levels. In these patients, lymphocytes showed increased DNA fragmentation and a significant reduction of glutathione peroxidase (GPx), and erythrocytes had an increased superoxide dismutase activity[8]. Other dated evidence on oxidative stress following viral infection demonstrated that Madin-Darby canine kidney cells infected with Sendai virus showed rapid loss of GSH without an increase in oxidized products. In this study, loss of GSH was mainly due to membrane damage upon virus fusion[9]. Indeed, the GSH cell content reduction can result from decreased GSH synthesis or increased consumption, degradation or transport/leakage, or a combination of these factors, as found in HIV-infected cells[2,10].

Such results have been confirmed and developed in the following years, and it is now known that GSH content reduction is the main mechanism of redox imbalance in viral-infected cells[11]. Indeed, GSH is an important nucleophilic scavenger and an enzyme-catalyzed antioxidant, with a major role in protecting from oxidative tissue injury[12].

The production of ROS and reactive nitrogen species (RNS) is increased in moderate and severe septic shock due to many viral diseases such as SARS-CoV[13,14]. This redox imbalance is associated with elevated expression of iNOS, NADP oxidases, COX-2, and xanthine oxidase, which activate transcription factors, NF-kB; an inflammatory response results, and its progression ensues[13,14].

It has been found that GSH levels are depleted in plasma, epithelial lining fluid, peripheral blood mononuclear cells, and monocytes in asymptomatic HIV-infected subjects and AIDS patients[15]. Moreover, clinical studies have shown that GSH deficiency is correlated with morbidity of AIDS[16]. GSH deficiency contributes to the induction of apoptosis in CD4+ T lymphocytes[8,10]. Decreased GSH levels have been shown to allow activation of NF-kB, with downstream events facilitating HIV expression, and this pathway was blocked by NAC supplementation[17].

Whereas GSH depletion has been associated with viral infections, an increase in GSH intracellular levels has been demonstrated to inhibit HIV replication by blocking NF-kB activation due to oxidative stress and interfering with HIV entry through induction of redox changes in the CD4 D2 domain[18]. Moreover, high levels of GSH inhibit the correct folding and stabilization of viral protein, thus preventing the production of infectious virus particles[5]. Finally, the virion-associated reverse transcriptase, which plays a crucial role in HIV replication, is inhibited in the presence of GSH[19].

In addition, studies on the influenza virus have demonstrated a facilitating role in oxidative stress. Briefly, GSH levels decrease during virus replication, as GSH modulates the life cycle of the influenza virus. This modulation is linked with the antiapoptotic protein Bcl-2, contributing to enhancing intracellular GSH concentrations. Finally, GSH inhibits influenza virus replication[20].

Progressive depletion of GSH with an oxidative stress state was observed during parainfluenza-1 Sendai virus infection, and the administration of exogenous GSH significantly inhibited virus replication[21]. Rhinovirus upregulates its cellular receptor, ICAM-1, in respiratory epithelial cells, and this mechanism depends on the activation of an NF-kB-binding element in the ICAM-1 promoter. GSH can significantly inhibit rhinovirus-induced ICAM-1 upregulation via inhibition of NF-kB activation[22,23].

During the recent pandemic, hospitalized patients with COVID-19 presented severe GSH deficiency, increased levels of thiobarbituric acid reactive substances expressing oxidative stress, and F2-isoprostane, indicative of oxidant damage, compared to uninfected controls. This oxidant damage was present at any age but worsened with increasing age[24].

Inflammation in Viral Infections

Inflammatory mechanisms have a major role in the pathophysiology of viral infections, as many pathological findings may suggest. For example, the pathology of ARDS in infections with SARS coronavirus or H5N1 avian influenza virus is characterized by an accumulation of inflammatory cells, edema, and a relevant increase in cytokines[25,26]. Patients with COVID-19 have a deregulated immune system with decreased lymphocyte count, elevated leukocyte count and neutrophil-lymphocyte-ratio,

8583

and reduced percentages of basophils, eosinophils, and monocytes. Levels of inflammatory cytokines are high, mainly in severe cases, and infection-related biomarkers are present[12].

Viral infections activate inflammation through different pathways, and several of these mechanisms are related to oxidative stress[11]. Proinflammatory cytokines are released by cells exposed to ROS, and, conversely, lipopolysaccharide (LPS) induces both intracellular accumulation of ROS and release of IL-1β, IL-6 and TNF-α. Inflammatory cytokines are regulated through a redox-dependent pathway independent from NF-kB activation and enhanced by GSH depletion[11].

NF-kB-dependent cytokine release is also relevant, as suggested by resistance to acid-induced acute lung injury (ALI) of mice mutant for Toll-like receptor 4 (TLR4), which is necessary for NF-κB activation[27]. The TLR4 Toll/IL-1 receptor domain-containing adaptor inducing the IFN-β (TRIF) pathway is involved in inducing cytokine production by macrophages and lung injury caused by oxidized phospholipids. These phospholipids are produced in the lungs of humans and animals infected with SARS, anthrax or H5N1[27].

