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# N-acetylcysteine - A Convenient Rationale for COVID-19 Consideration of Antiviral $H_2S$ for Inclusion in one of the ANTICOV or WHO Master Protocols

Ed J van Hezik MD
Senior Consultant Chest Physician, Waterfront at Sea – *a non-profit working group*
2235TD-10 Valkenburg ZH, The Netherlands

## Abstract

With the emerging mutations and new pandemic waves, there remains a need for an effective antiviral, administered safely and easily in the early treatment phase of SARS-CoV-2, despite the current roll-out of vaccines.
For antiviral options in COVID-19 two studies deserve our attention:

1--Ten consecutive severe COVID-19 cases, on ventilator as well ECMO support, all recovered completely and fairly rapid by high doses of N-acetylcysteine (NAC) without any mortality.

2--Another study found that serum $H_2S$ level is a prognostic marker in COVID-19 pneumonia. A low serum level $H_2S$ at admission or a decrease during infection significantly increased the risk of death in COVID-19 patients (n = 74).

Combining these two findings may give us even more options.
Stepwise we explore how $H_2S$ works in viral respiratory diseases and we focus on the targets in COVID-19: the cell entry (ACE2 receptor), the virus replication (RdRp, nsp12), and the escalation of inflammation to a lethal cytokine storm (NLRP3 inflammasome).
Finally, consider the question: How to administer $H_2S$? Dissecting the degradation of NAC shows how the endogenous $H_2S$ level can be generated and with which drugs. Already 13 well-documented human cases have successfully supported this approach. The antiviral application of the endogenous $H_2S$ provides a pathway to reactivate the collapsed innate immunity as a treatment regimen for COVID-19, in early out-patient as well as later clinical situations. Further randomized controlled trials are warranted, with a consideration of antiviral $H_2S$ for inclusion in one of the ANTICOV or WHO protocols.



**How to Cite:**

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## Introduction

The coronavirus disease 2019 (COVID-19) caused by the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has developed since December 2019 and caused a global pandemic with more than one million fatalities globally. Hallmarks of this type of coronavirus compared to older types are a high infectivity and more severe inflammation. During the ongoing Covid-19 pandemic, we have so far only found evidence of dexamethasone as a treatment (RECOVERY Collaborative Group, NEJM). Other antiviral candidates lacked evidence-based study results in a swirl of drug trials and also in the guidelines (Solidarity Therapeutics Trial WHO, Mehra MR 2020; Moynihan 2020; Boulware DR, 2020). The rapid development resulted in a number of promising vaccines, but the logistical allocation and danger of the current SARS-CoV-2 mutants may be prohibitive (Korber, 2020) and, moreover, obtaining a sustainable antibody response is still under investigation. There is no validated antiviral drug yet pending our vaccination turn, so in addition to timely steroids, supportive care, anticoagulants, and ventilator support, we should consider well-designed trials on this.

But is there some light at the end of the tunnel if we think more associatively about COVID-19 treatment? Indeed, SARS-CoV-2 contamination is usually mild at ~ 80%, but there are known risk factors that can cause deterioration, suggesting that a host factor is disturbed by these factors (such as hypertension, diabetes, obesity and older age). Via a screening of previous preclinical virus protection studies, hydrogen sulfide ($H_2S$) then comes to our attention.

In this article, we investigate why $H_2S$ supplementation may be beneficial for the treatment of COVID 19 disease. Next, it is important whether dissecting the breakdown of NAC can give us insights to modulate the level of $H_2S$.

In three steps we will therefore examine whether $H_2S$ 1) can be an antiviral host factor, 2) can be generated from N-acetylcysteine and 3) can act multi-targeted in SARS-CoV-2 infection.

## § Viewpoint 1 – $H_2S$: an antiviral host factor

Recently a study defined serum $H_2S$ a prognostic factor in COVID-19 (Renieris ,2020). In severe COVID-19 pneumonia cases (n=74) the authors found that for mortality :

-Low serum levels of $H_2S$ on day 1 had the best trade-off for sensitivity and specificity;
-Decrease in serum level $H_2S$ from day 1 to day 7 greater of 36% as the best discriminator.
-Mortality after 4 weeks was 32% ***vs*** 4.1% for suboptimal ***vs*** optimal level $H_2S$.
-Serum $H_2S$ was negatively associated with IL-6, Procalcitonin and CRP .
-The 4 weeks survivors are those who consume less of this $H_2S$.

