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Can Zinc correction in SARS-CoV-2 patients improve treatment outcomes?

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The ongoing severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic for which there is no established treatment available yet, has caused more than 68,000 deaths so far (https://www.worldometers.info/coronavirus/).

SARS-CoV-2 belongs to the family of coronaviruses and has a high genetic similarity to another family member SARS-CoV which caused the first major epidemic of 21st century[1,2].

SARS-CoV-2 has probably been zoonotically transmitted from their likely host bats to humans via an intermediate host, namely Malayan pangolins[3,4].

Following the SARS-CoV outbreak in 2003, an Italian group put forward a hypothesis about the efficacy of two old drugs: Chloroquine (CQ) and Hydroxychloroquine (HCQ), against SARS-CoV and its future emergents[5].

This hypothesis was shown to be relevant in-vitro, first by a Belgian-[6] and then a Canadian study[7].

Due to the high genetic similarity of SARS-CoV-2 and SARS-CoV, the hypothesis introduced by Savarino et al. and the further supportive in-vitro evidence served a rational ground for three different Chinese groups to test the efficacy of CQ or HCQ against SARS-CoV-2 in-vitro [8–10]. These studies showed promising in-vitro efficacy of CQ and HCQ against SARS-CoV-2[8–10].

Unfortunately, in the absence of sufficient clinical data on the (in)efficacy of CQ and HCQ in SARS-CoV-2 patients, the compassionate and off-label use of these medications is becoming politicized.

Herein, we underline some critical features of the CQ/HCQ mechanism of action concerning SARS-CoV-2.

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Moreover, we introduce a hypothesis on a probable link between zinc-deficiency/zinc correction and response to CQ/HCQ- and possibly other SARS-CoV-2 treatments.

SARS-CoV-2 outbreak and reincarnation of (Hydroxy)Chloroquine: ephemeral or long-lasting?

CQ and HCQ have experienced an adventurous journey since their synthesis and a life rich in repurposing, from anti-malaria agents to immunomodulatory medications of several auto-immune disorders[11].

Following the SARS-CoV epidemic in 2003, Savarino et.al[5] proposed even a new application for CQ and HCQ as potential anti-SARS agents yet.

Savarino et al. considered three lines of pre-SARS era evidence to base on their hypothesis.

HCQ and CQ are weak bases. They can be accumulated in the lysosomes and Golgi apparatus and thereby alkalizing their pH. Thus, HCQ and CQ can interfere with the pH-dependent endosome-mediated viral entry of several viruses[5,11].

On the other hand, it was known that CQ could interfere with post-translational modification of some enveloped viruses, their assembly, and budding within the endosomes and trans-Golgi vesicle networks[5,11].

As both of these mechanisms could be applied to SARS-CoV pathogenesis, Savarino et al. hypothesized that CQ/HCQ could have anti- SARS-CoV effects.

Besides these two antiviral capacities of CQ and HCQ, Savarino et al. keenly devoted their attention to another feature of these two drugs being their ability to relieve the excessive and damaging antiviral (among others) immune response. Indeed, CQ and HCQ can decrease the production of tumor necrosis factor-alpha (TNF-α) and interleukin-6, and downregulate both TNF receptors 1 and 2[5,11]. As such, CQ and HCQ mitigate macrophage response, monocyte activation, leukocyte extravasation and therefore alleviate the damaging immune response in various tissues, including lung[5,12,13].

The excessive and damaging immune response could also be one of the consequences of SARS-CoV infection. Therefore, Savarino et al. proposed that the immunomodulatory effect of CQ and HCQ could play an essential role in the battle against SARS-CoV and other emerging coronaviruses in the future[5].

Notably, today we learn that 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) [14,15].

With regards to cytokine storm and ARDS management, recently, Zhou et al. have underlined the HCQ capacity to repress CD154 expressing T-cells, among other inhibitory mechanisms[14].

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Both CQ and HCQ are reported with such an effect, but Zhou et al. recommend HCQ over CQ for SARS-CoV-2 treatment due to a better safety profile[14,16,17].

Interestingly, Zhou et al. have also highlighted another mechanism by which HCQ/CQ interferes with coronavirus binding to the host cells, a feature that can contribute to their anti-SARS-CoV-2 effects[14].