The contribution of TLR4 to the pathogenesis of COVID-19 is relevant, as its activation causes an excessive innate immune response. SARS-CoV-2 binds TLR4 and activates TLR4 signaling resulting in increased cell surface expression of ACE2, the binding site of SARS-CoV-2, and facilitation of virus entry. Furthermore, SARS-CoV-2 destroys the type II alveolar cells that secrete pulmonary surfactants, which normally block TLR4 in the lungs by decreasing the air/tissue surface tension; thus, alveolar cell destruction promotes ARDS and inflammation[28,29].

Complement may be involved in the pathogenesis of coronavirus infection disease. Activation of complement (sC5b-9 and C5a) was found in patients with COVID-19, with significantly higher plasma levels in the patients with severe disease than in those with moderate disease[11]. These findings agree with experimental data showing that C3 knockout mice infected with SARS-CoV have less lung disease than wild-type mice[30].

N-Acetylcysteine

NAC is a precursor of GSH, and many of its intracellular effects are mediated by GSH replenishment. At the intracellular level, NAC is deacetylated to yield L-cysteine (L-cys), which is the rate-limiting amino acid in GSH synthe-sis[11]. Increased availability of L-cys promotes the production of the rate-limiting enzyme glutamate-cysteine ligase (GCL), which may be activated to synthesize GSH[29].

NAC has long been used in clinical practice, since the 1960s, as a drug with mucolytic and antioxidant activity, for respiratory diseases and the treatment of acetaminophen poisoning. It has an excellent safety profile and better oral and topical bioavailability than GSH. Its ability to break the disulfide bonds is responsible for the depolymerization of mucin and mucolytic. Breaking disulfide bonds may also reduce the affinity of SARS-CoV-2 for the ACE2 sites, thereby reducing the entry of the viruses into the cell[29,31]. Intravenous NAC at doses as high as 150 mg/kg is standard practice as an approved antidote against acetaminophen intoxication, with efficacy up to 100%, if administered within 8 hours from ingestion[32].

Indeed, the replenishment of GSH in cells activates many mechanisms. Sulfhydryl groups (-SH) of GSH react with electrophilic metabolites in the cell nucleus, resulting in the binding of reactive DNA metabolites and blocking reactive intermediates[33]. Besides its intracellular antioxidant activity, mainly mediated by GSH replenishment, NAC is able to effectively regenerate the free form of Cys34 of human serum albumin, which represents the major and predominant extracellular antioxidant, by breaking the disulfide bond of the cysteinylated form of albumin in plasma[34].

Antioxidant activity is also exerted by inducing p53-mediated apoptosis[11]. Through the generation of L-cys, NAC acts as a hydrogen sulfide donor, which is a readily diffusible vasodilator and anti-inflammatory molecule[35].

A relevant mechanism in the antioxidant activity of NAC is due to the scavenging of ROS and especially of hypochlorous acid (HOCl) and •OH through the SH-groups. NAC molecules can also scavenge some RNS responsible for the oxidation of lipids, proteins, and DNA[11,36]. NAC inhibits the previously mentioned oxidative stress-mediated activation of NF-κB and the dependent pathways for the upregulation of proinflammatory cytokines[37].

NAC activates several anti-inflammatory mechanisms by inhibiting oxidative stress or acting on inflammation mediators. NAC downregulates inflammation and favors transcription of phase II enzyme genes, reducing the stimulation of Nrf2, a high sensitivity transcription factor involved in the cellular antioxidant response[38,39].

NAC was found to elicit an anti-inflammatory activity secondary to neurokinin A (NKA) reduc-

tion and secondarily of IL-6, thus modulating a vicious circle between oxidative stress and neurogenic inflammation[40]. By inhibiting EGFR, a tyrosine kinase involved in inflammation, α1-antitrypsin inactivation is decreased, thereby improving cell protection from inflammatory cell enzymes. In addition, via a GSH-mediated mechanism, NAC improves the structural conformational integrity of α1-antitrypsin and enhances α1-antitrypsin transcytosis, thus improving its cellular uptake and functions[37,41].

The mechanisms induced by NAC of decreased levels of IL-8, IL-6, ICAM, and the soluble alpha receptor for tumor necrosis p55 could be attributed to controlling the inflammatory immune response[42,43]. NAC was demonstrated to inhibit the stress-induced expression of mucins[44,45].

NAC has been demonstrated to exert also an H_2S-generating effect[46]. NAC can be desulfurated to H_2S, a gasotransmitter protective against oxidative stress, which in turn may be oxidized within mitochondria to generate sulfane sulfur species. It was hypothesized that H_2S might counteract SARS-CoV-2 through several targets: host receptors for viral entry, RNA-dependent RNA polymerase necessary for viral replication, and the TLR4 pathway and NLRP3 inflammasome, which plays an important role in the COVID-19 cytokine storm[46]. The main mechanisms activated by NAC in viral infections are depicted in Figure 1.

Preclinical Studies of Anti-Oxidants Activity in Viral Infections: Focus on NAC

Several preclinical studies suggested using NAC as an antioxidant in experimental viral infections[47]. The mechanisms of action of NAC on inflammation and oxidative stress have been demonstrated in *in vitro* and *in vivo* preclinical studies investigating possible targets for viral infection treatment.

