

In light of the SARS-CoV-2 pandemic: revisit of the evidence associating Zinc and anti-viral response

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Summary

Since the discovery of the first reported case with Zinc-deficiency in Iran¹ by Prasad et al. in 1961, the knowledge on Zinc has increased significantly. Zinc is the second most abundant common trace mineral in the human body, responsible for vital biological functions from cell growth and development to cell homeostasis and immune response^{2,3}. Up to a fifth of the global population is estimated to suffer from different degrees of Zinc deficiency⁴. In the western world, Zinc deficiency is more prevalent among the geriatric population³, vegans/vegetarians, and people with certain underlying conditions⁴ such as liver cirrhosis, inflammatory bowel disease, and various auto-immune disorders^{4,5}. The Zinc and Zinc deficiency has been associated with several infectious diseases^{2,3}.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) which is responsible for the ongoing pandemic belongs to the family of coronaviruses. SARS-CoV-2 has a high genetic similarity to another family member, SARS-CoV, which caused the first major epidemic of the 21st century^{6,7}. Currently there is no evidence linking the anti-SARS-CoV-2 response and the element Zinc.

Herein and in light of the SARS-CoV-2 pandemic, we marshal the evidence associating the element Zinc with the anti-viral and antibacterial immune response as well as the cytokine storm and lung injury. Such a revisit of the precedent evidence may inspire the further

investigation assessing the relationship between Zincemia status and the anti-viral response in SARS-CoV-2 patients.

Zinc deficiency hampers anti-viral and antibacterial immune response

Zinc deficiency is associated with increased risk of infectious diarrhea and pneumonia among children, as it is associated with an increased risk of developing *Staphylococcus aureus* pneumonia and *Streptococcus pneumoniae* tonsillitis infections among the elderly population^{3,8}. Zinc correction in children might be associated with decreased incidence and prevalence of pneumonia-related with these infections^{2,9,10}. The most recent meta-analysis investigating the pneumonia-preventive value of Zinc supplementation in children concludes that the Zinc correction in children is associated with 13% and 41% reduced incidence and prevalence of pneumonia, respectively⁹. Another meta-analysis, however, finds that Zinc correction does not significantly impact the mortality due to lower respiratory tract infection in children¹¹.

Elderly SARS-CoV-2 patients are those with the highest fatality rates, the very subgroup of patients who are at high risk for Zinc deficiency. Of note, pneumonia due to *Staphylococcus aureus* and pneumococcal infections are among the fatal co-infections of SARS-CoV-2¹², especially in the same subgroup of patients.

Toll-like receptors (TLR) are the major mediators of the innate anti-viral immune response¹³. TLR7/9-mediated virus recognition by plasmacytoid dendritic cells leads to a typical anti-viral IFN type I (IFN- β and IFN- α) response that can be applied to SARS-CoV-2 as well¹⁴⁻¹⁶. Interestingly, Zinc correction can restore IFN type I response in Zinc-deficient patients^{17,18}. In contrast, high Zinc levels can hamper IFN type III (IFN- λ 3) anti-viral response¹⁹, which also might influence anti-SARS-COV-2 response²⁰. This paradoxical effect highlights the delicacy of the possible Zinc role in the anti-viral immune response.

We question whether Zinc-deficient SARS-CoV-2 patients are at higher risk of mortality due to a weak or inefficient cellular and/or humoral immunity in response to SARS-CoV-2. Currently, there is no data available on such a correlation concerning SARS-CoV-2.

Zinc deficiency is associated with cytokine storm and lung injury

Zinc-deficiency not only hampers our immune system in terms of lack of efficient response when required⁸, but it can also be associated with an excessive and damaging response.

Zinc deficiency increases the risk of hyperinflammatory lung damage^{21,22} induced by polymicrobial sepsis^{23,24}, alcohol²⁵, hyperoxia²⁶, and ventilator²⁷ in mice and rats. Notably, Zinc deficiency is associated with an increased risk of acute respiratory distress syndrome (ARDS) in humans²⁷. One of the fatal complications of SARS-CoV-2 in a subgroup of patients is indeed the “Cytokine Storm” syndrome and the related acute respiratory distress syndrome (ARDS)^{16,28}. The majority of intracellular Zinc is protein-bound, but a tiny fraction is unbound and labile. The free Zinc is involved in cellular signal transduction together with Nitric-Oxide (NO). In pulmonary endothelial cells, NO-induced labile Zinc signaling has an indispensable role in hypoxic vasoconstriction, cytoprotection, and inhibition of cell-apoptosis^{29–32}. On the other hand, Zinc deficiency is associated with systemic oxidative stress and decreased Nitric-Oxide (NO) induced vasodilation^{32,33}.