This evidence suggests that the reduction of $H_2S$ bioavailability may be considered as an indicator of enhanced pro-inflammatory response and that the administration of exogenous $H_2S$ may be viewed as a pharmacological strategy to restore $H_2S$ plasma levels in order to counteract the severe consequences of COVID-19 infection (Renieris et al. 2020).
This also may give rise to a first statement that $H_2S$ is a Host Factor in COVID-19.

## About $H_2S$

$H_2S$ is endogenously produced out of sulfur amino acids (SAA) like cysteine, and it's level is very strictly regulated. After all, $H_2S$ was previously known as a poison.
$H_2S$ is produced from L-cysteine by cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE), but from 3-mercaptopyruvate (3MP) also mercaptopyruvate sulfurtransferase (3MST) produces $H_2S$ , which is produced from cysteine and α-ketoglutarate by cysteine aminotransferase (CAT). SAA and their derivatives transport sulfur through successive oxidation reactions,which then repeatedly also release $H_2S$.

$H_2S$ permeates all membranes freely as a gasotransmitter (like NO and CO) and unlike classical regulators of signal transduction, it acts independently of transmembrane receptors (Wang, 2002).Produced in mammalian tissues, $H_2S$ acts as biological mediator and signals many important physiological processesin humans. Marutani (2020) streamlines clearly the sulphur redox reactions.

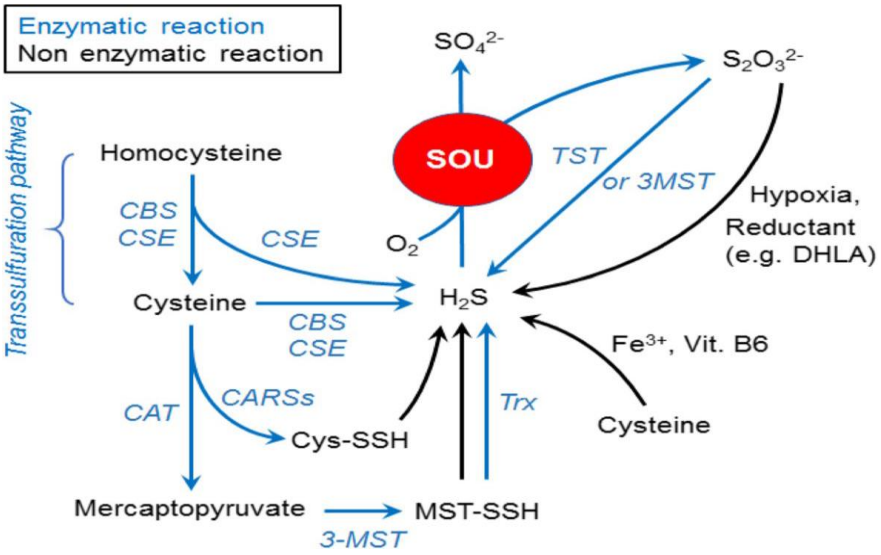



Figure 1- $H_2S$ release - Marutani et al (2020)

## $H_2S$ in Preclinical studies. *Anti-viral and anti-inflammatory effects*

In four preclinical studies the group of Casola* uncovered a critical protective role of $H_2S$ in RSV infection *in vitro* and *in vivo*, by modulating innate inflammatory responses and viral replication.
They used enzyme blocking, donor $H_2S$ and knock-out models and they reduced the viral replication and chemokine secretion by modulation of transcription factors nuclear factor (NF)-kB and the interferon regulatory factor (IRF)-3.
Casola and coworkers uncovered two mechanisms that were at play. Firstly the donor $H_2S$ treatment drastically reduced the secretion of the cytokines IL-6, TNF-α and G-CSF, as well as the chemokines IL-8, RANTES, IP-10, MCP-1 and MIP-1β from infected cells. Secondly airway epithelial cells infected with RSV display decreased ability to generate endogenous $H_2S$ and enhanced degradation of $H_2S$, indicating that the viral infection leads to changes in $H_2S$ cellular homeostasis or $H_2S$ depletion.
(***** Bazhanov,Casola, 2017;Bazhanov,Escaffre, 2017; Ivanciuc T, 2016;Li, Ma, Y.,2015).