Despite promising pre-clinical evidence and intellectual efforts on considering the CQ/HCQ as a potential therapy against SARS-CoV-2, the clinical evidence is still far from being conclusive[18–23].

Hypothesis: Zinc correction in SARS-CoV-2 patients improves HCQ/CQ and other treatments outcomes

Since the discovery of the first reported case with zinc-deficiency in Iran[24] by Prasad et al. in 1961, we have learned a lot about Zinc, and we have much more left to learn.

Zinc is the second most abundant common trace mineral in the human body, with vital biological functions from cell growth and development to cell homeostasis and immune response [25,26].

Up to a fifth of the global population is estimated to suffer from different degrees of Zinc deficiency[27]. In the western world, Zinc deficiency is more prevalent among the geriatric population[26] and vegans/vegetarians as well as among people with certain underlying conditions[27].

Notably, the early reports show that the elderly SARS-CoV-2 patients are among those with a higher fatality rate[28].

Herein, we summarize three lines of evidence that underscore the significance of Zinc and a possible link with the response to any anti-SARS-CoV-2 treatment, including CQ/HCQ therapy (figure 1).

Zinc deficiency hampers antiviral and antibacterial immune response

Zinc deficiency has been associated with increased risk of infectious diarrhea and pneumonia among children, while Zinc supplements were shown to have a corrective impact on both complications[29].

Moreover, zinc deficiency, whether mild or severe, can negatively impact different adult human organs, including the immune system[25,26,30].

Zinc deficiency is associated with increased risk of developing Staphylococcus aureus pneumonia and streptococcus pneumonia tonsillitis infections[30] among the elderly population.

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This risk can reduce, upon Zinc correction in the geriatric population[25]. Markedly, both of these respiratory system infections are reported among the fatal co-infections of SARS-CoV-2[31].

Additionally, zinc is associated with antiviral immune response in general, involving various viruses[27].

Not surprisingly, yet there is no data available on such a correlation concerning SARS-CoV-2.

It would be essential to learn whether, in particular, 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.

Further, the potential of combinatorial zinc correction and anti- SARS-CoV-2 treatments such as CQ/HCQ could be clinically explored.

Zinc deficiency is associated with cytokine storm

Zinc-deficiency not only hampers our immune system in terms of lack of efficient response when required[30], but it can also be associated with an irrelevant and damaging response.

Zinc levels among patients suffering from different auto-immune disorders appear to be lower than the healthy individuals[32].

Importantly, Zinc deficiency is associated with the excessive and tissue-damaging pro-inflammatory release of cytokines, including increased tumor necrosis factor-alpha (TNF-α)[33,34] and IL-6[35].

As previously mentioned, so far, the TNF-α and IL-6 have been reported among the usual suspects responsible for lung-damaging and fatal “Cytokine Strom” in a group of SARS-CoV-2 patients.

Notably, Zinc correction is associated with the down-regulation of tumor necrosis factor-alpha [30,33,34].

Concerning IL-6, however, the impact of Zinc might be rather qualitative than quantitative. One study reports that zinc deficiency is associated with increased IL-6 gene expression in mice[36], while another study on a human experimental model reports that zinc deficiency does not decrease IL-6 levels[37]. Nevertheless, it is reported that among the geriatric female population, a gene polymorphism that leads to an increased immune response-mediated release of Zinc is associated with decreased IL-6 levels[38]. On the other hand, zinc supplementation can even lead to the up-modulation of IL-6[39].

However, it seems to be a consensus that zinc correction inversely affects the IL-6-mediated response, which is, by nature, a pro-inflammatory response[35–37,40].

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Interestingly, zinc deficiency is reported to increase the risk of ventilator-induced injury in mice[41].

Moreover, zinc deficiency is associated with an increased risk of acute respiratory distress syndrome (ARDS) in human[41].

These associations merit special attention concerning SARS-CoV-2 patients management[15].

Therefore, we suggest that zinc deficiency should be investigated in a subgroup of SARS-CoV-2 patients at risk for developing hyperinflammatory complications[15].

Additionally, we suggest that zinc correction might cooperate with HCQ in reducing the risk of SARS-CoV-2-related tissue-damaging cytokine storm, and we believe that such a probable synergy worth clinical exploration.

