Short title: Zinc and COVID-19

Antiviral and Immunological Activity of Zinc and Possible Role in COVID-19

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Abstract
Zinc deficiency compromises its biological functions, its effect on the immune system and its antiviral activity, increasing vulnerability to infectious diseases. This narrative review aims at presenting and discussing functional aspects and possible mechanisms involved in the potential role of zinc in the immune response and antiviral activity for COVID-19 prevention and control. The searches were conducted in PubMed and Science Direct databases, using clinical trials, experimental studies in animals and humans, case-control studies, case series, letters to the editor, and review articles published in English, without restrictions on year of publication. Search approach was based on using the terms: "zinc", "COVID-19", "antiviral agents", "immunologic factors", and "respiratory tract infections". Literature shows the importance of zinc as an essential mineral immunomodulator with relevant antiviral activity in the body. Thus, although there is still a scarcity of studies evaluating zinc supplementation in patients with COVID-19, the results on the topic show the necessity of controlling zinc mineral deficiency, as well as maintaining its homeostasis in the body in order to strengthen the immune system and improve the prevention of highly-complex viral infections, such as that of the COVID-19.

Keywords: Zinc. COVID-19. Antiviral. Immunomodulator.
INTRODUCTION

Several micronutrients have been investigated worldwide to assess their role in the prevention and control of chronic diseases, such as diabetes mellitus, chronic kidney disease, and cancer (1–3). Zinc, in particular, is an essential mineral involved in several biological processes, participating in the metabolism of carbohydrates, lipids, and proteins, playing a relevant role as a cofactor, signaling molecule or structural element of biological components in the cells (4,5).

Certain studies raised awareness of the relevance of zinc in the prevention of infectious diseases of the respiratory tract, like the coronavirus infectious disease (COVID-19). Indeed, zinc is heavily investigated concerning its potential as a therapeutic target in the treatment of this infection because of performance of zinc in the immune response, regulating proliferation, differentiation, maturation, and function of leukocytes and lymphocytes, as well as further modulating the inflammatory response (6–9).

Furthermore, zinc has been shown to have an antiviral effect, inhibiting the interaction between the some virus and host cell and viral replication that could increase vulnerability to infectious diseases. The high prevalence of zinc deficiency worldwide should also be highlighted, mainly in high-risk groups including premature babies and elderly, making them more susceptible to viral infection (10,11).

Considering the importance of COVID-19 as an infectious disease of high prevalence and mortality rates, as well as the lack of clinical outcome that could identify the efficacy of zinc to treatment of COVID-19, this narrative review aims at presenting and discussing in detail immunological aspects and possible mechanisms underlying effects of zinc in COVID-19 prevention and control.

METHODS

Our research focused on selecting studies that confirmed the role of zinc in COVID-19 prevention and control. The searches were conducted in the PubMed and Science Direct databases, in June 2020. We used clinical trials, experimental studies in animals and humans, case-control studies, series of cases, letters to the editor, and review articles published in English, without restrictions in the year of publication. The search strategy was based on using the terms: “zinc”, “COVID-19”, “antiviral agents”, “immunologic factors”, and “respiratory tract infections”. The keyword combinations used during the search for articles are shown in Table 1.
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The research process was carried out independently by two authors and the articles included in the review were consensually selected. A third author was consulted in case of disagreement. In the first phase of the research, we analyzed the title, summary, and keywords of the articles and identified those that met the eligibility criteria. The articles selected in the first phase were analyzed by reading the full text and those eligible for the review were identified and included. In addition, we did manual search in reference list of eligible articles.

A total of 305 articles were identified by searching the PubMed (n = 176) and Science Direct (n = 129) databases. After the processes of selecting and removing duplicates, 59 were identified as eligible based on the title and summary. After reading in full, 16 studies were included. After that, 25 studies were included through the reference list search strategy of eligible articles, resulting in 41 studies included in this review (Figure 1). We conducted a new search in December 2020, and we included three more studies. Thus, this review included 44 studies.