Adding GSH in canine kidney cells or human small airway epithelial cells decreased the production of influenza virus particles upon infection. GSH inhibited the expression of viral matrix protein, caspase activation, and *Fas* upregulation. The same researchers found that administration of GSH with drinking water to BALB/c mice decreased the viral titer in the lung and trachea 4 days after intranasal inoculation of the mouse-adapted influenza A/X-31 strain[48].

NAC inhibited the replication of the H5N1 influenza A virus and proinflammatory molecule expression in adenocarcinoma human alveolar basal epithelial (A549) cells[2]. Zhang et al[49] demonstrated decreased production of proinflammatory molecules by NAC. In the lungs of BALB/c mice inoculated intranasally with A/swine/HeBei/012/2008/ H9N2 influenza virus, TLR4 protein and mRNA levels were reduced. Additionally, NAC inhibited pulmonary inflammation and edema as well as myeloperoxidase (MPO) activity, total cells, neutrophils, macrophages, TNF-α, IL-6, IL-1β, and chemokine (C-X-C motif) ligand-10 (CXCL-10) in the bronchoalveolar lavage fluid[49]. NAC inhibited the overexpression and increased release of MUC5AC, IL-8, IL-6, and TNF-α induced by influenza viruses A and B and with a respiratory syncytial virus in alveolar type II epithelial cells[4].

Some studies suggested a possible advantage of combining NAC with a different treatment. The administration of a suboptimal dose of NAC slightly impacted the survival in a murine model of lethal influenza infection. Similarly, the administration of oseltamivir partially improved the survival of infected mice. On the contrary, the combination of NAC and oseltamivir increased survival to 100%[50]. Similar results were obtained in mice infected intranasally with a lethal dose of influenza A virus APR/8 and treated with NAC alone or ribavirin alone, which were slightly effective, or with the combinations of the two drugs increasing the 14-day survival to 92%[51].

The Role of Pollution

The role of oxidative stress in viral infection pathophysiology has been confirmed by studies showing that air pollutants reduce host defenses, resulting in decreased resistance to respiratory infections. For example, exposure of mice to diesel exhaust during infection with influenza virus was associated with an increase in viral titers on days 4 and 8 post-infection. The bronchoalveolar lavage showed increased neutrophils and protein content, and IL-4 expression and production were significantly increased on days 1 and 4, while expression of the Th1 cytokines, IFN-γ, and IL-12p40 was decreased. Treatment with NAC prevented changes in cytokine expression and levels and lung inflammation, although diesel-enhanced virus titers were not changed[52]. Interestingly, treatment with high-dose NAC for 6 months was demonstrated to reduce exacerbation incidence and to improve symptoms and quality of life in patients with COPD[53].
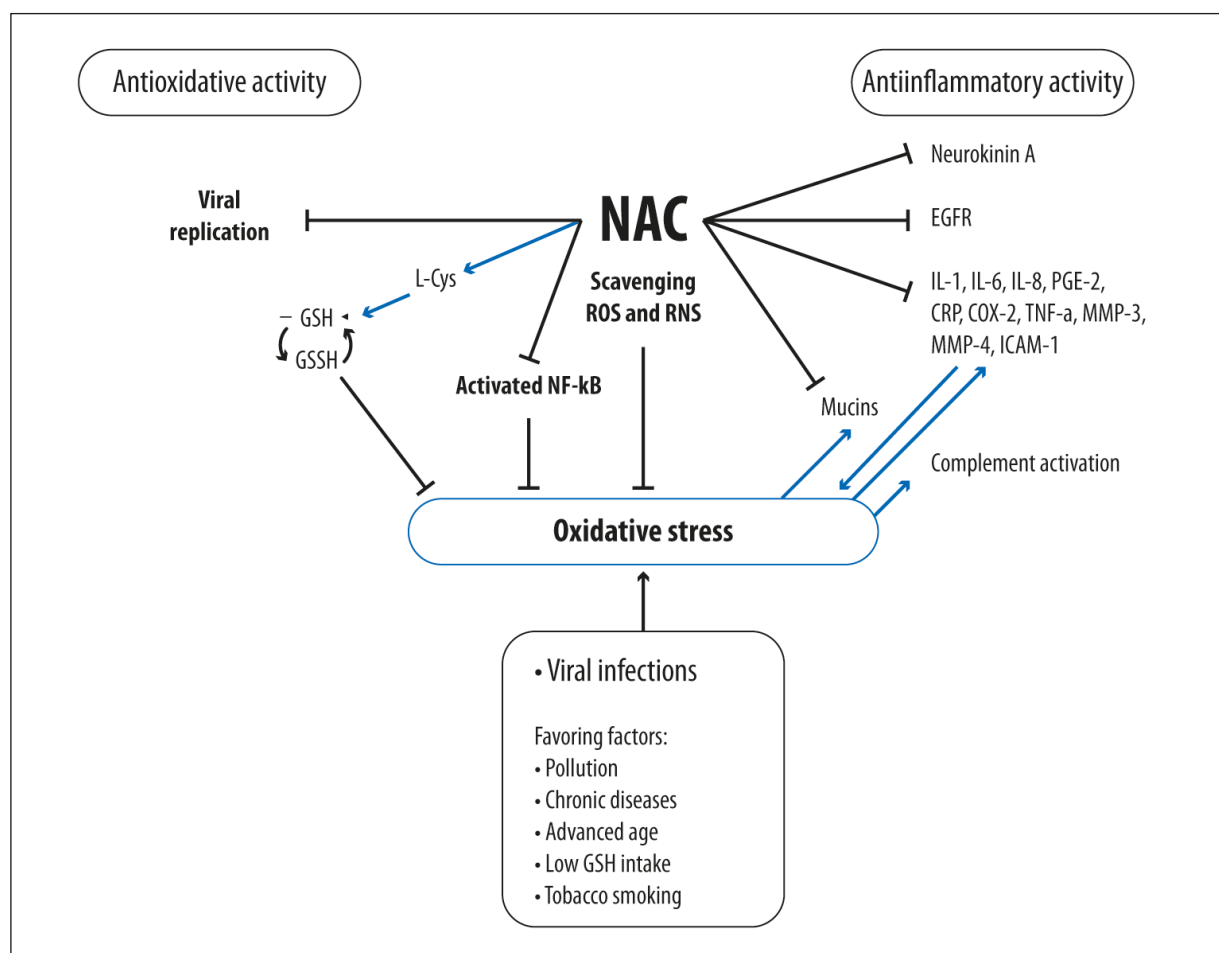
Figure 1. Targets and mechanisms of the protective activity of NAC during viral infections.