These well-designed preclinical studies provided a solid foundation for the antiviral and anti-inflammatory effects of $H_2S$. Therefore, additional studies were expected to elaborate on this. Currently the rationale for using $H_2S$ in COVID-19 is supported in several other studies, such as Yang (2020), Evgen'ev (2020), and Citi (2020).
The antiviral activity of a series of $H_2S$ releasing molecules and reference $H_2S$ donors (e.g., GYY4137 and sodium hydrosulfide), was first tested and this preliminary screen showed that most of the sulfur molecules provided a significant antiviral effect.
A disulfide compound (XM-01) was selected for further evaluation on both enveloped and non-enveloped viral strains, such as RSV, influenza virus (A / WSN / 33 strain) and rotavirus. As observed in previous GYY4137 studies, XM-01 showed antiviral effects on enveloped viruses only. Remarkably, no activity was observed on non-enveloped viruses such as rotavirus. Since the antiviral activity may be due to viral membrane changes, GYY4137 and its analogues may be useful against enveloped viruses especially at the time of viral entry into the host cell. Recently, a paper hypothesized that $H_2S$ may exhibit antiviral activity against SARS-CoV-2 by interfering with the ACE2 receptor and TMPRSS2 (Yang, 2020).

## § **Viewpoint 2** -- NAC generates $H_2S$

The above preclinical research leads us to suggest that it may be useful to generate $H_2S$ in mammals. Previous research suggests that NAC is able to do this: Zanardo et al (2006) using intravital microscopy in animals found anti inflammatory effects at the leuko-endothelial interface induced not only by donor $H_2S$ but also by NAC. Inhibition of the CSE enzyme reversed all these NAC effects. This suggested, for the first time to our knowledge, that NAC could generate $H_2S$.

Ezerina et al (2018) confirmed this effect of NAC, by dissecting in NAC the antioxidant effect from the $H_2S$-generating potential. NAC-derived cysteine has been shown to be desulfurated to generate hydrogen sulfide which is then oxidized in mitochondria to sulfane sulfur species. These sulfane sulfur species would be the actual substances responsible for mediating the antioxidant and cytoprotective effects we previously attributed to NAC. A different degradation pathway for NAC may be used, via cysteine and 3 MP, to generate $H_2S$ and sulfane sulfur species such as persulfides.(Ezerina, 2018; Cerda 2018 Zhao,2018; DiNicolantonio,2017); later Zuhra et al (2019) confirmed this finding and also Yadav et al (2020) suggest NAC, serving as a source of cysteine, could support MST activity. In SARS-CoV-2 infection, it may be appropriate to restore cysteine and $H_2S$ levels immediately in order to maintain the antiviral and anti-inflammatory effects; this can be achieved in two different ways:

### a) Taurine suppletion:

In the breakdown of cysteine, the CSE enzyme is strongly boosted by taurine.Taurine increases the expression of the $H_2S$-synthesizing enzymes CBS and CSE, and thereby it contributes strongly to increase (doubling) the endogenous $H_2S$ level in a human RCT (Sun,2016; Zaorska,2020). An animal study supports this principle (Zhao 2018). This option is apt if there is sufficient substrate like cysteine: otherwise consider option b).

**b) N-acetylcysteine suppletion:**
Important here is: (extracellular located) NAC in itself should not be considered a powerful antioxidant: its power is the targeted replenishment of the intracellular glutathione (GSH) stock in deficient cells and it is unlikely to be effective in cells already packed with GSH. This intracellular GSH stock is apt for the formation of cysteine. And, if necessary, this cysteine can be further desulphurized to produce $H_2S$ combined with a so-called sulfansulfur (the latter is the actual antioxidant). To investigate the antiviral therapy against the SARS-CoV-2 virus, we opt to use this endogenously produced $H_2S$, by administering the prodrug NAC. Therefore, it is possible now to conclude that treatment of cells with the Cys-prodrug NAC triggers endogenous $H_2S$ production.

**§ Viewpoint 3** -- $H_2S$ acts multi-targeted in SARS-CoV-2 infection.

**3a- Collaps of Innate Immunity and Amino acids** *Suppression-Exhaustion-Suppletion*
The components of the innate immune system act as first responders for the detection and clearance of viral infections. But many viral infections evade the host innate immune response, sometimes resulting in a complete Host Shut-Off (reviewed in detail by Kikkert, 2020). Then the virus will hijack the host's defenses by total capture of the cellular translation machinery for its own use.

In addition, the virus bypasses the type I interferon (IFN-I) response, which normally promotes an antiviral state in both the infected and neighboring cells, limiting viral replication and inducing apoptosis to protect the organism from virus spread. Indeed, coronaviruses have evolved multiple strategies to escape and to counteract innate detection and IFN-I production. Such efficient strategies allow the virus to replicate and disseminate in infected individuals without encountering the initial host defense.While many arms of the innate immune response are potently activated by COVID-19, in comparison to other respiratory viruses, SARS-CoV-2 infection drives a lower antiviral transcriptional response.This is marked by low IFN-I and IFN-III levels and elevated chemokine expression, suggesting that some aspects of the innate immune response to COVID-19 might actually benefit from a more careful amplification. (Blanco-Melo et al., 2020). The secretion of both *IFN-α* and *-β* were all reduced by donor $H_2S$ (Ivanciuc,2016).