COVID-19: ANTIVIRAL ACTIVITY AND MECHANISM OF ACTION OF ZINC

Zinc plays important physiological functions, in particular in neutralizing the activity of several viruses, such as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). This virus is part of the subfamily Orthocoronavirinae, family Coronaviridae, order Nidovirales, and kingdom Riboviria. It is characterized by single stranded RNA genome, positive direction, and a helically symmetric nucleocapsid (12). According to their genetic properties, coronaviruses are grouped into four genera: α-CoV, β-CoV, γ-CoV, and δ-CoV, SARS-CoV-2 belonging to β-CoV [7]. Another important aspect concerning the characteristics of coronaviruses is their structure, comprising at least four proteins: Spike (S), Envelope (E), Membrane (M) and nucleocapsid (N) (13).

It is worth mentioning that protein S mediates SARS-CoV-2 binding to receptors on the surface of the host cell, resulting in fusion and subsequent viral entry (13,14). Moreover, the protein S binding domain binds to the peptidase domain of the human angiotensin-converting enzyme 2 (ACE2), which acts as a virus receptor for SARS-CoV-2, enabling it to enter the host cell. This enzyme is a membrane protein expressed in several cells, like cells in the alveolar epithelium, trachea and bronchi, serous bronchial glands, as well as alveolar monocytes, macrophages, and pneumocytes (15).

The SARS-CoV-2 binding to ACE2 receptors triggers conformational changes in protein S, inducing its cleavage by transmembrane serine protease 2 (TMPRSS2). After that,
the virus is transported to the cytoplasm through endocytosis. The low pH inside the endosomes induces the activity of the host protease cathepsin-L, which cleaves protein S. The cleavage of protein S induces fusion of viral envelope and endosomal phospholipid membrane to release the viral genomic RNA from the positive strand in cell cytoplasm \(^{(7)}\).

In addition, the RNA-dependent viral RNA polymerase (RdRp), enzyme encoded in the SARS-CoV-2 genome, is essential for virus replicative cycle. Initially, the polyprotein precursor is formed from which the RdRp-containing subunit is proteolytically cleaved. Subsequently, RdRp is integrated into a membrane associated viral enzyme complex that drives the synthesis of negative chain RNA. The negative RNA strand is used as a template for viral mRNA synthesis. It is noteworthy that the RdRp enzyme has a deep groove as an active site for RNA polymerization \(^{(7)}\).

Possible antiviral therapies could be classified into two categories, depending on the target, either acting against the coronavirus itself or protecting the immune system \(^{(14)}\). The therapies against the coronavirus involve the inhibition of viral RNA synthesis by affecting the viral genetic material, inhibition of viral replication, and blocking viral enzymatic activity. In addition, certain therapies are based on blocking of virus interaction with human cell receptors or of the viral self-assembly process, through affecting structural proteins \(^{(14)}\).

Several \textit{in vitro} studies have evaluated the effectiveness of zinc as an antiviral agent. Although the involved mechanisms are not understood well enough in detail, the mechanism of antiviral activity of zinc are reportedly specific to each viral type, and zinc ion availability appears to play a significant role in its antiviral efficacy \(^{(16)}\). In addition, zinc deficiency has been associated with increased sensitivity to infectious diseases, including viral infections \(^{(17,18)}\).

Antiviral functions of zinc are based on inhibition of physical processes, as virus fixation, infection, and coating, as well as the inhibition of viral protease and polymerase enzymatic function. The increase in the intracellular zinc concentrations could interfere with the proteolytic processing of viral polyprotein, influencing its enveloping. Furthermore, high intracellular zinc concentrations may affect directly the viral protease (picornavirus, encephalomyocarditis, and polioviruses), and to alter the tertiary structure of the protein, as in the case of the encephalomyocarditis virus. In addition, zinc inhibits viral and host cellular membrane fusion, preventing viral infection \(^{(12,19-21)}\).

Another mechanism underlying antiviral activity of zinc refers to its ability to dose-dependently inhibit ACE2 enzymatic activity, that is, the higher the zinc concentration, the greater the enzymatic inhibition efficiency. Thus, the researchers suggest that zinc might
inhibit the interaction between SARS-CoV-2 protein S and ACE2, a recipient of the enveloped virus (15).

