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Should we supplement zinc in COVID-19 patients? Evidence from meta-analysis

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Short title: Should we supplement zinc in COVID-19 patients?

Key words: COVID-19, meta-analysis, SARS-CoV-2, zinc
What’s New?

Zinc supplementation was postulated to decrease mortality in COVID-19. We performed a meta-analysis to compare zinc vs. standard care in COVID-19. Zinc supplementation did not have any beneficial impact on the course of the disease. The zinc-supplemented group had a longer length of hospital stay. No evidence-based data supports routine zinc supplementation in COVID-19.
Abstract

Introduction: Preliminary retrospective reports showed that zinc supplementation may decrease mortality in COVID-19 patients, postulating the potential therapeutic efficacy of zinc in the management of the disease.

Objectives: We sought to summarize the studies published to date regarding the antiviral activity of zinc in COVID-19 patients.

Patients and Methods: A meta-analysis was performed to compare the outcomes of hospitalized patients receiving zinc supplementation and those treated with standard care. The primary outcome was survival to hospital discharge. Secondary outcomes were in-hospital mortality and length of stay in hospital or intensive care unit (ICU).

Results: Data relating to 1474 patients included in four studies were analyzed. Survival to hospital discharge was 56.8% in the zinc group, compared to 75.9% in the non-zinc group (P=0.88). In-hospital mortality was 22.3% in the zinc group, compared to 13.6% for the standard care group (P=0.16). Length of hospital stay was 7.7(3.7) days in the zinc group and 7.2(3.9) days in the standard treatment group (P<0.001). Length of ICU stay was 4.9(1.7) days in the zinc group and 5.8(1.9) days in the standard care group (P=0.009).

Conclusions: Zinc supplementation did not have any beneficial impact on the course of COVID-19 evaluated as survival to hospital discharge and in-hospital mortality. The zinc-supplemented group had longer hospital ICU lengths of stay. There is at present no evidence-based data to support routine zinc supplementation in COVID-19 patients.
Introduction

Ever since the pneumonia outbreak of unknown origin was reported in China in late 2019, which expanded all over the world, the global healthcare systems have been under severe pressure [1,2]. The new virus was called “severe acute respiratory syndrome coronavirus 2” (SARS-CoV-2) by the international committee of the Coronavirus Study Group (CSG) [3]. Due to the rapid spread of the disease, it was declared pandemic by the World Health Organization [4]. The major strain on healthcare systems exerted by the virus raised an urgent need for new treatment options to alleviate the SARS-CoV-2 pandemic. Efforts were made to administer agents known to restrict viral growth, including zinc. Zinc was shown to inhibit the in vitro reproduction of corona viruses [5] and to participate in the immunological response, especially involving T-lymphocytes [6,7]. Zinc’s mechanism of action is pleiotropic and associated with multiple pathways that participate in the inflammatory response. The baseline function of zinc is the reduction of the oxidative stress and inflammation [8]. Additionally, zinc is associated with the negative feedback loop decreasing the activity of nuclear factor kappa B, thus alleviating sepsis and excessive inflammation [9] and potentially reducing the cytokine storm – a phenomenon associated with severe COVID-19 [10]. Interestingly, the interaction between the angiotensin-converting enzyme-2 peptidase domain and SARS-CoV-2 spike protein, which is crucial for entry of the virus into the cell [11], is also modulated by the zinc levels [12].

Zinc supplementation was shown to decrease mortality in severe pneumonia [13]. At the very beginning of the COVID-19 pandemic, the idea of chloroquine derivatives as ionophores for intracellular uptake of zinc into lysosomes was postulated [14]. Thus, researchers have turned their interests to zinc as a promising agent in the management of COVID-19. To summarize the studies conducted hitherto into the antiviral activity of zinc in
COVID-19 patients, a meta-analysis was performed to compare the outcomes of the hospitalized patients receiving zinc supplementation and those treated with standard care.

The primary outcome was survival to hospital discharge. Secondary outcomes were in-hospital mortality, and length of stay in the hospital or intensive care unit (ICU).