Zinc deficiency might deprive SARS-CoV-2 patients of additive Zinc-(Hydroxy)Chloroquine anti-SARS effects

Several in-vitro studies had shown that zinc and zinc ionophores halt the RNA replication of different RNA viruses, including the ones involving human respiratory system[42–49].

This large piece of the evidence inspired Velthuis et al. .[50] to test a similar possibility in two RNA viruses, including SARS-CoV.

The group discovered that both a candidate zinc ionophore (pyrithione) and Zinc itself could inhibit SARS-CoV replication as singe agents and better in combination. However and possibly due to low cellular uptake of Zinc, the optimal zinc concentration to efficiently inhibit the SARS-CoV replication was relatively high[50].

Notably, Velthuis et al. .[50] could show that Zinc can 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 RdRp[50].

It deserves to be mentioned that a Chinese group who has unraveled the SARS-CoV-2 RdRp genome, could not observe zinc ions being chelated at the two candidate pockets within the RdRp of SARS-CoV-2 [51]. The group addresses the discrepancy in their technical approach compared to the precedent works on SARS-CoV [51].

Thanks to cancer research efforts, we learn a more exciting feature of CQ associated with Zinc. In 2014, a Chinese cancer study by Xue et al. reported that CQ increases zinc uptake in ovarian cancer cells and mediates zinc accumulation into the lysosomes of these cells [52].

In contrast, a Korean study conducted by Seo et al. .[53] partially contradicts findings of Xue et al., though in a different context and a separate cell line (adult retinal pigment epithelial cells).

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In contrast to the conclusion of Xue et al., Seo et al. .[53] reported that Chloroquine decreases the free zinc levels in lysosomes. However, they still observed some increased intracellular zinc levels upon CQ treatment compared to the control group.

Therefore, we acknowledge that whether CQ/HCQ are global zinc ionophores mediating intracellular uptake of Zinc by cells of different origins, at this stage, should remain an open question and the subject of further investigation.

On the other hand, even if CQ or HCQ does not turn out to be zinc ionophore, it would still be possible that Zinc can exert an anti-SARS replication effect independent of CQ/HCQ. Patients with zinc deficiency would likely be deprived of this additive effect.

If further data suggests that CQ/HCQ are zinc ionophores mediating zinc uptake into the SARS-CoV-2 infected cells, one can postulate combining zinc supplements with CQ/HCQ or at least zinc correction in zinc-deficient patients could be beneficial.

However, if the new data suggest that CQ/HCQ is interfering with zinc uptake into the SARS-CoV-2 infected cells or in an organelle such as lysosomes- in line with findings of Seo et al. combining zinc correction or zinc supplementation with CQ/HCQ might be even highly essential.

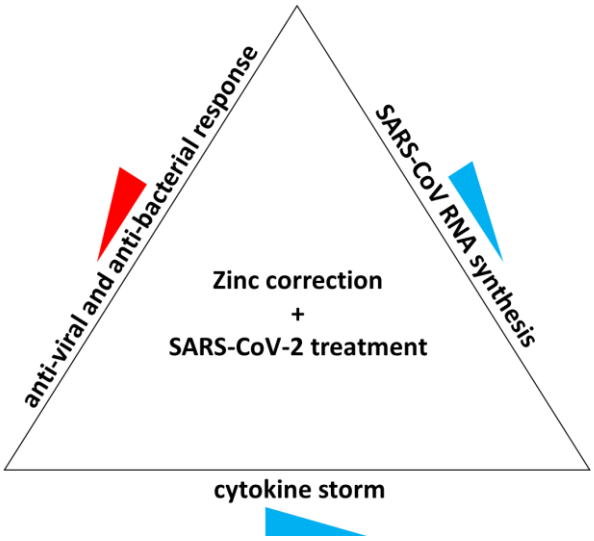

Figure 1 *Three lines of evidence supporting the inclusion of zinc correction in different anti-SARS-CoV-2 treatments including Hydroxychloroquine treatment*

Practicality

There is no consensus and widely accepted guideline concerning zinc deficiency diagnosis and correction. Radioisotopes are a decent diagnostic tool, but they are costly. New biomarkers such as Linoleic Acid are emerging[54–57]. However, considering the vast population involved with SARS-CoV-2 and the high economic Burdon of such approaches, we propose a simple and cheap

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method to select zinc-deficient SARS-CoV-2 patients and include zinc correction in their treatment package.