Ionophores, such as hydroxychloroquine, hinokitiol, pyrrolidine dithiocarbamate, and viral pyrithione, play an important role in the antiviral activity of zinc, stimulating zinc influx into cellular cytoplasm (12,16,19). Cell culture studies revealed that high concentrations of zinc and the supplementation of compounds that stimulate the cellular zinc influx inhibited the replication of several RNA viruses, including the influenza virus, respiratory syncytial virus, and various picornaviruses (12,16).

Zinc cations, especially in combination with the ionophore pyrithione, reportedly inhibit SARS-CoV-2 RNA polymerase activity, reducing viral replication. In addition, zinc inhibits the activity of the RdRp enzyme of SARS-CoV-2 also during the elongation phase of RNA synthesis. Thus, zinc ions appear to inhibit adequate proteolytic processing of replicase polyproteins and RdRp activity (9,16).

Concerning the efficacy of chloroquine ionophore against SARS-CoV-2, the results show that chloroquine increases the flow of zinc into cells. Derwand R and Scholz (22) supplemented zinc associated with chloroquine and hydroxychloroquine during the treatment of COVID-19, and they found an increase in intracellular zinc concentrations, mainly in lysosomes. In addition, it was also observed that the higher intracellular concentration of zinc, the greater its ability to inhibit the RdRp enzyme and, consequently, the intracellular replication of SARS-CoV-2. This potentially improved clinical outcomes of patients with COVID-19 treated with these drugs (22).

The antiviral activity of zinc could also be exerted through metallothioneins, low-molecular-weight enzymes that bind and transport zinc. Read et al. (23) demonstrated that the induction of these enzymes, particularly of subfamily 1 and 2 members, could inhibit the replication of the hepatitis C virus, and that the zinc antiviral activity could be mediated by mechanisms that involve metallothioneins. Figure 2 shows potential mechanisms underlying activity of zinc in therapy for COVID-19.

**IMMUNOMODULATOR ZINC ACTIVITY**

COVID-19 predominantly affects the respiratory system, resulting in pneumonia and acute respiratory distress syndrome (SDRA), leading the need for mechanical ventilation. Advanced age, SDRA, need for mechanical ventilation, and impaired immune system are related to higher COVID-19 mortality (9). Zinc acts as an important immunomodulator, as it regulates the proliferation, differentiation, maturation, and function of leukocytes and
lymphocytes, and it also modulates the inflammatory response. In addition, zinc supplementation in adults and children has a beneficial effect to reducing virus-induced symptoms and illness time, such as colds and flu (24, 25).

In acute phase response to infection, a systemic response coordinated by cytokines decreases concentrations of trace elements in plasma, including zinc. This is due to the fact that some pathogens need minerals for their growth, which contributes to reducing the levels of these minerals in plasma (26). During infection, polymorphonuclear leukocytes migrate by adhesion and chemotaxis to the infected tissue in response to inflammatory signals. As a defense mechanism, the body uses alternatives to maintain homeostasis, inducing phagocytosis, and subsequently, increased production of reactive oxygen species, mediated mainly by the high activity of NADPH oxidase (26,27).

One of the hallmarks of COVID-19 is an imbalanced immune response due to hyperinflammation, including a very rapid and enhance production of proinflammatory cytokines. During inflammatory response, zinc is redistributed to the tissues, resulting in serum hypozincemia. Thus, subjects with COVID-19 are at risk of zinc deficiency. Furthermore, in combination with the pre-existing suboptimal zinc supply, this will decrease serum zinc levels to critically low values and thereby significantly increase the susceptibility for co-infections with detrimental progression (28,29).

Zinc deficiency reduces the antibody production and impairs the innate immune system because of reduced natural killer cell activity, impaired monocyte cytokines production, impaired chemotaxis, and oxidative explosion of neutrophilic granulocytes (12,30). Zinc deficiency could also induce thymus atrophy, inducing altered production of thymic hormones, lymphopenia, cellular defects, and antibody-mediated responses that trigger increased infection rates and duration. This is because zinc deficiency reduces the number of peripheral and thymic T cells, their proliferation in response to phytohemagglutinin, and the functions of T cell cytotoxic auxiliaries (12,30).

