PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	A randomized trial to determine the effect of vitamin D and zinc supplementation for improving treatment outcomes among COVID-19 patients in India: trial protocol
AUTHORS	Sharma, Kamal Kant; Partap, Uttara; Mistry, Nerges; Marathe, Yogesh; Wang, Molin; Shaikh, Sanaa; D'Costa, Pradeep; Gupta, Gaurav; Bromage, Sabri; Hemler, Elena; Kain, Kevin; Dholakia, Yatin; Fawzi, Wafaie.

VERSION 1 – REVIEW

REVIEWER	Branka Djordjevic University of Nis Faculty of Medicine
REVIEW RETURNED	16-Feb-2022

GENERAL COMMENTS	In general, the protocol (bmjopen-2022-061301) is well written and in good English. However, the results of the study might be country- or region-specific. Strong points 1. The study has been registered in an appropriate trial registry before the recruitment of participants. The required ethics approval was obtained, as well as, the participants' consent. 2. The study started recruiting in April 2021 (ongoing). 3. Scientific background and explanation of rationale are sound. Vitamin D deficiency is recognized as a public health problem in India, as stated in recent publications(10.4103/jfmpc.jfmpc_78_18, 10.1038/s41430-020-0558-y). The same goes for zinc (10.1177/0379572118825176). High bolus doses of vitamin D (less than 500000 IU) and up to 40 mg of zinc are generally considered safe in adults. 4. Data on the randomization method are provided. Interventions for each group are described sufficiently well to enable replication of the study. Primary and secondary outcome measures are defined, including their assessments. Information on planned statistical analyses and sample size calculation is provided. Weak points (minor flaws that need clarification) 1. The method used to generate the random allocation sequence and methods used for allocation sequence concealment before enrollment should be explained in more detail. Who are “authorized study team members”? Were the "trained site hospital staff members" those who enrolled participants, who assigned participants to interventions, or both? Unblinding procedures should be described as well. 2. Eligibility criteria for participants have changed two months after the recruitment started. I agree with the authors that the change in criteria would increase the generalizability of the results. It would be

	beneficial if the authors could discuss possible consequences (if any) of eligibility criteria change in the course of the trial (e.g. removed inclusion criterion of SpO2 ≥90, and removed exclusion criterion of recent daily multivitamin use). 3. The study collected both self-reported information and information that could be objectively measured. How do you assure uniform criteria for obtaining self-reported information (e.g. duration of symptoms)? Are there any definitions or grading? Is there a protocol for a telephone interview? Please provide more details (a questionnaire is mentioned in Data and sample management). 4. I believe that participants get therapy aside from protocol interventions. How do you secure that important non-protocol interventions are balanced across intervention groups (line 176; stratification mentioned)? Potential non-protocol interventions should be presented in the protocol. What about the patients who did not adhere to therapy (if any)? Please provide more details. 5. References regarding vitamin D deficiency in India could be updated since there are more recent articles.

REVIEWER	David Meltzer The University of Chicago
REVIEW RETURNED	19-Feb-2022

GENERAL COMMENTS	P3 L5: the statement that there are no standardized approaches to address SARS-COV-2 infection is incorrect. Preventive, not preventative, is the correct word. P3 L18: followed up is an imprecise descriptive term P3 L21: the outcome of resolution of all fever, cough and shortness of breath may not be ideal in the sense that these symptoms could be of very different importance to patients P4 L13: Is the fact that all studies are in India a limit to generalizability? P5 L35: Reference 15 is to a meta-analysis, which I do not believe included meta-analytic results related to symptom duration P6 L26-32: why are the secondary aims defined as the effects of vitamin D or zinc supplementation? Why not and/or? P6 L57-59: The change in design to include outpatients presumably altered power calculations by lowering risk. Was desired sample size adjusted upwards based on this change? P7 L21: Was data collected on multivitamin use after it was eliminated as an exclusion criterion? P8 L50-58: the rationale for these dosages should be provided. I especially like the decision to include bolus as well as daily doses of vitamin D and think that should be explained P9 L10: It could be argued that vitamin D supplementation at low doses is already widely recommended for bone health so that a lower daily dose might be justified. P9 L29: It would be good to describe Indian national guidelines that are being referred to here. P9 L41-58 There is a general lack of specificity in describing the measurements in this section, for example what survey interests, specific questions, etc.. A reference to table 1 in this paragraph would be helpful. Table 1 it self is lacking specifics with respect to how items are measures. P10 L1-10 It is not clear over what period respondents are asked to report their symptoms when interviewed. Is it over the past 3 days? P10, L15: Why are levels of symptoms, but changes in biomarkers, the right set of measures? P13 L35: What distributional assumptions about time to recovery are being made?