Inhibition of Viral Infection and Replication by NAC

Binding of the receptor-binding domain (RBD) to ACE2 is necessary for SARS-CoV-2 binding to the cell surface, and the Cys-488 in the RBD spike is required for SARS-CoV-2 spike functions and infectivity. NAC inhibits the virus entry through this pathway, which may represent a target for early COVID-19 therapy[31]. Molecular dynamic simulations showed that the binding affinity of SARS-CoV-2 to ACE2 was significantly impaired when all the disulfide bonds of both ACE2 and SARS-CoV/CoV-2 spike proteins were reduced to thiol groups, adding to molecular bases for the severity of COVID-19 infection due to oxidative stress[54].

The antioxidant activity of GSH interferes with the late stages of HIV replication in chronically infected macrophages by inhibiting HIV envelope glycoproteins (gp120), a known reservoir of the virus in the body[5]. Both GSH and NAC inhibited the induction of HIV-1 expression in a chronically infected promonocytic cell line (U1/HIV) and human primary cultured monocyte/macrophages. The two compounds decreased HIV-1 p24 antigen levels and the reverse transcriptase activity[5].

Clinical Experiences with NAC in Viral Infections

Although large clinical trials are not available, several clinical experiences confirm the evidence from preclinical studies and suggest a role for NAC in treating viral infections. The administration of GSH concomitantly with standard treatment relieved the dyspnea associated with COVID-19 pneumonia in two patients[55]. In patients with septic shock, a shorter mechanical ventilation time and fewer days of ICU stay were necessary upon treatment with NAC[43]. The administration of

NAC (2×600 mg/day for 8 weeks) to peritoneal dialysis patients in a placebo-controlled study reduced plasma levels of inflammatory markers, including complement (C3)[56,57]. In humans, during influenza infection, NAC inhibited the induction of proinflammatory cytokines through endosomal TLR3/hemagglutinin (HA)-induced ROS-dependent NF-κB activation[58].

A total of 262 subjects (78% were aged ≥65 years and 62% suffered from non-respiratory chronic-degenerative diseases) were randomized to receive either placebo or oral NAC 600 mg twice daily for 6 months. The group receiving NAC experienced a significantly decreased frequency of influenza-like episodes, severity, and length of time confined to bed. Subjects with influenza syndrome had reduced symptoms in the NAC group. Nevertheless, the frequency of seroconversion towards the A/H1N1 Singapore 6/86 influenza virus was similar in the two groups. Only 25% of the virus-infected subjects under NAC treatment developed a symptomatic form, *versus* 79% in the placebo group[59].

Recently, the addition of NAC solution for inhalation to oseltamivir for the treatment of viral infection was associated with a significantly shorter time to the resolution of cough, sore throat, fever, cold sweat and fatigue compared to patients receiving oseltamivir alone ($p<0.05$). Moreover, the levels of inflammatory factor lymphocyte ratio (LY), C-reactive protein (CRP) and procalcitonin (PCT) were lower in patients treated with NAC ($p<0.05$)[60].

Experiences in COVID-19

A retrospective study compared standard of care alone versus standard of care and additional NAC 600 mg bid orally for 14 days, in patients hospitalized with moderate or severe COVID-19 pneumonia. Compared to the control group, treatment with oral NAC led to significantly lower rates of progression to severe respiratory failure ($p<0.01$), significantly lower 14- and 28-day mortality ($p<0.001$ and $p<0.01$ respectively), and progressively improved PO_2/FiO_2 ratio and decreased white blood cell, CRP, D-dimers and LDH levels[61].