Zinc uptake and absorption:

The Recommended Dietary Allowance (RDA) of zinc intake is 11mg and 8mg/day for adult males and females, respectively[58].

According to an analysis of the National Health and Nutrition Examination Survey (NAHNES III), 35%-45% of adults above 60 years old have daily zinc intake below the recommended average [59].

The zinc transport protein family members that are apically positioned on the cell surface mediate zinc uptake.
Foods with a good source of Zinc include meat, fish, shellfish, legumes, nuts, seeds, eggs, and whole-grain cereals[60].
Phytate, the hexaphosphate ester of inositol, due to its high polarity, strongly binds to divalent Zinc, thereby preventing its absorption[61,62]. Due to phytate-rich content, the zinc-bioavailability of vegan/vegetarian diets are less compared to omnivore diets[62].

Nevertheless, unrefined phytate containing food such as whole-grain bread, despite higher phytate content, has higher zinc-bioavailability compared to refined ones like white bread, which have poor zinc content[62].

Evaluation of zinc status: Serum or plasma Zinc

Plasma and serum zinc concentration are the most frequently used biomarkers to establish zinc status[63].
Clinical effects of zinc deficiency can be existing despite normal zinc plasma concentration[63].
Conditions that lead to hypoalbuminemia also reduce plasma zinc concentrations, as Zinc is bound to the albumin in the plasma[64]. Therefore, it is essential to include albumin along with plasma zinc test and consider albumin-normalized zinc values to determine the zinc cut-off[65].

Infections, fever, contraceptives, and pregnancy lower the plasma zinc while starvation and catabolism increase it[63].

In particular and concerning SARS-CoV-2 patients, the status of two widely used biomarkers of inflammation[31,66]; c-reactive protein and procalcitonin, is linked to zinc status.

Interestingly, both these markers are inversely associated with serum zinc levels[67,68], other evidence that zinc might have anti-hyper inflammatory effects in SARS-CoV-2 patients.

The interpretation of the concentration of plasma zinc must, therefore, take into account all the confounding factors[69].
Plasma zinc concentrations dose-dependently respond to supplementation in patients with a low or moderate baseline, independent of gender and age[69].

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On the other hand, Zinc deprivation results in a reduction in the plasma zinc concentrations.

The fact that plasma zinc concentrations are normally-distributed among healthy populations makes it useful as a zinc-deficiency marker [63].

Frank T. et al. concluded that as plasma and urinary zinc concentrations are probably more precise indicators currently available, as opposed to indirect ones such as stunting, anemia, or iron deficiency. They suggest that direct indicators can be used to estimate the prevalence of zinc deficiency in populations [57].

Cutoffs:
A revisit of the NHANES II data suggests cutoffs of serum zinc concentrations for assessing the fasting zinc status at 70 and 74 µg/dL for females and males over ten years old, respectively[70].

Treatment:

Long term zinc supplementation should be very well-weighed[71] as the therapeutic window of chronic zinc supplementation can relatively narrow.

In contrast, short term zinc supplementation may be less of a concern[26].

The proposed dose of zinc (Znso4) injection for parenteral nutrition in metabolically stable adult patients is 3 mg/day [72].

The maximum level of the excipient used in FDA-approved oral zinc tablets (excluding extended-release forms) is 25 mg[73].

The recommended oral daily dose for zinc correction in adults is 10-30 mg/day[26,74].

Various forms of zinc supplements exist, including zinc gluconate, zinc citrate, zinc sulfate, and zinc acetate.
Rita Wegmüller et al. study demonstrated that zinc citrate could be as active as zinc gluconate in the prevention of zinc deficiency.

The high zinc content of zinc citrate, acceptable quality perception for the patients, the comparable bioavailability, and its low cost[75] may make it a relevant form to be considered for zinc correction in zinc-deficient SARS-CoV-2 patients.

Conclusion

Taken all together, we believe there is a large piece of evidence that links antiviral response, zinc deficiency, and in particular, HC/HCQ therapy. At this stage, this evidence is far from being conclusive. However, such an indispensable and intriguing hint in the precedent literature

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justifies the clinical exploration of the possible link between zinc deficiency and response to HC/HCQ and other treatments in SARS-CoV-2 patients.

Author Contributions

A.N and G.V developed the idea together and co-drafted the manuscript.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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