VERSION 1 – AUTHOR RESPONSE

Reviewer 1

Strong points
1. The study has been registered in an appropriate trial registry before the recruitment of participants. The required ethics approval was obtained, as well as, the participants' consent.
2. The study started recruiting in April 2021 (ongoing).
3. Scientific background and explanation of rationale are sound. Vitamin D deficiency is recognized as a public health problem in India, as stated in recent publications(10.4103/jfmpc.jfmpc_78_18, 10.1038/s41430-020-0558-y). The same goes for zinc (10.1177/0379572118825176). High bolus doses of vitamin D (less than 500000 IU) and up to 40 mg of zinc are generally considered safe in adults.
4. Data on the randomization method are provided. Interventions for each group are described sufficiently well to enable replication of the study. Primary and secondary outcome measures are defined, including their assessments. Information on planned statistical analyses and sample size calculation is provided.
Response: We thank the Reviewer for their positive feedback highlighting the strong points of this study.

1. The method used to generate the random allocation sequence and methods used for allocation sequence concealment before enrollment should be explained in more detail. Who are "authorized study team members"? Were the "trained site hospital staff members" those who enrolled participants, who assigned participants to interventions, or both? Unblinding procedures should be described as well.
Response: We thank the Reviewer for these comments, and appreciate that the randomization method may not have been entirely clear. Block randomization (blocks of 20), stratified by hospital, was used to generate the random allocation sequence for this study. The randomization sequence was prepared by the study statistician. In the resulting list, each participant randomization ID was assigned a regimen code, with the actual regimen known only to the manufacturer and accessible to the statistician in a currently unopened, sealed envelope. Supplement bottles and envelopes are labelled using these regimen codes by the manufacturer, so as to maintain blinding of participants and all research staff, including investigators. As each participant enters the trial, they are assigned the next randomization ID, and provided their corresponding supplement bolus envelope and daily supplement bottle. Unblinding of the study staff with respect to this allocation will occur once the analyses described in the manuscript are completed.

We have now added further detail to this end in the relevant sections (text reproduced below; underlined text denotes changes – page numbering refers to the revised, tracked manuscript draft).

Randomization and blinding, p7:
"Participants are assigned randomly to one of four groups: (1) vitamin D, (2) zinc, (3) vitamin D and zinc, or (4) placebo. For randomization, a computer-generated list was prepared by the study statistician, according to a randomization sequence in blocks of 20 and stratified by follow up clinic. The randomization list assigns each participant randomization identifier (ID) to a regimen code, with the actual regimen known only to the manufacturer and accessible to the statistician in a currently unopened, sealed envelope. Supplement bottles and envelopes are pre-labelled with codes, and active tablets and placebo are indistinguishable, so that participants and all research staff including investigators remain blinded. At each site, each participant entering the trial is given the next available randomization ID, and is provided their corresponding regimen based on the assigned regimen code."

Data analysis, p13:
"Data analysis
Planned analyses will initially be undertaken in blinded fashion (comparing coded treatment groups); unblinding of investigators and research staff with respect to treatment allocation will only occur once analyses are completed."

To note, the reference to "authorized study team members" in the manuscript is not within the context of randomization, but within the context of data management, where we outline that study data may only be accessed by authorized study personnel.

Data and sample management, p13:
“All data collected as part of this trial are entered into password-protected android electronic tablets, with pre-programmed questionnaires using ODK.[31] All data are automatically and directly uploaded from the tablets onto a secure electronic server, and entered into a password-protected database *accessible only to authorised study team members*.”

Additionally, we use “trained hospital site staff members” to refer to members of appointed study staff at each hospital site who undertake enrolment procedures as well as other data and sample collection procedures. As described above, these study members (as with all study members with the exception of the statistician) are not involved in the randomization process, and are blinded with respect to treatment allocation.

Study procedures, p6:
“Potential participants are *approached by trained site hospital staff members* when they present to site hospitals. Site hospital staff members undergo intensive training and refresher training in order to ensure that potential participants are able to make an informed decision regarding participation.”

2. Eligibility criteria for participants have changed two months after the recruitment started. I agree with the authors that the change in criteria would increase the generalizability of the results. It would be beneficial if the authors could discuss possible consequences (if any) of eligibility criteria change in the course of the trial (e.g. removed inclusion criterion of SpO2 ≥90, and removed exclusion criterion of recent daily multivitamin use).
Response: We agree with the Reviewer that it may be helpful to consider some of the implications of changing the eligibility criteria during the course of the trial. Doing this could result in distinct study sub-populations before and after the change (in this case, with regards to severity of illness and with regards to baseline nutritional status), which may have different responses to the intervention. Importantly, we changed our eligibility criteria early in the study when few participants were in the trial, meaning that we anticipate that the majority of participants will have been enrolled under the updated criteria, and there will not be two large pre-change and post-change subgroups. We have now added some brief wording around this in the Eligibility criteria section to this end.

Eligibility criteria, p6:
“To capture the greatest possible number and range of symptomatic COVID-19 cases and increase generalizability, we made the following alterations to our eligibility criteria from June 2021 (within 2 months of recruitment commencement): (1) added inclusion criterion of individuals with Rapid Antigen Test-confirmed SARS-COV-2 infection (with confirmatory PCR tests performed subsequently on all such enrolled individuals), (2) removed inclusion criterion of SpO2 ≥90, and (3) removed exclusion criterion of recent daily multivitamin use. Since this change was made early, when few (<6% of target population) participants were enrolled in the trial, we anticipate that the majority of the final study population will have been enrolled under the updated, broader criteria.”