Another retrospective study on 1,083 patients hospitalized for COVID-19 pneumonia found that hospital stay was shorter in patients receiving NAC administered at a dosage of 300 mg intravenous TID, switched to 600 mg per os BID once it reached clinical stability and continued until discharge. However, no impact of NAC on short- and long-term outcomes, including in-hospital mortality, ICU admission, impairment of lung diffusing capacity for carbon monoxide, and chest X-ray alterations at the 6-month follow-up, was present[62].

The use of oral NAC at doses of 600 mg every 8 hours in addition to other treatments was associated with significantly lower mortality (OR 0.56; 95% CI: 0.47-0.67) in a community-based study of 19,208 patients hospitalized with a diagnosis of COVID-19, despite these patients being older, more frequently male and with more comorbidities. On the contrary, there were no significant differences with the use of NAC on the mean duration of hospitalization, admission to the intensive care unit or use of invasive mechanical ventilation[63].

Significantly improved respiratory parameters with a shorter hospital stay were found in a case-control study, including 46 patients with confirmed COVID-19 receiving NAC therapy at a daily dose of 1200-1800 mg intravenously within 10 days ($p=0.01$)[64].

Conclusions

Based on the evidence presented in this review, many scientific data underlined as oxidative stress could play a central role in promoting inflammation related to different diseases, including viral infections.

Viral infections are associated with increased production of ROS and decreased activity of antioxidant systems, with activation of a vicious circle where host defenses are reduced. The viral load is progressively increased, resulting in infectious disease. Several biological mechanisms are involved and may represent targets for therapeutic interventions.

We have reported different and interesting evidence supporting the possible use of an antioxidant as an add-on treatment during viral infections. Particularly, NAC could be used as an adjunctive treatment for respiratory viral infections. NAC is a safe drug with several action mechanisms and may be easily administered per os. Current evidence shows that NAC may interfere with virus link to cell receptors and with inflammation and oxidative stress induced by the infection. In addition, NAC may be protective against factors facilitating viral infections, such as pollution.

All these points allow the clinicians to have a drug that is easy to use, safe and well tolerated, opening an interesting perspective regarding the management of viral infections.

Thanks to the capacity of NAC to generate GSH in the lung by oral administration and the

possibility to modulate the dosage, this drug is a good option for clinical practice.

NAC is not an antiviral drug but could be used as an add-on treatment to obtain a good clinical answer thanks to a modulation of different biological systems that are correlated with oxidative stress during disease history and progression.

In conclusion, an adjunctive antioxidant treatment may improve the outcomes of antiviral therapies. Current evidence supports the rationale for this practice and some clinical experience showed encouraging results with the administration of NAC.

Authors' Contributions
Study conception and design: P. Santus; manuscript drafting: all authors equally contributed; approval to submit: all authors.

References

1) Di Marco F, Foti G, Corsico AG. Where are we with the use of N-acetylcysteine as a preventive and adjuvant treatment for COVID-19? Eur Rev Med Pharmacol Sci 2022; 26: 715-721.

2) Micheletto C, Izquierdo JL, Avdeev SN, Rada Escobar RA, Pacheco Gallego MC. N-acetylcysteine as a therapeutic approach to post-COVID-19 pulmonary fibrosis adjunctive treatment. Eur Rev Med Pharmacol Sci 2022; 26: 4872-4880.

3) Geiler J, Michaelis M, Naczk P, Leutz A, Langer K, Doerr HW, Cinatl J Jr. N-acetyl-L-cysteine (NAC) inhibits virus replication and expression of proinflammatory molecules in A549 cells infected with highly pathogenic H5N1 influenza A virus. Biochem Pharmacol 2010; 79: 413–420.

4) Mata M, Morcillo E, Gimeno C, Cortijo J. N-acetyl-L-cysteine (NAC) inhibit mucin synthesis and proinflammatory mediators in alveolar type II epithelial cells infected with influenza virus A and B and with respiratory syncytial virus (RSV). Biochem Pharmacol 2011; 82: 548-555.

5) Palamara AT, Di Francesco P, Ciriolo MR, Buè C, Lafavia E, Rotilio G, Garaci E. Cocaine increases Sendai virus replication in cultured epithelial cells: critical role of the intracellular redox status. Biochem Biophys Res Commun 1996; 228: 579-585.

6) Ho WZ, Douglas SD. Glutathione and N-acetylcysteine suppression of human immunodeficiency virus replication in human monocyte/macrophages in vitro. AIDS Res Hum Retroviruses 1992; 8: 1249-1253.

7) Khomich OA, Kochetkov SN, Bartosch B, Ivanov AV. Redox biology of respiratory viral infections. Viruses 2018; 10: 392.

8) Gil L, Martínez G, González I, Tarinas A, Alvarez A, Giuliani A, Molina R, Tápanes R, Pérez J, León OS. Contribution to characterization of oxidative stress in HIV/AIDS patients. Pharmacol Res 2003; 47: 217-224.