Associated with this, zinc deficiency acts indirectly, reducing the levels of active serum thymulin, a zinc-dependent hormone that regulates the differentiation of immature T cells in the thymus and the function of mature peripheral T cells (12,30). Most antigens are dependent on T cells and, therefore, during zinc deficiency, the production of antigens is compromised, and the body becomes unable to respond with the synthesis of antibodies in response to neoantigens (31).

Zinc deficiency also could impair the immune system because zinc induces the production of interferon-α and interferon-γ by leukocytes, enhancing its antiviral activity (31).
It induces cellular resistance to apoptosis by inhibiting caspase 3, 6, and 9, and increases the Bcl-2/Bax ratio, which could contribute to increasing T cell amounts (32). Thus, zinc contributes to enhance immune response to viral infection. (12,30).

Moreover, zinc is essential for the barrier function of mucosal epithelium due to its antioxidant and anti-inflammatory activity. Zinc also regulates tight junction proteins that are important for the maintenance of mucosal membrane integrity. However, reduction of mucosal integrity and loss of tight junction cohesion aggravates viral inflammation (33,34).

Figure 3 shows the possible antiviral and immunomodulatory effects of zinc.

ZINC SUPPLEMENTATION STUDIES

Studies of zinc supplementation have been done to evaluate immunomodulatory zinc activity and antiviral zinc action. Hasegawa et al. (35) examined the ability of human neutrophils to produce reactive oxygen species to investigate the effects of zinc on nonspecific immune functions. They stimulated neutrophils with opsonized zymosan and phorbol myristate acetate in presence of 1 to 10$^{-3}$ mmol/L of zinc chloride in vitro. Their results suggested that zinc activates protein kinase C and promotes myeloperoxidase degranulation, suggesting that zinc supplementation beyond physiological doses improves neutrophil functional activity. Thus, the authors suggested that zinc is essential for optimal functioning of non-specific immunity.

In order to better understand the zinc activity on thymic function and immune homeostasis, Lovino, Mazziotta, Carulli (36) conducted a prospective clinical study using high doses of oral zinc (600 mg/day zinc sulfate heptahydrate) to improve immune reconstitution after hematopoietic stem cell transplantation. Patients received zinc supplementation by postoperative day 5 to day 100 after the transplantation. The authors found an increase in CD4+ lymphocytes and T lymphocyte receptor only in the group treated with zinc, and they concluded that high-dose zinc supplementation is safe and could improve thymic reconstitution after hematopoietic stem cell transplantation.

Mariani et al. (37) evaluated the zinc activity in modulating the immune response, in balance of T helper 1 and 2 cells and in low-grade systemic inflammation during aging. Researchers found that the supplementation with 10 mg/day of zinc aspartate for 48 days increased the zinc concentration in blood, and it increased plasma IL-6, monocyte chemotactic protein 1 (MCP-1), and lytic activity of natural killer cells in elderly individuals with zinc plasma concentrations below 11 μmol/L.
In a study by Ganatra et al. (38), supplementation with 10 mg/kg of zinc gluconate solution was able to suppress neutrophil recruitment, inflammatory response, and subsequent lung injury after polymicrobial sepsis in rats. In addition, this study mentioned that zinc had modulating effects of the inflammatory cascade, proved by low serum concentrations of IL-2, IL-6 and IL-1β, reducing hyperinflammatory response to infectious agents.

Zinc deficiency is also associated with increased susceptibility to infectious diseases due to activity of several pathogens, including viruses (39,40). In such cases, zinc administration at sufficient therapeutic doses has the potential to improve or restore immune cell function (12). Mossad et al. (41) tested the effectiveness of zinc gluconate in reducing the duration of symptoms caused by common cold in 100 individuals. Patients received one lozenge every 2 hours while awake, containing 13.3 mg of zinc in the form of zinc gluconate, as long as they had cold symptoms. The main results found were less time to complete the resolution of symptoms, fewer days with cough, headache and hoarseness in the group supplemented with zinc.