3. The study collected both self-reported information and information that could be objectively measured. How do you assure uniform criteria for obtaining self-reported information (e.g. duration of symptoms)? Are there any definitions or grading? Is there a protocol for a telephone interview
? Please provide more details (a questionnaire is mentioned in Data and sample management).
Response: We thank the Reviewer for this comment. Data on symptoms are collected using the same questions at multiple time points throughout the study: (1) whether the participant has experienced X symptom today, and (2) How many days in total the participant has experienced X symptom, including today. As noted in the manuscript, data collection on symptoms occurs at baseline, daily while the participant is in hospital, every three days once the participant has left the hospital, and at the 8-week and 12-week follow ups. As noted by the Reviewer and described in the manuscript, all data collected in this study are collected using structured pre-programmed questionnaires on electronic tablets. We have now added further data to this end in the manuscript, including further details on some other study measures in response to comments from Reviewer 2.

Study outcomes and follow up, p9-10:
“The primary outcome of the study is time to resolution of all of the following symptoms: (1) fever, (2) cough and (3) shortness of breath. These symptoms are most commonly reported among COVID-19

patients, including in Indian populations,[46,47] and have also been assessed as part of studies examining vitamin D and zinc in respiratory illnesses [17]. These and additional symptoms (including fatigue, headache, loss of smell and taste and sore throat) are captured on multiple time points, including baseline, daily hospital follow ups for admitted patients, telephone follow ups every three days after leaving the hospital until 8 weeks post-enrolment, the 8-week clinical assessment, and finally at a 12-week assessment call. Data on symptoms are collected using the same structured questions at each time point: (1) whether the participant has experienced X symptom today, and if so, (2) how many days in total including today the participant has experienced X symptom. Staff conducting in-person and telephone follow ups are trained uniformly using a standardised telephone script with regards to collecting this information. Metrics of individual symptoms and combination of symptoms are used to identify the time point of resolution symptoms from baseline."

4. I believe that participants get therapy aside from protocol interventions. How do you secure that important non-protocol interventions are balanced across intervention groups (line 176; stratification mentioned)? Potential non-protocol interventions should be presented in the protocol. What about the patients who did not adhere to therapy (if any)? Please provide more details.

Response: We agree with the Reviewer that a clearer outline of these aspects would strengthen the manuscript.

Regarding non-protocol interventions, we anticipate that these will be balanced across groups due to randomization. At the same time, we record information on all medications and additional supplements prescribed to the participant (1) at baseline, (2) throughout their hospital stay, if admitted, and (3) at 8 weeks. We also collect information daily on other key interventions if the participant is hospitalized, including aspects such as the need for non-invasive ventilation or dialysis. This information will enable us to assess balancing of non-protocol interventions. Some of this information is already contained in the text and Table 1, and we have now amended our manuscript to add further relevant detail.

Baseline data and sample collection, p7:
"Following informed consent, participants undergo baseline data and sample collection, including recording of key background and clinical information as follows:

- Screening and background: the initial screening form is extended to collect information including participants' demographic background, socio-economic status, and health and prevention behaviours (smoking and drinking), and COVID-19 vaccination status
- Clinical baseline: clinical and physical measures are collected alongside information on COVID-19 symptoms, vital signs, blood investigations, medical conditions, treatment and medications including those prescribed for COVID-19, nutritional supplement use, complications, and medical history"

Study outcomes and follow up, p9-10:
"Following baseline assessment and provision of supplements, participants are regularly followed up as described below and in Table 1:

- Daily hospital follow up: Daily assessment of COVID-19 symptoms, vital signs, complications, medical conditions and study supplement compliance is recorded for hospitalised participants. Any new prescribed medications and supplements are also recorded alongside other interventions such as need for non-invasive ventilation or dialysis. Symptoms are specifically asked to participants; other measures are asked, observed assessed, or abstracted from the participants' records.

[…]

- 8-week clinical assessment: After completion of study supplements at 8 weeks, information is gathered on results of a clinical and physical examination, COVID-19 symptoms, compliance with regimen (including direct questioning and pill count), vital signs, blood investigations (from a collected blood sample), medical conditions, treatment and medications, use of any other nutritional supplements, updates to COVID-19 vaccination status, complications, and history. This assessment is conducted in person at the hospital, or at a location convenient to the participant where privacy can be ensured (including an option to collect some information via telephone if an in-person visit is not possible). Symptoms are specifically asked to participants; other measures are asked, observed, assessed, or abstracted from the participants' records."

Please also refer to tracked changes made in Table 1, p11-12.

Planned analyses, p13-14:
"An intent-to-treat analysis will be used as the primary analytic strategy. […] Additional collected information, including data on prescribed medications and other treatments, will enable an assessment of whether important factors including non-protocol interventions are balanced across intervention groups."