Zinc concentrations among patients suffering from different auto-immune disorders appear to be lower than those observed in healthy individuals³⁴. Zinc deficiency is also associated with the excessive and tissue-damaging pro-inflammatory release of cytokines, including increased TNF- α ^{35,36} and IL-6³⁷. Excessive TNF- α and IL-6 are incriminated for lung-damage and the fatal “Cytokine Storm” in a subgroup of SARS-CoV-2 patients. In contrast, Zinc correction is associated with the down-regulation of TNF- α ^{8,35,36}.

However, concerning the effect of Zinc levels on IL-6 levels, there is a discrepancy in the literature. One study reports that Zinc deficiency is associated with increased IL-6 gene expression in mice³⁸, while another study on a human experimental model reports that Zinc deficiency does not alter IL-6 levels¹⁸. Nevertheless, among the geriatric female population, a gene polymorphism that leads to an increased immune response-mediated release of Zinc is associated with decreased levels of IL-6³⁹. On the other hand, Zinc supplementation can even lead to the up-regulation of IL-6 levels⁴⁰. Despite the dilemma on the quantitative association of Zinc and IL-6, Zinc correction inversely affects the IL-6-mediated response and signaling^{18,37,38,41}, which is often^{18,37,38,41} but not always⁴² considered as a pro-inflammatory response.

Altogether, these observations merit special attention concerning SARS-CoV-2 prevention and patients management²⁸.

Zinc has direct anti-viral activity

Several *in-vitro* studies have shown that Zinc and Zinc ionophores halt the RNA replication of different RNA viruses, including the ones that can infect the human respiratory system^{43–50}.

Initially, in 1984, Eby et al. show that Zinc gluconate lozenges could be effective in reducing the common colds associated with viral infections of the upper respiratory tract⁵¹. Since then, further studies resulted in varying and conflicting conclusions on the efficacy of Zinc supplementation in the treatment of common colds⁵². Reanalysis of the reports from 1984 to 1992 by George Eby on the efficacy of zinc lozenges in the treatment of common colds revealed the importance of Zinc

lozenge formulation and type of zinc ion bioavailability in determining its efficacy⁵³. Only the Zinc lozenges which were releasing positive Zinc ions at the physiological pH such as gluconate or acetate forms and not the ones with negative or neutral ion bioavailability were effective in shortening the disease period. Zinc lozenges with negative Zinc ion bioavailability even adversely increased the common cold duration⁵³. The same author later put forward a hypothesis that the chemical formulation of the Zinc lozenge form determines its efficacy against common colds. Another subsequent Eby's reanalysis of twelve precedent reports suggested that his hypothesis could explain the varying results among the reports and additionally underscored the significance of a minimal dose needed for Zinc efficacy. Eby recommends an initial dose of 200mg for Zinc followed by 100-150mg daily doses for seven days during common colds treatment⁵⁴ and names the lower dose of 75mg a "candy" instead of a drug. A further meta-analysis by Eby also leads to similar conclusions⁵⁵. A meta-analysis by Hemila further concluded no superiority of gluconate vs. acetate form and no significantly greater efficacy of doses above 100mg⁵². A recent Finnish randomized controlled trial conducted by Hemila et al. finds no benefit of zinc acetate in treating the common cold⁵⁶. Interestingly, Hemila et al. administered the Zinc lozenge at the dose of 78 mg per day zinc for five days, a dosage, and a duration in total disagreement with Eby's recommendation⁵⁶. As concluded by Hemila et al., further investigation is justified to determine the efficacy and optimal dose and duration of Zinc in treating common colds.

Velthuis et al. have shown that a candidate Zinc ionophore (pyrithione) and Zinc itself could inhibit SARS-CoV replication as single agents and better when combined. Notably, Velthuis et al.⁵⁷ showed that Zinc could directly disrupt the initiation of RNA synthesis by the SARS-CoV RNA-dependent RNA polymerase (RdRp) *in-vitro*, possibly via interacting with two Zinc-binding pockets present in the SARS-CoV RdRp⁵⁷. However, likely due to the low cellular uptake of Zinc, the optimal Zinc concentration to efficiently inhibit the SARS-CoV replication was relatively high⁵⁷. Furthermore, a Chinese group who unraveled the SARS-CoV-2 RdRp protein structure⁵⁸ observed Zinc ions being chelated at the two candidate pockets within the RdRp of SARS-CoV-2 as well.