**Patients and Methods**

This systematic review and meta-analysis were carried out following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines and the recommendations of the Cochrane Collaboration [15].

**Search strategy**

Two authors (L.S. and M.P.) independently searched PubMed, Scopus and the Cochrane Library for articles published in English from the inception of the databases until 10 February 2021. The search was performed using the following terms: “SARS-CoV-2” OR “COVID-19” OR “coronavirus” AND “ZINC”. The references listed in the identified articles were also reviewed. A manual search for the related articles was conducted in order to identify all eligible studies and achieve minimal publication bias.

**Inclusion and exclusion criteria**

Studies included in this meta-analysis fulfilled the following PICOS criteria: (1) participants: patients with a confirmed diagnosis of COVID-19; (2) intervention: zinc supplementation; (3) comparison: standard care; (4) outcomes: detailed information for survival; (5) study design: randomized controlled trials, quasi-randomized or observational studies comparing the effect of zinc and standard care on patient outcomes. Studies were excluded if they were reviews, animal studies, case reports, letters, conference or poster abstracts or articles not containing original data.

**Study selection**
The studies were independently screened by two authors (L.S. and M.P.), looking at the study titles and abstracts for potential eligibility. After reviewing the full texts, eligible studies were included according to the previously determined study inclusion criteria. Discrepancies in the selection of articles were resolved by consensus with a third reviewer (J.S.).

**Data extraction**

Raw data were extracted using a standardized, premade form. Care was taken to avoid including data from duplicate publications. In the event of any suspected data discrepancies, the relevant author was contacted directly. Data extracted from eligible studies included the following characteristics: study and year, country, type of participants, number of participants, types of therapy, mortality rate, adverse event occurrence as well as length of stay in hospital or intensive care unit (ICU).

**Risk of bias assessment**

Two investigators (L.S. and A.G.) independently extracted individual study data and assessed studies for risk of bias. Any disagreements were discussed and resolved in a consensus meeting with the third reviewer (M.J.J.). The ROBINS-I tool was used to assess risk of bias in non-randomized trials [16] and the RoB 2 tool to assess risk of bias in randomized studies [17]. The Robins application was used to visualize risk of bias assessments [18]. The scale has seven main domains (confounding, participant selection, classification of interventions, deviation from intended interventions, missing data, outcome measurement, and selection of reported results) and assigns one point for each of the following four judgments: critical, serious, moderate, and low. The review authors’ judgments about each risk of bias item are provided in Figures S1–S4 (Supplementary Materials).

**Statistical analysis**

Statistical analysis was performed in Review Manager, ver. 5.4 (Nordic Cochrane Center, The Cochrane Collaboration, Copenhagen, Denmark). The Mantel-Haenszel method was used to
analyze dichotomous outcomes, and results are reported as risk difference (RD) with a 95% confidence interval (CI) and two-tailed P-values. Continuous outcome differences were analyzed using an inverse variance model with a 95% CI, and values are reported as mean differences (MD). When the continuous outcome in a study was reported as median, range, and interquartile range, the means and standard deviations were estimated using the formula described by Hozo et al. [19]. Heterogeneity in each analysis was quantified by the tau-squared and I-squared statistics. Heterogeneity was detected with the chi-squared test with n – 1 degrees of freedom, which was expressed as I². Values of I² > 50% and > 75% were considered to indicate, respectively, moderate and significant heterogeneity among studies. A P-value less than 0.05 was considered statistically significant.

**Results**

*Characteristics of the studies included in the meta-analysis*

Figure 1 shows a flow diagram summarizing the literature search. The search of electronic databases initially identified 217 articles for review. Of those, 194 studies were excluded as being unrelated. The remaining 23 articles were carefully examined to determine whether they met the authors’ inclusion criteria. Ultimately, 4 articles that met the inclusion criteria and contained the necessary data for the planned comparison were identified [20-23].