Regarding participants not adhering to therapy (which we understand to mean compliance/adherence with the intervention): (1) in hospital, supplements are consumed daily under supervision of study staff (and this is recorded during the follow up visit), (2) adherence to supplementation is assessed by direct questioning at every telephone follow up and counseling regarding the importance of adherence if the participant mentions they have not taken the supplement, and (3) compliance is assessed objectively via pill count at 8 weeks. As mentioned in our Data analysis section, our primary analytical strategy will be intent-to-treat, so for these analyses, we will compare participants by assigned intervention rather than adherence to assigned intervention. We have now added some further details around existing text to this end in the manuscript.

Intervention, p8:
"Participants receive a pre-labelled daily supplement bottle with 60 tablets, and an envelope which contains three vitamin D3/placebo bolus tablets to be consumed at baseline under supervision of site hospital staff. Following the bolus dose, participants are instructed to take supplements daily for 8 weeks. Participants are observed taking supplements daily while in hospital or contacted regularly via telephone after leaving the hospital to ensure compliance. Research staff identify barriers to compliance and aim to address these via appropriate counselling, and assess compliance at 8 weeks via direct questioning and pill count."

Table 1, p11-12: please refer to updates in tracked changes

Planned analyses, p13:
"An *intent-to-treat analysis will be used as the primary analytic strategy*. Time to primary outcome will be compared between participants randomized to vitamin D vs. no vitamin D and zinc vs. no zinc using Cox regression."

5. References regarding vitamin D deficiency in India could be updated since there are more recent articles.
Response: we have now added more updated references on vitamin D deficiency in India; these are noted below for ease of reference.

Introduction, p4:
"Observational and experimental evidence link vitamin D to an array of communicable and non-communicable diseases.[7] Vitamin D deficiency (VDD; serum vitamin D <20 ng/ml) [8] is common in urban and rural India despite the country's sunny climate, due to environmental, sociological, and biological factors,[9,10] including skin pigmentation and cultural practices related to clothing and sun exposure."

References, p19:
"9 Misra P, Srivastava R, Misra A, et al. Vitamin D status of adult females residing in Ballabgarh health and demographic surveillance system: A community-based study. Indian Journal of Public Health 2017;61:194. doi:10.4103/ijph.IJPH_176_16
10 Suryanarayana P, Arlappa N, Sai Santhosh V, et al. Prevalence of vitamin D deficiency and its associated factors among the urban elderly population in Hyderabad metropolitan city, South India. Annals of Human Biology 2018;45:133–9. doi:10.1080/03014460.2018.1425479"

Reviewer 2

1. P3 L5: the statement that there are no standardized approaches to address SARS-COV-2 infection is incorrect. Preventive, not preventative, is the correct word.

Response: We have now (1) clarified the opening statement of our Abstract, and (2) changed the word “preventative” to “preventive” as noted by the Reviewer – as reproduced in blue text below (underlined text indicates changes; page numbering refers to the revised, tracked manuscript draft).

Abstract, p2:
“Introduction: Presently, there are few population-level strategies to address SARS-COV-2 infection except preventive measures such as vaccination. Micronutrient deficiency, particularly vitamin D and zinc deficiency, has been associated with dysregulated host responses, and may play an important role in COVID-19.”

2. P3 L18: followed up is an imprecise descriptive term
Response: We thank the Reviewer for this point, and have now replaced the term “followed up”.

Abstract, p2:
“Methods and analysis: […] Participants undergo a detailed assessment at baseline and at 8 weeks, and are monitored daily in hospital or every three days after leaving the hospital to assess symptoms and other clinical measures.”

3. P3 L21: the outcome of resolution of all fever, cough and shortness of breath may not be ideal in the sense that these symptoms could be of very different importance to patients
Response: We agree with the Reviewer that individuals may give different importance to the various symptoms of COVID-19. Given that fever, cough and shortness of breath are most commonly reported among individuals with COVID-19 including as reported in Indian populations (1,2), and have also been assessed in other studies examining the role of vitamin D and zinc in respiratory illnesses (3), we aimed to similarly examine these outcomes as the primary outcomes in this trial. Additionally, as part of this trial, we are collecting information on a number of other symptoms: fatigue, headache, loss of smell, loss of taste, diarrhea, anorexia, sore throat, nasal congestion, nausea and vomiting, and as part of our questionnaires we provide participants the option to report any additional symptoms they have experienced. We would like to also examine the effect of supplementation on these symptoms – however, these are not among our current planned primary or secondary analyses.

We appreciate that this may be important to note in the manuscript, and have therefore added further wording to this end in the Methods and analysis section.

Study outcomes and follow up, p9:
“The primary outcome of the study is time to resolution of all of the following symptoms: (1) fever, (2) cough and (3) shortness of breath. These symptoms are commonly reported among COVID-19 patients, including in Indian populations,[33,34] and have also been assessed as part of studies examining vitamin D and zinc in respiratory illnesses [17]. These and additional symptoms (including fatigue, headache, loss of smell and taste and sore throat) are captured on multiple time points […]”

Table 1, p11-12: Please refer to footnote outlining symptoms

To note, we ask simple, directed and consistent questions about each symptom at multiple time points during the study, to ensure that we capture all possible information on each symptom, regardless of severity or importance to the participant.