9) Ciriolo MR, Palamara AT, Incerpi S, Lafavia E, Buè MC, De Vito P, Garaci E, Rotilio G. Loss of GSH, oxidative stress, and decrease of intracellular pH as sequential steps in viral infection. J Biol Chem 1997; 272: 2700-2708.

10) Fraternale A, Paoletti MF, Casabianca A, Nencioni L, Garaci E, Palamara AT, Magnani M. GSH and analogs in antiviral therapy. Mol Aspects Med 2009; 30: 99-110.

11) De Flora S, Balansky R, La Maestra S. Rationale for the use of N-acetylcysteine in both prevention and adjuvant therapy of COVID-19. FASEB J 2020; 34: 13185-13193.

12) Soto ME, Guarner-Lans V, Soria-Castro E, Manzano Pech L, Pérez-Torres I. Is anti-oxidant therapy a useful complementary measure for Covid-19 treatment? An algorithm for its application. Medicina (Kaunas) 2020; 56: 386.

13) Pérez-Torres I, Manzano-Pech L, Rubio-Ruíz ME, Soto ME, Guarner-Lans V. Nitrosative stress and its association with cardiometabolic disorders. Molecules 2020; 25: 2555.

14) Liu Z, Ying Y. The inhibitory effect of curcumin on virus-induced cytokine storm and its potential use in the associated severe pneumonia. Front Cell Dev Biol 2020; 8: 479.

15) Buhl R, Jaffe HA, Holroyd KJ, Wells FB, Mastrangeli A, Saltini C, Cantin AM, Crystal RG. Systemic glutathione deficiency in symptom-free HIV-seropositive individuals. Lancet 1989; 2: 1294-1298.

16) Herzenberg LA, De Rosa SC, Dubs JG, Roederer M, Anderson MT, Ela SW, Deresinski SC, Herzen-

berg LA. Glutathione deficiency is associated with impaired survival in HIV disease. Proc Natl Acad Sci USA 1997; 94: 1967-1972.

17) Staal FJ, Roederer M, Herzenberg LA, Herzenberg LA. Intracellular thiols regulate activation of nuclear factor kappa B and transcription of human immunodeficiency virus. Proc Natl Acad Sci USA 1990; 87: 9943-9947.

18) Matthias LJ, Yam PT, Jiang XM, Vandegraaff N, Li P, Poumbourios P, Donoghue N, Hogg PJ. Disulfide exchange in domain 2 of CD4 is required for entry of HIV-1. Nat Immunol 2002; 3: 727-732.

19) Kameoka M, Okada Y, Tobiume M, Kimura T, Ikuta K. Intracellular glutathione as a possible direct blocker of HIV type 1 reverse transcription. AIDS Res Hum Retroviruses 1996; 12: 1635-1638.

20) Nencioni L, Iuvara A, Aquilano K, Ciriolo MR, Cozzolino F, Rotilio G, Garaci E, Palamara AT. Influenza A virus replication is dependent on an antioxidant pathway that involves GSH and Bcl-2. FASEB J 2003; 17: 758-760.

21) Garaci E, Palamara AT, Di Francesco P, Favalli C, Ciriolo MR, Rotilio G. Glutathione inhibits replication and expression of viral proteins in cultured cells infected with Sendai virus. Biochem Biophys Res Commun 1992; 188: 1090-1096.

22) Papi A, Johnston SL. Respiratory epithelial cell expression of vascular cell adhesion molecule-1 and its up-regulation by rhinovirus infection via NF-kappaB and GATA transcription factors. J Biol Chem 1999; 274: 30041-30051.

23) Papi A, Papadopoulos NG, Stanciu LA, Bellettato CM, Pinamonti S, Degitz K, Holgate ST, Johnston SL. Reducing agents inhibit rhinovirus-induced up-regulation of the rhinovirus receptor intercellular adhesion molecule-1 (ICAM-1) in respiratory epithelial cells. FASEB J 2002; 16: 1934-1936.

24) Kumar P, Osahon O, Vides DB, Hanania N, Minard CG, Sekhar RV. Severe glutathione deficiency, oxidative stress and oxidant damage in adults hospitalized with COVID-19: Implications for GlyNAC (glycine and N-acetylcysteine) supplementation. Anti-oxidants (Basel) 2021; 11: 50.

25) Lew TW, Kwek TK, Tai D, Earnest A, Loo S, Singh K, Kwan KM, Chan Y, Yim CF, Bek SL, Kor AC, Yap WS, Chelliah YR, Lai YC, Goh SK. Acute respiratory distress syndrome in critically ill patients with severe acute respiratory syndrome. JAMA 2003; 290: 374-380.

26) Beigel JH, Farrar J, Han AM, Hayden FG, Hyer R, de Jong MD, Lochindarat S, Nguyen TK, Nguyen TH, Tran TH, Nicoll A, Touch S, Yuen KY; Writing Committee of the World Health Organization (WHO) Consultation on Human Influenza A/H5. Avian influenza A (H5N1) infection in humans. N Engl J Med 2005; 353: 1374-1385.