A study conducted by Sempitéregui et al. (42) aimed evaluate how zinc supplementation affect the respiratory tract disease, immunity, and growth in malnourished children. It demonstrated that zinc sulfate supplementation of 10 mg/day for 8 weeks reduced the incidence of fever, cough, and respiratory tract secretions, reinforcing the importance of zinc in immunity against respiratory infections.

Results by Suara et al. (43) research suggest that antiviral activity of zinc against the respiratory syncytial virus, the main cause of pediatric lower respiratory tract disease. Authors verified in vitro inhibitory effect of three zinc salts (acetate, lactate, and zinc sulfate) on respiratory syncytial virus replication at concentrations ranging from 1 to 10 mM or 10 to 100 µM, and they found that the inhibitory effect of zinc salts was dependent on its concentration, especially zinc in the form of sulfate.

In the context of morbidity and mortality due to COVID-19, zinc deficiency might be relevant to the negative outcome in patients with severe disease, including elderly patients with hypertension, diabetes mellitus, coronary heart disease, or chronic obstructive pulmonary disease (25). It has also been demonstrated that zinc could act synergistically when co-administered with standard antiviral therapy (39).

Velthuis et al. (16) demonstrated that the combination of zinc with its ionophore pyrithione, at low concentrations (2 µM Zn2+ and 2 µM pyrithione) inhibited the replication of SARS-coronavirus (SARS-CoV) and equine arteritis virus (EAV) in cell culture. This study also showed that an activity assay for the multiprotein replication and transcription
complex, isolated from cells infected with SARS-CoV or EAV, allowed to eliminate the need to use pyrithione to transport zinc across the plasma membrane, and that zinc efficiently inhibited the RNA synthesis activity of the RTCs of both viruses.

In the case report by Finzi (6), including 4 patients diagnosed with SARS-CoV-2, one male and three females, aged between 26–63 years, patients were supplemented with zinc in combination with hydroxychloroquine. In that study, two participants were supplemented with zinc citrate tablets (23 mg elemental zinc), a patient with zinc citrate/zinc gluconate (23 mg) and a patient with zinc acetate (15 mg), and individuals started zinc therapy at different times during the course of the disease. All participants exhibited significant improvement in the symptoms of the disease after a day of therapy using high doses of zinc associated with the drug, suggesting that zinc therapy has a beneficial effect on clinical recovery.

In order to investigate the role of zinc in elderly patients hospitalized with COVID-19, Yao et al. (18) carried out a retrospective study to evaluate the survival of patients treated with 440 mg of zinc sulfate (100 mg of elemental zinc). Results obtained from the study did not reveal an association between zinc supplementation and the survival rate in the group of elderly people evaluated.

In this perspective, Derwad et al. (44) evaluated the effect of early supplementation with 220 mg of zinc sulfate (50 mg of elemental zinc) and other associated drugs. There was a significant reduction in hospitalization and mortality in patients undergoing triple intervention (zinc plus low-dose hydroxychloroquine and azithromycin). However, more research is necessary to demonstrate the efficacy of zinc supplementation to increase survival in patients with COVID-19.

Table 2 shows studies that evaluated the effect of zinc supplementation associated with therapeutic drugs in patients with confirmed COVID-19.

Although studies have already shown benefits of zinc in infections and respiratory diseases, beneficial effects of zinc supplementation in patients with COVID-19 and the definition of dosage and use duration are still being consolidated. Thus, considering the role in immune function and the potential to decrease coronavirus replication, zinc has been extensively investigated as a treatment strategy for patients with COVID-19 (45-47).

On the other hand, zinc supplementation may not be useful in conditions of zinc sufficiency (48,49). Results of studies that investigated the effects of zinc supplementation on patients with infection with the human immunodeficiency virus (HIV), for example, are contradictory because the different zinc status of the patients. They suggest that while
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moderate zinc supplementation to zinc-deficient subjects can advance their immune responses, it may have harmful effects when given to zinc-sufficient ones. This fact underlining the potential benefits of monitoring the zinc status of the patients with viral infections like COVID-19.