Study outcomes and follow up, p9-10:
“The primary outcome of the study is time to resolution of all of the following symptoms: (1) fever, (2) cough and (3) shortness of breath. […] Data on symptoms are collected using the same structured questions at each time point: (1) whether the participant has experienced X symptom today, and if so, (2) how many days in total including today the participant has experienced X symptom. Staff conducting in-person and telephone follow ups are trained uniformly using a standardised telephone script with regards to collecting this information. Metrics of individual symptoms and combination of symptoms are used to identify the time point of resolution symptoms from baseline.”

4. P4 L13: Is the fact that all studies are in India a limit to generalizability?
Response: We thank the Reviewer for noting this point from the Key Messages. The wording intended to point out that to our knowledge, few other trials on this topic are based in South Asia (rather than

that all studies are based in South Asia). We have now amended the wording to clarify our point, alongside some other changes based on comments from the Editor.

Strengths and limitations, p3:
"• The setting of this study in India enables applicability of findings to the wider South Asia region, where evidence on this topic remains scarce despite a notable recent burden of COVID-19 and high prevalence of micronutrient deficiency."

5. P5 L35: Reference 15 is to a meta-analysis, which I do not believe included meta-analytic results related to symptom duration
Response: We thank the Reviewer for catching this error, and have now amended that reference.

Please refer to reference 16 (earlier reference 15) in p19, reproduced below:
"16 Zhou J, Du J, Huang L, et al. Preventive Effects of Vitamin D on Seasonal Influenza A in Infants: A Multicenter, Randomized, Open, Controlled Clinical Trial. The Pediatric Infectious Disease Journal 2018;37:749–54. doi:10.1097/INF.0000000000001890"

6. P6 L26-32: why are the secondary aims defined as the effects of vitamin D or zinc supplementation? Why not and/or?
Response: We understand the Reviewer's point that it may be valuable to understand the combined effect of supplementation with both vitamin D and zinc on our primary outcomes. The main aim of this trial, as outlined in the primary and secondary objectives, is to examine the effect of each single treatment. However, as outlined in the Data analysis section of the manuscript, we will examine effect modification of each treatment by the other – which will enable an understanding of combined effects (although notably, we may be underpowered to detect effect modification based on our sample size).

Data analysis, p13:
"Planned analyses
An intent-to-treat analysis will be used as the primary analytic strategy. Time to primary outcome will be compared between participants randomized to vitamin D vs. no vitamin D and zinc vs. no zinc using Cox regression. *We will investigate effect modification of either treatment effect by the other, and by third variables collected at baseline (including, anthropometric status, and vitamin D status).* Effect modification will be assessed by including interaction terms in Cox regression models, and statistical significance assessed via likelihood ratio tests."

Strengths and limitations, p3:
"• One limitation to the study design is that with the current sample size, the statistical power to detect modification of the effects of each supplement by other factors may be limited."

7. P6 L57-59: The change in design to include outpatients presumably altered power calculations by lowering risk. Was desired sample size adjusted upwards based on this change?
Response: We thank the Reviewer for noting this, and agree that the change to include outpatients would lead to the inclusion of participants who may have milder symptoms and therefore a shorter overall time to recovery – which may affect the power of the study to detect an effect. Additionally, around the same time, we also removed our inclusion criterion of SpO2≥90 and exclusion criterion of multivitamin use, thereby also opening the study to participants who may have more severe symptoms at baseline, and those with better micronutrient status at baseline. Since it is difficult to estimate the combined effect on the distribution of time to recovery on this basis (and it will likely depend upon the relative proportions of participants with these characteristics that we will recruit), we have not changed the power calculations. Additionally, we record characteristics such as inpatient or outpatient status and baseline SpO2 and multivitamin use, so we will be able to examine the potential effects of these during analysis. We agree that the potential implications of these changes on the power should be noted, and have therefore made some edits to the relevant statements in the Data analysis section.

Statistical power calculations, p14:
"We calculated power for detecting specified hazard ratios associated with vitamin D or zinc given a specified true effect of the other treatment. Assuming average time to recovery of 22.2 days,[39] and a low (5%) rate of loss to follow-up, enrolment of 700 patients will yield the statistical power estimates

in the Table 2. This analysis indicates that we will have at least 80% power to detect a moderate (25-30%) effect of either treatment, given a maximum 30% true effect of the other treatment. We did not further adjust our power calculations and desired sample size following changes to our eligibility criteria, which may result in the inclusion of participants with symptoms that are both more severe (SpO2 <90) and less severe (outpatients) at baseline. "

8. P7 L21: Was data collected on multivitamin use after it was eliminated as an exclusion criterion?
Response: As part of this study, we do collect data on multivitamin (and other nutritional supplements use) use at baseline, during all hospital follow up visits, and also at 8 weeks. We have now made this clear in the Methods and analysis section.

Baseline data and sample collection, p7:
"Following informed consent, participants undergo baseline data and sample collection, including recording of key background and clinical information as follows:
[…]
- Clinical baseline: clinical and physical measures are collected alongside information on COVID-19 symptoms, vital signs, blood investigations, medical conditions, treatment and medications including those prescribed for COVID-19, nutritional supplement use, complications, and medical history."