Also, significant dysregulation of monocytes and macrophages seems to be a feature of severe COVID-19, apart from a decrease in T cell levels, a significantly higher neutrophil-to-lymphocyte ratio (NLR) and depleted peripheral NK cell counts. The number of total T cells, CD4+ and CD8+ T cells were dramatically reduced in COVID-19 patients and correlated negatively with survival (Vardhana,2020). COVID-19 patients show significantly higher levels of the exhausted marker PD-1 (Yang ,2020). T cell numbers in patients were negatively correlated to serum IL-6, IL-10, and TNF-α concentration, but $H_2S$ can positively impact these concentrations. It reduced IL-6 and TNF-α-induced NF-κB activation. $H_2S$ also potentiates the T cell activation (Miller 2012).

On the other hand, SARS-CoV-2 infection caused an acute depletion of the sulfur-containing amino acids (SAA) as a function of oxidant stress or inflammation-induced proteolysis. Indeed, cysteine and taurine levels tended to decrease (Thomas 2020), especially with moderately high IL-6. In contrast, the oxidized forms of SAA (methionine sulfoxide, cystine) increased. Altogether, this acutely reduces the bioavailability of cysteine as a substrate for $H_2S$.

To compensate for this also acutely, NAC treatment safely replenishes whole blood GSH and T-cell GSH, as seen in HIV-infected persons with the same depletion of SAA (De Rosa, 2000, Dröge, 1993). GSH acts as a supply of cysteine. A rapid response of changes in cysteine levels was observed within hours of NAC supplementation by Stipanuk (2009).

**3b- NLRP3 Inflammasome** *A Potential Drug Target In COVID-19*

Although, innate immune mechanisms such as an optimal activation of the NLRP3 inflammasome plays an important role in antiviral host defenses, its aberrant activation and downstream mediators often lead to pathological tissue injury during infection.(Costa 2019) Also, infection with SARS-CoV is known to induce a storm of pro-inflammatory cytokines, especially IL-1β, IL-6, and TNF. These play an important role in the progression of tissue inflammation causing acute respiratory distress syndrome ARDS and often leads to death. Hydrogen sulfide inhibits NLRP3 inflammasome activation and reduces cytokine production .(Castelblanco 2018).

**3c -The vascular compartment** *ARDS - ACE-2 expression - coagulation - CVD*

$H_2S$ modulates leukocyte-mediated inflammation. In the leuko-endothelial interface it interferes with leukoadhesion and leukoinfiltration (Zanardo 2006). In COVID-19 this could have some relevance for the complicating variant ARDS (Gattinoni,2020) or SIRS and other complicating vascular problems like myocardial infarction ,stroke, thrombosis and embolia (Klok,2020; Wang, 2018).The ACE2 receptor is not only necessary for the viral entry in the AECs, but the ACE2 receptor is also expressed in the vascular system.(Hamming,2004).
SARS-CoV infection reduces ACE2 expression in lung cells and that loss of pulmonary ACE2 function is associated with acute lung injury. As virus-induced ACE2 downregulation may be important for disease pathology, then on the contrary ACE2 upregulation by $H_2S$ (as shown by Lin, 2017) may attenuate the acute lung injury. And at last, a deficiency of $H_2S$-producing enzymes results in hypertension, and administration of $H_2S$ donors lowers blood pressure and protects against organ damage in the experimental setting. (van Goor, 2016).

**3d - Pharmaceutical aspects of N-Acetylcysteine** *disulfide bonds*

High Dosing: NAC is used safely for some 30 years in case of acetaminophen intoxication. N-Acetylcysteine may be applied as tablet, intravenously or by nebulization. Its mostly mentioned anti-oxidant effect was dissected in 2018 from its $H_2S$ generating effect in a breakthrough study (Ezerina,2018; Cerda,2018), later confirmed by two other studies. NAC easily penetrates cells where it is deacetylated to yield L-cysteine thereby promoting GSH synthesis. Therefore, NAC works per se in the extracellular environment and as a precursor of GSH inside cells. Accordingly, all its intracellular effects are mediated by GSH replenishment (de Flora 2020).

NAC has mucolytic properties on sputum by breaking disulfide bonds in the mucus (Otu, 2018), while $H_2S$ shows only antiviral effects on enveloped viruses, which use a fusion protein for the cell entry (Citi, 2020).