Excess dietary zinc could also impair immune response by inhibiting T-lymphocyte and B-lymphocyte function, reducing intracellular pathogen destruction in macrophages or inducing an overload of regulatory T-cells. Therefore, a balanced zinc homeostasis is critical for adequate immune functions. Similarly, Matwald, Rink reported that zinc supplementation excess was able to reduce the expression of interferon-γ, reducing the expression of the interferon 1 regulatory factor in regulatory T cells. Thus, the reduction of the expression of this cytokine results in immunological impairment, since it reduces the defense capacity against pathogens with high viral activity.

Studies that evaluate the effect of supplementation dietary zinc on non-specific immunity are controversial. Morgan et al. reported that supplementation of zinc gluconate is able to reduce the infiltration of neutrophils into the airways and the release of TNF-α by inhibiting NF-kB-dependent transcription of inflammatory genes, enhancing its antiviral activity and inflammation. In contrast, Wessels, Maywald, Rink explain that excessive doses of zinc could affect the immune response, because brings about overload of regulatory T cells, direct activations of macrophages, and suppression of T and B cell function, reducing the immune response to viral infection.

Furthermore, acute exposure to high doses of zinc could induce disorders of the gastrointestinal tract, including nausea, vomiting, loss of appetite, epigastric pain, diarrhea, in addition to headache and fatigue. Chronic zinc toxicity could include lethargy, copper deficiency and severe iron deficiency anemia. Excessive zinc levels are cytotoxic and have been shown to induce higher mortality in experimental studies. The risk of developing adverse effects can limit tolerance and long-term use of zinc.

Researches that evaluate zinc supplementation as a therapeutic strategy and prophylaxis in groups at risk for COVID-19 are necessary because zinc is an economical option and simple to use. Some clinical trials on zinc supplementation alone and in combination with other drugs such as chloroquine have already been registered. It is important to highlight that the COVID-19 Treatment Guidelines recommends against using zinc supplementation above the recommended dietary allowance for the prevention of COVID-19, except in a clinical trial.
CONCLUSION

Literature shows the role of zinc as an essential mineral to several biological functions in the body, its action as an immunomodulator nutrient, and its antiviral activity. Thus, although there is still a lack of studies evaluating zinc supplementation in patients with COVID-19, the results on the topic show the need to control zinc deficiency, as well as maintaining its homeostasis in the body in order to strengthen the immune system and improve the prevention of highly-complex viral infections, such as that of the COVID-19.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS’ CONTRIBUTIONS:
DNM, KJCC and BJSAF conceived the idea of study. ARSO, JBSM, SRM, LRS, TMSD and BEPC searched databases, screened the articles, and extracted data. All authors drafted the manuscript. DNM, JBSM, KJC and ARSO revised the manuscript.
REFERENCES


Table 1: Keyword combinations used in the search for articles*.

<table>
<thead>
<tr>
<th></th>
<th>Combination</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>zinc AND (COVID-19 or 2019 novel coronavirus disease or COVID19 or COVID-19 pandemic or SARS-CoV-2 infection or COVID-19 virus disease or 2019 novel coronavirus infection or 2019-nCoV infection or coronavirus disease 2019 or coronavirus disease-19 or 2019-nCoV disease or COVID-19 virus infection)</td>
</tr>
<tr>
<td>2</td>
<td>Zinc AND (Antiviral Agents or Agents, Antiviral or Antivirals or Antiviral Drugs or Drugs, Antiviral) AND (Respiratory Tract Infections or Infection, Respiratory Tract or Respiratory Tract Infection or Infections, Respiratory or Respiratory Infections or Infections, Respiratory Tract or Upper Respiratory Tract Infections or Infections, Upper Respiratory Tract or Infections, Upper Respiratory or Respiratory Infection, Upper or Upper Respiratory Infection)</td>
</tr>
<tr>
<td>3</td>
<td>Zinc AND (Immunologic Factors or Immune Factors or Factors, Immune or Immunological Factors or Factors, Immunological or Factors, Immunologic or Immunomodulators or Biological Response Modifiers or Biomodulators or Modifiers, Biological Response or Response Modifiers, Biological) AND (Respiratory Tract Infections or Infection, Respiratory Tract or Respiratory Tract Infection or Infections, Respiratory or Respiratory Infections or Infections, Respiratory Tract or Upper Respiratory Tract Infections or Infections, Upper Respiratory Tract or Infections, Upper Respiratory or Respiratory Infection, Upper or Upper Respiratory Infection)</td>
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* Since the most articles included in this review were published before the Covid-19 pandemic, articles related to other respiratory diseases were included for a better understanding of involved mechanisms.
Table 2: Studies that evaluated the effect of zinc supplementation associated with therapeutic drugs in patients with confirmed COVID-19.