Study outcomes and follow up, p9-10:
"- Daily hospital follow up: Daily assessment of COVID-19 symptoms, vital signs, complications, medical conditions and study supplement compliance is recorded for hospitalised participants. Any new prescribed medications and supplements are also recorded alongside other interventions such as need for non-invasive ventilation or dialysis.
[…]
- 8-week clinical assessment: After completion of study supplements at 8 weeks, information is gathered on results of a clinical and physical examination, COVID-19 symptoms, compliance with regimen (including direct questioning and pill count), vital signs, blood investigations (from a collected blood sample), medical conditions, treatment and medications, use of any other nutritional supplements, updates to COVID-19 vaccination status, complications, and history."

Please also refer to tracked changes made in Table 1, p11-12.

9. P8 L50-58: the rationale for these dosages should be provided. I especially like the decision to include bolus as well as daily doses of vitamin D and think that should be explained
Response: We agree with the Reviewer that a rationale for the dosages chosen would be beneficial. Our primary motivation for the chosen dosages was to quickly achieve and maintain sufficient vitamin D and zinc levels among participants. We have now outlined this in greater detail in the Methods and analysis section.

Intervention, p8:
"We selected vitamin D3 as it has been shown to be more effective in raising and maintaining high levels of circulating 25(OH)D than vitamin D2 [33,34]. A bolus dose followed by daily doses was chosen to boost vitamin D levels quickly and safely within the first few days and maintain levels thereafter. Previous studies have indicated the efficacy of large oral doses (>200 000 IU bolus, and 1,700-2,000 IU per day) in increasing and sustaining blood 25(OH)D concentrations, with very low risk of side effects [35–41]. The 40 mg dosage of zinc is understood to be sufficiently high to assess efficacy, while remaining within the Institute of Medicine's tolerable upper intake level for adults [42]. A placebo was chosen as the comparator group given that there is currently no widespread consensus on the use of any nutritional supplement as part of standard or routine treatment for COVID-19.[17]"

10. P9 L10: It could be argued that vitamin D supplementation at low doses is already widely recommended for bone health so that a lower daily dose might be justified.
Response: We thank the Reviewer for this comment. As mentioned in our response to point 9 above, our primary motivation for choosing the higher dosage for vitamin D supplementation was to ensure that we quickly achieve and maintain sufficient vitamin D levels, based on the previous literature. However, we do agree that it may be interesting to examine the utility of lower doses of vitamin D in future studies.

Intervention, p8:
"We selected vitamin D3 as it has been shown to be more effective in raising and maintaining high levels of circulating 25(OH)D than vitamin D2 [33,34]. A bolus dose followed by daily doses was chosen to boost vitamin D levels quickly and safely within the first few days and maintain levels thereafter. Previous studies have indicated the efficacy of large oral doses (>200 000 IU bolus, and 1,700-2,000 IU per day) in increasing and sustaining blood 25(OH)D concentrations, with very low risk of side effects [35–41]. The 40 mg dosage of zinc is understood to be sufficiently high to assess efficacy, while remaining within the Institute of Medicine's tolerable upper intake level for adults [42]. A placebo was chosen as the comparator group given that there is currently no widespread consensus on the use of any nutritional supplement as part of standard or routine treatment for COVID-19.[17]"

11. P9 L29: It would be good to describe Indian national guidelines that are being referred to here.
Response: We thank the Reviewer for this comment. Since the national guidelines are being continually updated, we have provided a brief statement to this end in the manuscript, along with references to the national guidelines.

Intervention, p8-9:
"All participants are provided with care and treatment consistent with Indian national guidelines, and are encouraged to visit the study clinics seven days a week for medical attention if they feel unwell. Indian national guidelines have evolved during the pandemic, and currently consist of appropriate treatment (which may include oxygen support, respiratory support, anti-inflammatory or immunomodulatory therapy, and anticoagulation therapy) according to disease severity; discharge of admitted patients from the hospital upon resolution of symptoms and sufficient oxygen saturation (SpO2 > 93%) for three days; and self-monitoring during home isolation [43–45]."

References, p20-21:
"43 AIIMS/ICMR National Task Force/Joint Monitoring Group. Clinical Guidelines for Management of Adult COVID-19 Patients: Revised on 14/01/2022. New Delhi: : Ministry of Health and Family Welfare, Government of India https://www.mohfw.gov.in/pdf/ClinicalGuidanceforManagementofAdultCovid19Patientsupdatedason17thJanuary2022.pdf (accessed 28 Feb 2022).
44 MOHFW, GOI. Clinical Management Protocol for COVID-19 (In Adults) - Version 6 (24.05.21). New Delhi: : Ministry of Health and Family Welfare, Government of India 2021. https://www.mohfw.gov.in/pdf/UpdatedDetailedClinicalManagementProtocolforCOVID19adultsdated24052021.pdf (accessed 28 Feb 2022).
45 MOHFW, GOI. Revised Discharge Policy for COVID-19: Updated on 9th January 2022. New Delhi: : Ministry of Health and Family Welfare, Government of India https://www.mohfw.gov.in/pdf/RevisedDischargePolicyforCOVID19updatedon9thJanuary2022.pdf (accessed 28 Feb 2022)."