Despite the absence of conclusive clinical data on the efficacy of two old drugs⁵⁹; Chloroquine (CQ) and Hydroxychloroquine (HCQ), in SARS-CoV-2 patients, the off-label use of these medications has become widespread.

Zn and (H)CQ might interact at the cellular level. Xue et al. have shown that CQ increases Zinc uptake in ovarian cancer cells and mediates Zinc accumulation into the lysosomes of these cells⁶⁰. In contrast, a study⁶¹ by Seo et al. partially contradicts these findings reporting that CQ decreases the free Zinc levels in lysosomes in adult retinal pigment epithelial cells. However, they still observed some increased intracellular Zinc levels upon CQ treatment compared to the control group. If HCQ is a Zinc ionophore, then increasing lysosomal Zinc uptake in the epithelial cells of the respiratory system may contribute to its anti-SARS-CoV2 mechanism of action.

However, if conversely, HCQ interferes with Zinc lysosomal uptake, it would be interesting to learn whether increasing Zinc plasma concentration can counteract this adverse effect.

Whether (H)CQ are global Zinc ionophores or rather interfere with Zinc uptake into the cells or organelles should remain an open question and the subject of further investigation at this stage.

In figure 1, we summarize three lines of evidence concerning the probable link between Zinc correction and the response to anti-viral treatments that might be applied to SARS-CoV-2 (figure 1).

The practicality of Zinc correction

Until today, no consensus and accepted guidelines concerning Zinc deficiency diagnosis and correction has been reached. Radioisotopes can be a sensitive diagnostic tool, but they are costly and exposes patients to radioactive substances. New biomarkers such as Linoleic Acid are emerging⁶²⁻⁶⁵. However, considering the vast population involved with SARS-CoV-2 and the high economic burden of such approaches, we propose a cheap and straightforward method to evaluate Zn status. The modalities of Zn supplementation are also provided.

Evaluation of Zinc status: Serum or plasma Zinc

Plasma and serum Zinc concentration are the most frequently used biomarkers to establish the Zinc status of a patient⁶⁶. Plasma Zinc concentrations respond in a dose-dependent fashion to supplementation in patients with a low or moderate baseline, independent of gender and age⁶⁷. Consequently, Zinc deprivation results in a reduction of the plasma Zinc concentrations. Zinc deficiency is defined as serum Zinc concentrations below 70 and 74 $\mu\text{g}/\text{dL}$ for females and males over ten years old, respectively⁶⁸.

Nevertheless, several other conditions also affect plasma Zinc concentrations, including hypoalbuminemia, infections, fever, contraceptives, and pregnancy. Concerning SARS-CoV-2 patients, the status of two widely used biomarkers of inflammation^{69,70}, C-reactive protein, and procalcitonin, are inversely associated with serum Zinc levels^{71,72}. The interpretation of the concentration of plasma Zinc must, therefore, take into account all the confounding factors⁶⁷.

Treatment:

The proposed dose of Zinc (ZnSO_4) injection for parenteral nutrition in metabolically stable adult patients is 3 mg/day⁷³. The recommended oral daily dose for Zinc correction in adults is 10-30 mg/day^{3,74}. The maximum level of the excipient used in FDA-approved oral Zinc tablets (excluding extended-release forms) is 25 mg⁷⁵.

Long term Zinc supplementation nevertheless should be very well-weighed⁷⁶ as the therapeutic

window of chronic Zinc supplementation is relatively narrow (reviewed here⁷⁶). In contrast, short term Zinc supplementation may be less of a concern³⁷⁶.

Conclusion

Some evidence links the anti-viral response and Zinc deficiency.

The possible link between Zinc deficiency, clinical evolution, and response to anti-SARS-CoV2 treatments, particularly in those at risk of developing hyperinflammatory complications, deserves further investigation.

Author Contributions

A.N and G.V developed the initial idea and co-drafted the first draft under M.H supervision. D.D critically revised the manuscript and contributed to authoring the revised version. M.M revised the manuscript and contributed to authoring the revised version. C.G critically revised the manuscript and contributed to authoring the revised version. M.H supervised the manuscript drafting and critically revised the manuscript and co-authored the final version.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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Legends

Figure 1 Three lines of evidence associating Zinc and anti-viral response