The ability to break disulfide bonds may be important to this fusion process, as the SARS coronavirus peak S2 domain is flanked by cysteine residues C822 and C833 and this domain is required for membrane fusion activation. FP2 has some effect on the membrane sequence. Two cysteines (C822 and C833) within FP2 are considered an internal disulfide bond, giving this domain a loop structure. It is questionable whether these disulfide bonds will resist the local $H_2S$ and / or NAC in the membrane fusion region (Madu, 2009).
Lai et al tested whether such a disulfide bond could play a role in the FP2-mediated membrane ordering. Indeed they found that in the presence of 5 mM dithiothreitol (DTT), a reducing agent that removes disulfide bonds, the membrane-ordering effect of FP2 was abrogated (Lai, 2017).

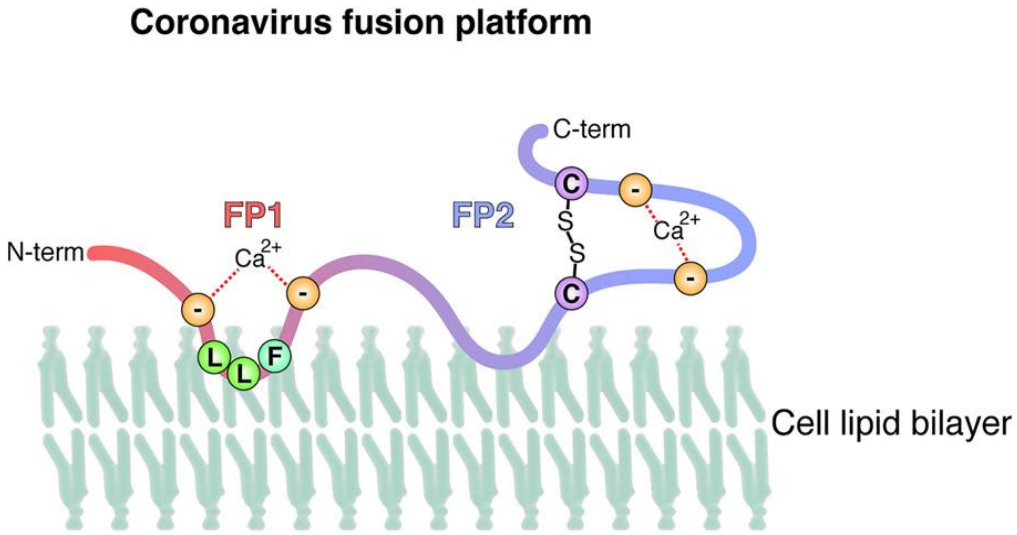



**Fig 2. Receptor – Fusion peptide - target for NAC and $H_2S$ (Madu)**

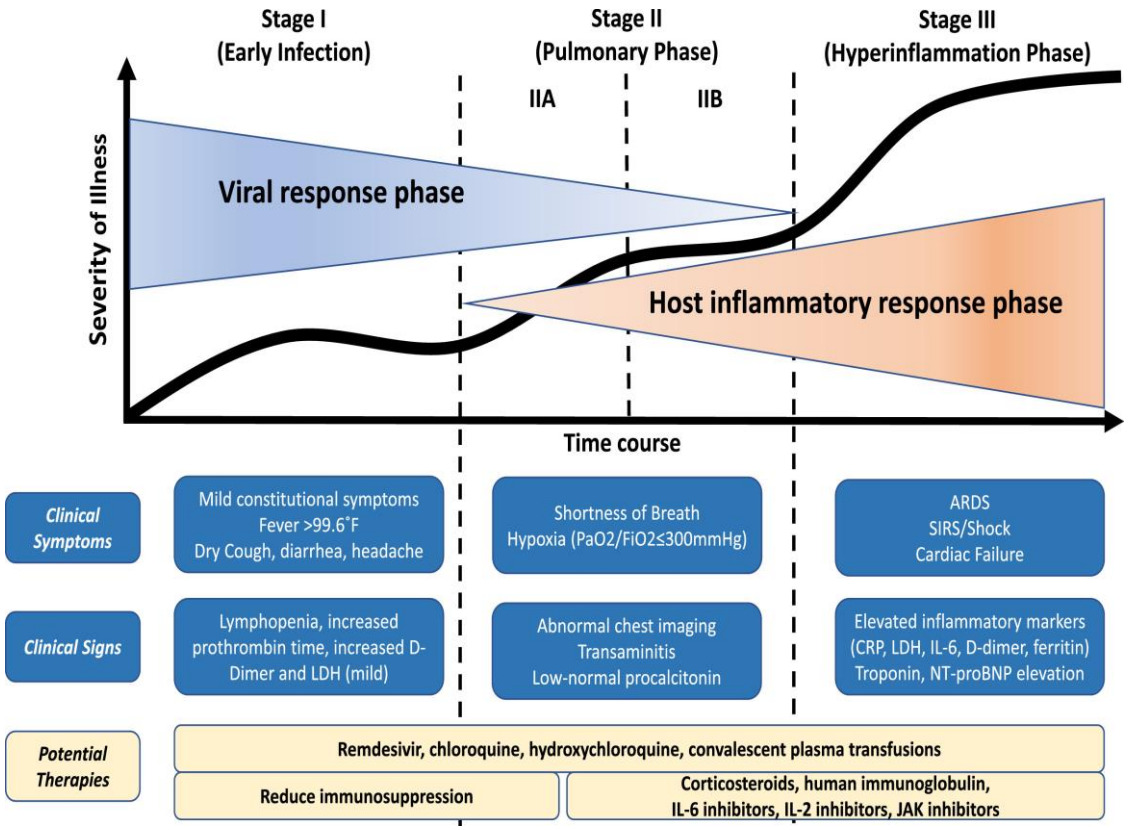
Not only at the cell entry (ACE2 recptor) but also in the cytosol it makes sense to consider a same antiviral effect. The RNA-dependent RNA polymerase (RdRp), also named nsp12 may be a target in SARS-CoV-2. In the cytosol this is the central component of the coronaviral replication and transcription machinery.
Recently Gao et al (2020) were able to identify an N-terminal β hairpin (D29 to K50) which inserts into the groove clamped by the NiRAN domain and the palm subdomain in the RdRp domain and forms a set of close contacts to stabilize the overall structure . In the absence of DTT they showed C301 to C306 and C487 to C645 to form disulfide bonds. But - in the presence of DTT - chelated zinc ions were present in the same location as that observed in SARS-CoV. So it is doubtful whether the disulfide bonds in the nsp12-nsp7-nsp8 complex of the SARS-CoV-2 virus will resist the local effects of $H_2S$ and/or NAC.
Note that $H_2S$ functions as a gasotransmitter and is not bothered by membranes.