<table>
<thead>
<tr>
<th>Article</th>
<th>Methodology</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Velthuis et al. (^{(16)})</td>
<td>2 µM Zn2+ and 2 µM pyrithione Cell culture</td>
<td>Inhibited SARS-CoV replication.</td>
</tr>
<tr>
<td>Finzi (^{(6)})</td>
<td>N = 4 Group A (n = 2): 23 mg elemental zinc Group B (n = 1): 23 mg zinc citrate/zinc gluconate Group C (n = 1): 15 mg zinc acetate</td>
<td>Significant improvement in symptoms of COVID-19 after one day of therapy using high doses of zinc associated with hydroxychloroquine.</td>
</tr>
<tr>
<td>Yao et al. (^{(18)})</td>
<td>N = 196 elderly patients Dose: 440 mg of zinc sulfate (100 mg of elemental zinc)</td>
<td>No association between zinc supplementation and survival rate in elderly people.</td>
</tr>
<tr>
<td>Carlucci et al. (^{(45)})</td>
<td>N = 411 Drugs + 220 mg of zinc sulfate (50 mg of elemental zinc) Treatment: 5 days</td>
<td>Decreased: mortality, need for ICU care, need for ventilation. High probability of returning home.</td>
</tr>
<tr>
<td>Derwand; Scholz; Zelenko (^{(44)})</td>
<td>N = 141 Drugs + 220 mg of zinc sulfate (50 mg of elemental zinc) Treatment: 5 days</td>
<td>Significantly fewer hospitalizations.</td>
</tr>
<tr>
<td>Sattar et al. (^{(46)})</td>
<td>N = 3 Drugs + 220 mg of zinc sulfate (50 mg of elemental zinc) Treatment: 5 days</td>
<td>All patients were recovered.</td>
</tr>
</tbody>
</table>
Legend of figures

Articles identified through database searches (n=305)
  Pubmed (n=176); ScienceDirect (n=129)

Articles deleted after initial selection (n=244)
  PubMed (n=122)
    Irrelevant articles (n=96)
    Animal studies (n=16)
    Joint supplementation (n=6)
  Science Direct (n=122)
    Irrelevant articles (n=122)
    Animal studies (n=0)
    Joint supplementation (n=6)

Selected articles after reading in full (n=16)

Studies included through references of eligible articles (n=26)

Studies included after a new search in December 2020 and April 2021 (n=11)

Studies included in the review (n=53)

**Figure 1:** Diagram of study selection.
Figure 2: Potential mechanisms of zinc in COVID-19 therapy. The spike proteins of SARS-CoV-2 bind to ACE2 receptors. The virion then releases the RNA genome into the cell and translation of structural and non-structural proteins follows. ORF1a and ORF1ab are translated to produce pp1a and pp1ab polyproteins, which are cleaved by the proteases that are encoded by ORF1a to yield non-structural proteins. This is followed by assembly and budding into the lumen of the ERGIC. Virions are then released from the infected cell through exocytosis. Zinc might also possess antiviral activity through inhibition of RdRp and reduction in template binding. Indirect evidence also indicates that Zinc might decrease ACE2 activity, known to be the receptor for SARS-CoV-2, contributing to inhibit the fusion of SARS-CoV-2 in the cell membrane. ACE2: Angiotensin-Converting Enzyme 2; Rough ER: Rough Endoplasmic Reticulum; ERGIC: Endoplasmic Reticulum Golgi Intermediate Compartment; Zn: Zinc.
Figure 3: Antiviral and immunomodulator effects of zinc. ACE: angiotensin-converting enzyme; IFN: interferon.