In total, 761 patients were treated with zinc supplementation, and 712 with standard care. Three of the studies were conducted in the USA and one in Egypt. The details of the selected trials are summarized in Table 1. The methodology characteristics and comorbidities of patients in the included studies are presented in Tables S1 and S2, respectively (Supplementary Materials). The mean age of the patients treated with zinc was 59.7 (standard deviation, SD, 15.4), compared with 58.6 (17.2) years in the standard care group (MD= -0.89; 95% CI: -5.27, 3.48; P=0.69; I²=88%).
Outcomes

Survival to hospital discharge was reported in two studies and was 56.8% in the zinc group, compared to 75.9% in the non-zinc group (RD=0.01; 95% CI: -0.07, 0.08; P=0.88; I² = 0%, Figure 2). Three studies reported in-hospital mortality, which was 22.3% in the zinc group, compared to 13.6% in the standard care group (RD=-0.03; 95% CI: -0.09, 0.03; P=0.16; I²=45%; Figure 3).

The length of stay in hospital was 7.7(3.7) days in the zinc group and 7.2(3.9) days in the standard treatment group (MD=0.30; 95% CI: 0.18, 0.41; P<0.001; I²=0%; Figure S5, Supplementary Materials). The length of ICU stay was reported only by Carlucci et al. and was 4.9(1.7) days in the zinc group and 5.8(1.9) days in the standard care group (MD=-0.90; 95% CI: -1.58, -0.22; P=0.009).

Discussion

The main finding of this meta-analysis is that zinc supplementation has no positive impact on the course of COVID-19, evaluated in terms of survival to hospital discharge and in-hospital mortality. The zinc-supplemented group had a significantly longer hospital ICU length of stay, although the latter result might be questionable due to the considerable discrepancy in the size of the analyzed groups and the predominant role of the study by Carlucci et al. in this synthesis (weight: 99.5%) [21]. Our findings contradict the unambiguous advantages of zinc antiviral activity in clinical practice, both in monotherapy and in combination with hydroxychloroquine/chloroquine, which increases intracellular zinc transport. The National Institute of Health recommends a daily dose of zinc of up to 8 mg for adult women and 11 mg for men [24], while the dose used in clinical trials ranges up to 50 mg daily [25]. Overdosing of zinc causes nausea, vomiting, diarrhea, lethargy and disorders of copper metabolism [26], further discouraging zinc supplementation in COVID-19 patients.
The Carlucci et al. study was the only one to find that the addition of zinc sulfate decreased mortality or transfer to hospice, but only in the case of patients who were never admitted to the ICU [21]. In this study, patients treated with zinc sulfate had higher baseline absolute lymphocyte counts, which is in agreement with previous finding that zinc stimulated lymphocytogenesis [27]. Based on the results, it might be hypothesized that zinc is a useful tool in preventing progression of the disease, but once it progresses to the cytokine storm, the zinc activity is no longer viable [28]. However, owing to the retrospective nature of the study by Carlucci et al., caution needs to be exercised in drawing conclusions. The following randomized controlled trials failed to reveal any beneficial role for zinc in the therapeutic management of COVID-19, compared with standard of care [20,22].

The hypothesis between zinc supplementation and COVID-19 outcomes was based on the results of a retrospective study in a relatively small cohort of 275 patients that showed the median blood zinc level to be significantly lower in COVID-19 patients with poor clinical outcome than in patients with good clinical outcome (840 mg/L versus 970 mg/L; p<0.0001) [29]. Based on the results of this meta-analysis, despite some evidence that low zinc levels are associated with a worse prognosis, at present there is no evidence-based data to support the concept of zinc supplementation in COVID-19 patients. We acknowledge the limitations of our analysis. First, two studies offered retrospective data, while only two offered prospective data. Also, there was a small number of enrolled individuals in our analysis. Furthermore, there are notable differences in zinc formulation supplements that greatly affect absorption of the zinc and might not be mutually compared. For example, oral zinc picolinate supplementation has different absorption compared to zinc citrate, sulphate or gluconate. Hence, our results should be interpreted with caution. For a definitive answer to this question, it would be necessary to conduct some double-blind placebo-controlled trials.
Conclusions

Despite early evidence supporting the potential benefits of zinc supplementation in COVID-19 patients, which was based on retrospective studies and in vitro findings, the effectiveness of zinc supplementation in the treatment of COVID-19 was not proven in this meta-analysis.