**3e -- Timing of the treatment** *initial virusload and subsequent inflammation*

Treatment timing is very important when this NAC-Taurine model is combined with other antiviral treatments (e.g., tocilizumab; dexametasone); their order depends on the stage of the disease (Siddiqi, 2020).

In one study, only high-dose NAC was given alone and started too late, without any clinical benefit (Alencar 2020).

CoV-2 infection initially has a extremely high viral load at the onset of symptoms, so the early antiviral (e.g. NAC & nebulizer) component is important. If symptoms are worsening around 7-10 days, adding dexametasone to NAC should be considered to control or reverse the progressive inflammation.
One thing remains to be emphasized: use NAC only when necessary, especially with an enveloped virus (Corona, Influenza), no maintenance.
Start only with an incipient viral infection or preventively with a nearby viral threat.
Chronic intake of NAC / $H_2S$ is not recommended.




----------N-Acetylcysteine----------|----------added Dexametason----------

**Figure 3 -The Timing of Treatment in COVID-19** (Siddiqi,2020)

## § 4 - Is $H_2S$ clinical relevant in COVID-19 ?

**4a-** *A prognostic factor in severe COVID*

A Greek study was published defining serum $H_2S$ a prognostic factor in COVID-19.(Renieris ,2020). In COVID-19 pneumonia cases (n=74) authors found that **for mortality** :
-Low serum levels of $H_2S$ on day 1 had the best trade-off for sensitivity and specificity;
-A decrease in level $H_2S$ from day 1 to day 7 greater of 36% as the best discriminator.
- The mortality after 4 weeks was 32% *vs* 4.1% for suboptimal *vs* optimal level $H_2S$.
- Serum $H_2S$ was negatively associated with IL-6, Procalcitonin and CRP .
- The 4 weeks survivors are those who consume less of this $H_2S$.
This leads to considerations for exogenous $H_2S$ supplementation as a treatment strategy.

### 4 b – Checking some risk factors in COVID-19 , related to low $H_2S$

Risk factors in COVID-19 are known in general. Nevertheless, a number of risk factors for a serious course have been found that may suggest a relationship with $H_2S$ :

***Gender risk***: more men then women affected: Firstly it may relate to the ACE2 gene, only located on the X chromosome. Otherwise oestrogen boosts expression of CSE in the vasculature ,so boosting generation of $H_2S$ (Li ,2017).