27) Imai Y, Kuba K, Neely GG, Yaghubian-Malhami R, Perkmann T, van Loo G, Ermolaeva M, Veldhuizen R, Leung YH, Wang H, Liu H, Sun Y, Pasparakis M, Kopf M, Mech C, Bavari S, Peiris JS, Slutsky AS, Akira S, Hultqvist M, Holmdahl R, Nicholls J, Jiang C, Binder CJ, Penninger JM. Identification of oxidative stress and Toll-like receptor 4 signaling as a key pathway of acute lung injury. Cell 2008; 133: 235-249.

28) Aboudounya MM, Heads RJ. COVID-19 and Toll-like receptor 4 (TLR4): SARS-CoV-2 may bind and activate TLR4 to increase ACE2 expression, facilitating entry and causing hyperinflammation. Mediators Inflamm 2021; 2021: 8874339.

29) Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, Schiergens TS, Herrler G, Wu NH, Nitsche A, Müller MA, Drosten C, Pöhlmann S. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell 2020; 181: 271-280.e8.

30) Cugno M, Meroni PL, Gualtierotti R, Griffini S, Grovetti E, Torri A, Panigada M, Aliberti S, Blasi F, Tedesco F, Peyvandi F. Complement activation in patients with COVID-19: A novel therapeutic target. J Allergy Clin Immunol 2020; 146: 215-217.

31) Murae M, Shimizu Y, Yamamoto Y, Kobayashi A, Houri M, Inoue T, Irie T, Gemba R, Kondo Y, Nakano Y, Miyazaki S, Yamada D, Saitoh A, Ishii I, Onodera T, Takahashi Y, Wakita T, Fukasawa M, Noguchi K. The function of SARS-CoV-2 spike protein is impaired by disulfide-bond disruption with mutation at cysteine-488 and by thiol-reactive N-acetyl-cysteine and glutathione. Biochem Biophys Res Commun 2022; 597: 30-36.

32) Ershad M, Wermuth HR, Vearrier D. N Acetylcysteine. In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2020.

33) De Flora S, Izzotti A, D'Agostini F, Balansky RM, Balansky RM. Mechanisms of N-acetylcysteine in the prevention of DNA damage and cancer, with special reference to smoking-related end-points. Carcinogenesis 2001; 22: 999-1013.

34) Altomare A, Baron G, Brioschi M, et al. N-acetyl-cysteine regenerates albumin Cys34 by a thiol-disulfide breaking mechanism: An explanation of its extracellular anti-oxidant activity. Anti-oxidants (Basel) 2020; 9: 367.

35) Aldini G, Altomare A, Baron G, Vistoli G, Carini M, Borsani L, Sergio F. N-Acetylcysteine as an antioxidant and disulphide breaking agent: the reasons why. Free Radic Res 2018; 52: 751-762.

36) Tardiolo G, Bramanti P, Mazzon E. Overview on the effects of N-acetylcysteine in neurodegenerative diseases. Molecules 2018; 23: pii: E3305.

37) Sadowska AM. N-Acetylcysteine mucolysis in the management of chronic obstructive pulmonary disease. Ther Adv Respir Dis 2012; 6: 127-135.

38) Zhou Y, Wang HD, Zhou XM, Fang J, Zhu L, Ding K. N-acetylcysteine amide provides neuroprotection via Nrf2-ARE pathway in a mouse model of traumatic brain injury. Drug Des Devel Ther 2018; 2018: 4117-4127.

39) Zhao N, Guo FF, Xie KQ, Zeng T. Targeting Nrf-2 is a promising intervention approach for the prevention of ethanol-induced liver disease. Cell Mol Life Sci 2018; 75: 3143-3157.

40) Calzetta L, Matera MG, Rogliani P, Cazzola M. Multifaceted activity of N-acetyl-l-cysteine in

chronic obstructive pulmonary disease. Expert Rev Respir Med 2018; 12: 693-708.

41) Patel K, Cheng G, Rucker L, Bazzle G, Nadig S, Atkinson C. N-acetylcysteine potentiates the protective effects of α-1-antitrypsin in a mouse model of orthotopic lung transplantation. J Heart Lung Transpl 2017; 36: S371.

42) Rahman I, MacNee W. Regulation of redox glutathione levels and gene transcription in lung inflammation: therapeutic approaches. Free Radic Biol Med. 2000;28(9):1405-20.

43) Koksel O, Cinel I, Tamer L, Cinel L, Ozdulger A, Kanik A, Ercan B, Oral U. N-acetylcysteine inhibits peroxynitrite-mediated damage in oleic acid-induced lung injury. Pulm Pharmacol Ther 2004; 17: 263-270.

44) Xu X, Li Q, Li L, Zeng M, Zhou X, Cheng Z. Endoplasmic reticulum stress/XBP1 promotes airway mucin secretion under the influence of neutrophil elastase. Int J Mol Med. 2021; 47: 81.

45) Menicagli L. Pulmonary covid fibrosis a new pharmaceutic approach. Int J Prev Med 2021; 12: 35.