**Contribution statement:** Conceptualization, L.S., M.P., T.M.; methodology, L.S., A.G.; software, L.S., M.J.J.; validation, T.M., F.W.P.; formal analysis, L.S. and J.S.; investigation, L.S., A.G. and K.P.; resources, L.S., A.G., M.J.J. and K.J.F.; data curation, L.S.; writing—original draft preparation, L.S., M.P., T.M.; visualization, L.S., A.G.; supervision, L.S. M.J.J. and K.J.F.; project administration, L.S. All authors have read and agreed to the published version of the manuscript.

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**Conflicts of interest:** The authors declare no conflict of interest.

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Table 1. Characteristics of the included studies. NS = Not specified.

| Parameter     | Treatment            | Abd-Elsalam et al. 2020 | Carlucci et al. 2020 | Thomas et al. 2020          | Yao et al. 2020          |
|---------------|----------------------|-------------------------|----------------------|-----------------------------|--------------------------|
|               |                      | Country                 | Study design         |                             |                          |
| Country       | -                    | Egypt                   | Randomized controlled study | USA                       | Prospective randomized clinical open-label trial | USA | Single-institution retrospective study |
| Study design  | -                    |                         | Retrospective analysis |                             |                          |
| Number of patients | Zinc Standard Care                          | 96                      | 411                   | 58                          | 196                      |
|               |                      | 95                      | 521                   | 50                          |                          |
| Age, years    | Zinc Standard Care                          | 43.48 (14.62)           | 63.19 (15.18)         | 44.1 (14.8)                 | 65 (4)                   |
|               | Standard Care                                  | 43.64 (13.17)           | 61.83 (15.97)         | 42 (14.6)                   | 71 (7.5)                 |
| Body Mass Index | Zinc Standard Care                          | NS                      | 29.4 (1.3)            | 30.4 (3.4)                  | 28.8 (1.1)               |
|               | Standard Care                                  | NS                      | 29.4 (1.2)            | 31.5 (3.6)                  | 26.2 (2)                 |
Figure 1. Flow diagram showing stages of database searching and study selection as per Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines.

Figure 2. Forest plot of survival to hospital discharge of zinc vs. non-zinc group. The center of each square represents the weighted risk difference for individual trials, and the corresponding horizontal line stands for a 95% confidence interval. The diamonds represent pooled results. Legend: CI = Confidence Interval; M-H = Mantel–Haenszel model.
**Figure 3.** Forest plot of in-hospital mortality of zinc vs. non-zinc group. The center of each square represents the weighted risk difference for individual trials, and the corresponding horizontal line stands for a 95% confidence interval. The diamonds represent pooled results.

Legend: CI = Confidence Interval; M-H = Mantel–Haenszel model.

| Study or Subgroup | Zinc | Standard care | Risk Difference M-H, Fixed, 95% CI |
|-------------------|------|---------------|-----------------------------------|
| Events            | Total| Events        | Total               | Weight | 95% CI       |
| Abd-ElSalam 2020  | 5    | 96            | 5                   | 95     | -0.00 [-0.06, 0.06] |
| Thomas 2021       | 0    | 58            | 0                   | 50     | 0.00 [-0.04, 0.04]  |
| Yao 2021          | 73   | 196           | 21                  | 46     | -0.08 [-0.24, 0.07] |
| **Total (95% CI)**| **350**| **191**      | 100.0%             | -0.03 [-0.09, 0.03] |

Total events 78 26
Heterogeneity: $\chi^2 = 3.61, df = 2 (P = 0.16); I^2 = 45$
Test for overall effect: $Z = 0.92 (P = 0.36)$

Zinc Standard care