***Hypertension :*** a deficiency of $H_2S$-producing enzymes (van Goor, 2016).

***Diabetes***: the lower $H_2S$ blood values (Jain, 2010).

***Obesity***: blood levels of $H_2S$ were twice as low vs normal (Whiteman, 2010).

***Advanced age***: the efficiency of glutathione synthesis and glutathione tissue levels decline with age. This age-related deficit in GSH can be corrected with supplemental NAC (Sekhar, 2011). From that GSH stock , cysteine generates $H_2S$.

***Young patients, ages 2-15 yrs:*** Multisystem inflammatory syndrome in children (MIS-C) is a serious condition that appears to be linked to coronavirus disease 2019 (COV*ID-19)* with inflammation in multiple organ systems and features of Kawasaki disease following to SARS-CoV-2 infection; in the acute phase, plasma $H_2S$ is low.(Datillo, 2020)

***Beneficial course in infants and toddlers***: taurine in breast milk or legally added to bottled milk, this increases the $H_2S$ synthesis. (DiNicolantonio, 2017).

**4c Successful Case reports**: N =13

- Ibrahim et al (2020) described 10 consecutive patients with severe COVID (10 on ventilator and 9 on ECMO) with good clinical response on high dose NAC, with corresponding decrease in CRP and ferritine. No mortality here, quite unexpected (expected 37.4% ,Barbaro ,Lancet ,2020). Steroids were also administered.

- Horowitz (2020) described in 2 COVID patients immediate improvement after suppletion with glutathione (GSH) and NAC. Worth to mention: NAC is a direct precursor of GSH. Glutathione inhibits viral replication in mice (Amatore 2019).

- Puyo (2020) successfully treated one critical ill COVID patient with high dose NAC intravenously at 75 mg/kg over 4 hours, then 35 mg/kg over 16 hours, followed by 17 mg/kg over 24 hours on Day 2. Also HCQ was given on day 1 and day 2. Detailed clinical parameters showed fast improvement, although a complicating tromboembolic activation was seen.

**4d - Trials in progress** (as off 5-aug-2020)**:**

Additional research on NAC in COVID is started in 3 clinical trials :(USA and China):

NCT04419025 ; Boston Cambridge Health Alliance
NCT04279197 ; Shanghai ShuGuang Hospital
NCT04374461 ; Memorial Sloan Kettering Cancer Center NY

Caution- If only NAC alone is started and too late (later then 7-10 days after the onset of symptoms) and no course of steroids is added around that time, then the clinical result will be negative (Alencar 2020). Siddiqi (2020) proposed a more logical sequence in medication.

**4 d - NAC used in other (NON-Corona) viral states** *influenza and HIV-1*

1-- In influenza, NAC was effective with a lower incidence and much lower disease burden in a predominantly elderly cohort (De Flora, 1997). Oral N-acetylcysteine did not prevent viral infection, but at the same rate of seroconversion, it greatly reduced the incidence of clinically significant H1N1 disease ,the severity of local and systemic symptoms and length of time confined to bed. ( No symptoms with NAC 75% vs with placebo 21%)

2-- Severe H1N1 Influenza pneumosepsis was treated with high dose NAC (100 mg/kg) continuous 3 days. It showed (twice times) a fast improvement in weaning, oxygenation and CRP (Lai, 2010). However different influenza strains show different effects of NAC (Garigliany, 2011).

3-- in HIV-1 patients: Lower concentration in blood of cystine**,** tryptophan, and methionine were suppleted with oral NAC (Hortin, 1994). The concentrations of cysteine and glutathione increases in mononuclear cells of patients with HIV infection (de Quay, 1992).

Sadly no $H_2S$ was measured in these studies.

## Discussion

This review reveals a role for endogenous hydrogen sulfide ($H_2S$) as a fundamental defense mechanism against viral infections, guided by these three viewpoints: 1) $H_2S$ acts as an antiviral host factor; 2) $H_2S$ can be generated by N-acetylcysteine (NAC) and taurine; 3) $H_2S$ acts multi-targeted in SARS-CoV-2 infection.

1- Based on the studies discussed above (in vitro, in vivo, preclinical and clinical), $H_2S$ emerged as a host factor for viral infections, while in addition an adequate serum $H_2S$ level was attributed a role as a prognostic marker of COVID- 19 pneumonia. With corona, the clinical course is usually 80% mild; 15% have desaturation requiring hospitalization and 5% require IC care.
This natural remedy suggests that an antiviral host factor is active here, but some risk factors disrupt the natural course: Gender risk, diabetes, obesity, advanced age, as opposed to a favorable course infants and toddlers. This natural remedy seems to be a sufficient level of $H_2S$. This has already been checked in section 4 b.