12. P9 L41-58 There is a general lack of specificity in describing the measurements in this section, for example what survey interests, specific questions, etc.. A reference to table 1 in this paragraph would be helpful. Table 1 it self is lacking specifics with respect to how items are measures.
Response: We thank the Reviewer for this comment. We have now added more detail on how the measures noted were collected, including specific questions for outcomes in the text, and a signal in Table 1 as to the method of collection. We also appreciate that a reference to Table 1 earlier on in the section would be helpful, and have therefore added this in.

Study outcomes and follow up, p9-10:
"Study outcomes and follow up
Following baseline assessment and provision of supplements, participants are regularly followed up as described below and in Table 1:
- Daily hospital follow up: Daily assessment of COVID-19 symptoms, vital signs, complications, medical conditions and study supplement compliance is recorded for hospitalised participants. Any new prescribed medications and supplements are also recorded alongside other interventions such as need for non-invasive ventilation or dialysis. Symptoms are specifically asked to participants; other measures are asked, observed, assessed, or abstracted from the participants' records.

- Telephone follow up: Assessment of COVID-19 symptoms, supplement compliance and adverse events is conducted in a follow up call every three days after leaving the hospital for all participants. All information is self-reported by participants.
- 8-week clinical assessment: After completion of study supplements at 8 weeks, information is gathered on results of a clinical and physical examination, COVID-19 symptoms, compliance with regimen (including direct questioning and pill count), vital signs, blood investigations (from a collected blood sample), medical conditions, treatment and medications, use of any other nutritional supplements, updates to COVID-19 vaccination status, complications, and history. This assessment is conducted in person at the hospital, or at a location convenient to the participant where privacy can be ensured (including an option to collect some information via telephone if an in-person visit is not possible). Symptoms are specifically asked to participants; other measures are asked, observed, assessed, or abstracted from the participants' records.
- 12-week telephone follow up: A final assessment is conducted of long-term COVID-19 symptoms, and any updates to COVID-19 vaccination status. All information is self-reported by participants.

All data are collected using standardized questionnaire forms on electronic tablets,[32] as described above.

The primary outcome of the study is time to resolution of all of the following symptoms: (1) fever, (2) cough and (3) shortness of breath. […] Data on symptoms are collected using the same structured questions at each time point: (1) whether the participant has experienced X symptom today, and if so, (2) how many days in total including today the participant has experienced X symptom. Staff conducting in-person and telephone follow ups are trained uniformly using a standardised telephone script with regards to collecting this information. Metrics of individual symptoms and combination of symptoms are used to identify the time point of resolution symptoms from baseline."

Please also refer to tracked changes made in Table 1, p11-12.

13. P10 L1-10 It is not clear over what period respondents are asked to report their symptoms when interviewed. Is it over the past 3 days?

Response: We appreciate that it would be good to have clear information on how data on symptoms were collected, and have now added further detail to this end in the Methods and analysis section. Briefly, at each follow up visit or call, participants are asked whether they have had the symptom today and if yes, how many days in total including today they have had the symptom.

Study outcomes and follow up, p9-10:

"The primary outcome of the study is time to resolution of all of the following symptoms: (1) fever, (2) cough and (3) shortness of breath. […] Data on symptoms are collected using the same structured questions at each time point: (1) whether the participant has experienced X symptom today, and if so, (2) how many days in total including today the participant has experienced X symptom. Staff conducting in-person and telephone follow ups are trained uniformly using a standardised telephone script with regards to collecting this information. Metrics of individual symptoms and combination of symptoms are used to identify the time point of resolution symptoms from baseline."

14. P10, L15: Why are levels of symptoms, but changes in biomarkers, the right set of measures?

Response: We understand from the Reviewer's comment that it is important to justify why we have noted changes in biomarkers but not changes in symptoms as our outcomes. To note, the referenced section was inaccurately worded with respect to the biomarkers outcome, which is blood biomarker levels (rather than strictly changes in blood biomarker levels from baseline to endline), and we thank the Reviewer for raising this. As such, our analyses will seek to assess the difference in biomarker levels associated with vitamin D or zinc supplementation, by comparing blood biomarker levels at 8 weeks across groups. As described in the manuscript, we will consider accounting for baseline blood biomarker levels in these analyses, based on whether our randomization method appears to be successful with regards to these characteristics.

We have now made slight edits to the relevant section to reflect the exact outcome that we intended to note, and that is reflected in the ClinicalTrials.gov registry record (https://clinicaltrials.gov/ct2/show/NCT04641195).

Study outcomes and follow up, p10:

“Secondary outcomes include duration of hospital stay, need for assisted ventilation, individual symptoms duration, all-cause mortality, occurrence of other severe adverse events and blood biomarker levels, including […].”

15. P13 L35: What distributional assumptions about time to recovery are being made
Response: We thank the Reviewer for this comment. The methodology that we adopted for the statistical power calculation assumes that the endpoint is an exponentially distributed time measurement. We have now added this detail into the analysis section.