46) Bourgonje AR, Offringa AK, van Eijk LE, Abdulle AE, Hillebrands JL, van der Voort PHJ, van Goor H, van Hezik EJ. N-acetylcysteine and hydrogen sulfide in coronavirus disease 2019. Antioxid Redox Signal 2021; 35: 1207-1225.

47) Patel VJ, Biswas Roy S, Mehta HJ, Joo M, Sadikot RT. Alternative and natural therapies for acute lung injury and acute respiratory distress syndrome. Biomed Res Int 2018; 2018: 2476824.

48) Cai J, Chen Y, Seth S, Furukawa S, Compans RW, Jones DP. Inhibition of influenza infection by glutathione. Free Radic Biol Med 2003; 34: 928-936.

49) Zhang RH, Li CH, Wang CL, Xu MJ, Xu T, Wei D, Liu BJ, Wang GH, Tian SF. N-acetyl-l-cysteine (NAC) protects against H9N2 swine influenza virus-induced acute lung injury. Int Immunopharmacol 2014; 22: 1-8.

50) Garozzo A, Tempera G, Ungheri D, Timpanaro R, Castro A. N-acetylcysteine synergizes with oseltamivir in protecting mice from lethal influenza infection. Int J Immunopathol Pharmacol 2007; 20: 349-354.

51) Ghezzi P, Ungheri D. Synergistic combination of N-acetylcysteine and ribavirin to protect from lethal influenza viral infection in a mouse model. Int J Immunopathol Pharmacol 2004; 17: 99-102.

52) Gowdy KM, Krantz QT, King C, Boykin E, Jaspers I, Linak WP, Gilmour MI. Role of oxidative stress on diesel-enhanced influenza infection in mice. Part Fibre Toxicol 2010; 7: 34.

53) Kolarov V, Kotur Stevuljević J, Ilić M, Bogdan M, Tušek B, Agic A, Dugajlić M, Tot Vereš K, Kutlešić Stević S, Zvezdin B. Factorial analysis of N-acetylcysteine and propolis treatment effects on symptoms, life quality and exacerbations in patients with

Chronic Obstructive Pulmonary Disease (COPD): a randomized, double-blind, placebo-controlled trial. Eur Rev Med Pharmacol Sci 2022; 26: 3192-3199.

54) Hati S, Bhattacharyya S. Impact of thiol-disulfide balance on the binding of COVID-19 spike protein with angiotensin-converting enzyme 2 receptor. ACS Omega 2020; 5: 16292-16298.

55) Horowitz RI, Freeman PR, Bruzzese J. Efficacy of glutathione therapy in relieving dyspnea associated with COVID-19 pneumonia: A report of 2 cases. Respir Med Case Rep 2020; 30: 101063.

56) Maglakelidze N, Manto KM, Craig TJ. A Review: Does Complement or the Contact System Have a Role in Protection or Pathogenesis of COVID-19? Pulm Ther 2020; 6: 169-176.

57) Purwanto B, Prasetyo DH. Effect of oral N-acetylcysteine treatment on immune system in continuous ambulatory peritoneal dialysis patients. Acta Med Indones 2020; 44: 140-144.

58) Lai KY, Wing Yiu GNG, Cheng FF. The W-shaped mortality-age distribution of novel H1N1 influenza virus helps reconstruct the second wave of pandemic 1918 Spanish flu. J Pulm Respir Med 2015; 5: 2.

59) De Flora S, Grassi C, Carati L. Attenuation of influenza-like symptomatology and improvement of cell-mediated immunity with long-term N-acetylcysteine treatment. Eur Respir J 1997; 10: 1535-1541.

60) Cao M, Huang L, Lu P. Effect analysis of acetylcysteine solution for inhalation combined with oseltamivir phosphate in the treatment of patients with viral pneumonia. J Med Theor Pract 2022; 35: 1497-1501.

61) Assimakopoulos SF, Aretha D, Komninos D, Dimitropoulou D, Lagadinou M, Leonidou L, Oikonomou I, Mouzaki A, Marangos M. N-acetyl-cysteine reduces the risk for mechanical ventilation and mortality in patients with COVID-19 pneumonia: a two-center retrospective cohort study. Infect Dis (Lond) 2021; 53: 847-854.

62) Faverio P, Rebora P, Rossi E, Del Giudice S, Montanelli F, Garzillo L, Busnelli S, Luppi F, Valsecchi MG, Pesci A. Impact of N-Acetylcysteine on SARS-CoV-2 pneumonia and its sequelae: results from a large cohort study. ERJ Open Res 2021; 2022; 8: 00542-2021.

63) Izquierdo JL, Soriano JB, González Y, Lumbreras S, Ancochea J, Echeverry C, Rodríguez JM. Use of N-acetylcysteine at high doses as an oral treatment for patients hospitalized with COVID-19. Sci Prog 2022; 105: 368504221074574.

64) Avdeev SN, Gaynitdinova VV, Merzhoeva ZM, Berikkhanov ZG. N-acetylcysteine for the treatment of COVID-19 among hospitalized patients. J Infect 2022; 84: 94-118.