2- The possibility that NAC could act as an $H_2S$ donor was for the first time demonstrated by Zanardo (2006), later on by Ezerina (2018) and then endorsed by others (2019, 2020). This finding has since been somewhat overlooked or ignored, with much more emphasis on the often cited "antioxidant" activity (which is actually related to only the sulfane-sulfur component) and less emphasis on the associated $H_2S$ release by NAC derived cysteine. Indeed, the COVID-19 treatment can be viewed from two sides:
Yang (2020) viewed it from a '$H_2S$ standpoint', while Poe & Corn (2020) and De Flora (2020) worked from a 'NAC as an anti-oxidant' position.
In fact we can conclude that they are all looking at the same processes and we are not surprised that the results are the same or match flawlessly. Both pathways (antioxidant and $H_2S$) signal via oxidation reactions with protein cysteine sulfur and both produce identical effector responses (Olson 2020).

3-Multiple targets can be used by $H_2S$ against the coronavirus, such as the cell entry, the virus replication and the escalation of inflammation to a cytokine storm, which were also targets explored in the recent drug trials mentionned in the introduction.

The attack of the virus acutely collapses both the immune cells of the innate immunity and the (supply of) sulfur amino acids. The latter ensures that $H_2S$ decreases very quickly. On the other hand, to correct this, also the generation of endogenous $H_2S$ appears to be a very dynamic, fast-acting process (evidenced by the case reports) and this suggests that, from a safety standpoint, $H_2S$ will immediately degrade once it has fulfilled its function, which also explains why measuring $H_2S$ remains a challenge.

But even in severe cases of COVID-19, a fast, high dose of NAC appears to have sufficient effect for supplementation, according to the clinical success in the patient cases. This safety is also evidenced by the relatively low serum and tissue concentrations measured *in vivo* versus the high and fast concentrations by the artificial $H_2S$ donors in the previous *in vitro* studies. Endogenous $H_2S$ generation is apparently safe, but further intensive dose finding

studies are warranted, especially when administered in the high dose range. The rationale for using $H_2S$ in COVID-19 is supported in several 4 other studies, like Yang; Citi ; Evgen'ev; Datillo (all in 2020), but in those studies the use of $H_2S$ still has not yet been linked to NAC as a $H_2S$ donor.

From another viewpoint, Poe & Corn (2020) and De Flora (2020) investigated the rationale for the use of NAC in COVID-19, but yet again without taking into account the $H_2S$ generating capacity of NAC.

Also the timing of combination in treatment is very important and is determined by the ratio of the viral load to inflammation.By starting antiviral therapy in a timely manner and then containing the inflammatory process at an early stage, it is possible to prevent the disease from developing and causing severe damage during the inflammation phase.(Siddiqi, 2020). NAC only, and started too late was without clinical benefit (Alencar 2020).
Preferentially the viral killing NAC (& nebulizer) therapy is introduced in an early phase. Steroids [eg dexametason] in the second phase, follows the evidence of the Recovery trial. The large safety margin of NAC and the various options in its administration make it possible to scale up with the time course and severity of the SARS-CoV-2 infection, starting with prevention in case of (suspected) virus contact, via home medication [home confinement for isolation or for quarantaine] to hospital or ICU ward application. A proposal for therapy is provided on researchgate.net/publication/345982081 (22). The repurposed drug NAC used here, is over-the-counter, very safe, without side effects, and cheap, making it very feasible for conducting Randomized Controlled clinical trials.

Of Course, it is also clear that not one drug alone, will give a sufficient effect on COVID-19. An intelligent combination of different drugs may prove necessary, but even if it is not possible to fully suppress the virus, also a mild course with less hospitalizations or ICU admissions, will suffice.
The drug NAC as a endogenous $H_2S$ generator to reactivate the collapsed innate immunity, it deserves consideration for inclusion in one of the ANTICOV or WHO master protocols, and may always be additive to any supportive care and can be combined with other antiviral schemes at the discretion of the treating physician.

## Conclusion

The described process of endogenously generating $H_2S$ provides a multi-targeted antiviral Host Factor in COVID-19 infection by reactivation of the collapsed innate immunity. Application of this generated $H_2S$ as a pharmacologic antiviral is already sustained by the successful outcomes in quite a dozen case studies with very severe COVID pneumonia. We may assume that also the milder phases of COVID-19 may be treatable with this antiviral host factor, maybe even preventively, avoiding unwanted socio-economic measures and health care overload. Randomized controlled clinical trials are needed.

## Competing Interests

The author declares that he has no relevant or material financial interests that relate to the research described in this paper. No funding. ICMJE Form for Disclosure of Potential Conflicts of Interest 14-april-2020 Dr. van HEZIK has nothing to disclose.

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