Statistical power calculations, p14:
“We based power analysis on the primary outcome of time from onset of disease to clinical recovery, using methodology for survival times, which assumes exponential distribution of the time to recovery.[51]”

VERSION 2 – REVIEW

REVIEWER	Branka Djordjevic University of Nis Faculty of Medicine
REVIEW RETURNED	23-Mar-2022

GENERAL COMMENTS	I have no further concerns.

REVIEWER	David Meltzer The University of Chicago
REVIEW RETURNED	09-Apr-2022

GENERAL COMMENTS	Overall I think this is an excellent exposition of a well designed protocol. I have a few minor questions. Are their any issues about the potential to successfully blind people to their receipt of these treatments vs. placebo. I believe zinc has a distinctive taste. Did you consider collecting any data on sun exposure? For the clinical measurements, when during the course of care are various measures recorded? For the section on statistical power, I would like more explanation of what is meant be the "two for one" power advantage.

VERSION 2 – AUTHOR RESPONSE

Reviewer: 2

Overall I think this is an excellent exposition of a well designed protocol. I have a few minor questions.
Response: We thank the Reviewer for their positive comments, and hope we have addressed all outstanding concerns, below.

1. Are their any issues about the potential to successfully blind people to their receipt of these treatments vs. placebo. I believe zinc has a distinctive taste.
Response: We thank the Reviewer for making this point. As part of this study, supplements were manufactured to be identical in size, shape, and color, and were similar in smell. The supplement tablets are designed to be swallowed with water, and we therefore do not expect that participants will have an extended opportunity to taste these. While it is possible that participants may purposefully (or incidentally) taste the supplement tablets and attempt to link this to information about what zinc or

vitamin D supplements may taste like (whether it is information they previously hold, or information that they actively seek), we consider the likelihood of this to be very low.

2. Did you consider collecting any data on sun exposure?
Response: We thank the Reviewer for this comment. We do not collect data on sun exposure as part of this study. We agree that this would have some influence on vitamin D levels in the study population, although we expect that distributions of measures of sun exposure would be similar across treatment groups due to randomization. The fact that we cannot test this is indeed a limitation, and we have now added comments in this regard in the Discussion.

Discussion, p16:
"We report here the protocol of a 2x2 factorial randomized controlled trial, designed to generate evidence on the effect of vitamin D and zinc on COVID-19 progression. […]. We have taken steps to mitigate any anticipated effects of this, including broadening our eligibility criteria as described previously, and rigorous training of site hospital staff to help improve recruitment of eligible individuals. Another limitation is that we may not be able to ascertain differences in distribution of sun exposure (as a source of vitamin D) across treatment groups, although we would expect this to be similar due to randomization. […]."

3. For the clinical measurements, when during the course of care are various measures recorded?
Response: We understand from the Reviewer's comment that further clarification is required as to when clinical measurements (fourth row in Table 1) were recorded while participants were hospitalized. These measurements are recorded in study visits that are separate from (and conducted after) daily hospital inpatient ward rounds. We opted for this approach to ensure that study-related visits did not interfere in the care of the participant, and to ensure that all routine measurements and investigations for the day are captured. We have now added this additional detail in the Methods and Analysis section.

Study outcomes and follow up, p9:
"Daily hospital follow up: Daily assessment of COVID-19 symptoms, vital signs, complications, medical conditions and study supplement compliance is recorded for hospitalised participants. Any new prescribed medications and supplements are also recorded alongside other interventions such as need for non-invasive ventilation or dialysis. Symptoms are specifically asked to participants; other measures are asked, observed, assessed, or abstracted from the participants' records. Clinical measurements are recorded in study-specific visits that are conducted independently after ward rounds, to minimize interference in care and ensure all relevant information for the day is noted."

4. For the section on statistical power, I would like more explanation of what is meant be the "two for one" power advantage.
Response: We thank the Reviewer for this comment. By the "two-for-one" power advantage, we intend to refer to the potential sample size efficiency that factorial trials present - whereby the total number of participants needed to test each treatment is less using a factorial design than it would be using two separate trials. It may be justifiable to assume this advantage for a factorial trial where the endpoints for each intervention are different, since each intervention would be expected to not affect the endpoint for the other intervention. However, for trials with a single endpoint (such as the current study), each intervention would affect the same endpoint. As a result, the frequency of events of interest in the study may be reduced in groups where both interventions are administered (1). This may drive up sample size requirements (or reduce power). We have now added a brief note to this end in the Data analysis section.

Statistical power calculations, p14:
"With a single endpoint for both interventions, the factorial design does not provide a "two-for-one" power advantage, where the total number of participants required to test two treatments is lower using a single factorial trial compared with two parallel group trials.[49]"

Additional minor edit
We have made a minor update to the funding information in the manuscript – this has also been updated in the manuscript submission system.

Funding, p16:
“This trial is supported by the Canadian Institutes of Health Research, Operating Grant: COVID-19 Rapid Research Funding Opportunity – Therapeutics, application number: 447092 and the Canada Research Chair program (to KCK). SB was supported by the National Institutes of Health (grant D43 TW010543).”