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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

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Manuscripts

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5 2 **supplementation for improving treatment outcomes among COVID-19**
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7 3 **patients in India: trial protocol**
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ABSTRACT

Introduction: Presently, there are no standardised strategies to address SARS-COV-2 infection except preventative 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.

Methods and analysis: We have designed a 2x2 factorial, randomized, double-blind, multi-centre placebo-controlled trial to evaluate the effect of vitamin D and zinc on COVID-19 outcomes in Maharashtra, India. COVID-19 positive individuals are recruited from hospitals in Mumbai and Pune. Participants are provided (1) vitamin D3 bolus (180,000 IU) maintained by daily dose of 2000 IU, and/or (2) zinc gluconate (40 mg daily), versus placebo for 8 weeks. Participants undergo a detailed assessment at baseline and at 8 weeks, and are followed up daily in hospital or every three days after leaving the hospital to monitor symptoms and other clinical measures. A final follow up telephone call occurs 12 weeks post-enrolment to assess long-term outcomes. The primary outcome of the study is time to recovery, defined as time to resolution of all of fever, cough and shortness of breath. Secondary outcomes include: duration of hospital stay, all-cause mortality, necessity of assisted ventilation, change in blood biomarker levels, and individual symptoms duration. Participant recruitment commenced on April 2021.

Ethics and dissemination: Ethical approval was obtained from institutional ethical committees of all participating institutions. The study findings will be presented in peer-reviewed medical journals.

Trial Registration number: NCT04641195, CTRI/2021/04/032593, HMSC (GOI)-2021-0060

STRENGTHS AND LIMITATIONS OF THIS STUDY

- To our knowledge, this is the first factorial trial designed primarily to assess the effect of vitamin D (high-bolus dose maintained by daily doses) and zinc gluconate in COVID-19. A few other trials have been based in South Asia – this is key given the notable recent burden of COVID-19 and high prevalence of micronutrient deficiency in this region.
- The randomized, doubled-blind, placebo-controlled design of this trial will enable a better understanding of the role of vitamin D and zinc in COVID-19, informing relevant recommendations and action. The location of the study in two large cities in India will facilitate more generalizable results.
- With frequent follow up of participants, this study collects information across a range of domains including sociodemographic and clinical measures, and biomarker data, which will allow for a detailed investigation of the effect of supplementation on disease progression.
- This study is powered to detect a modest effect (25-30%) of either treatment on the primary outcome. 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.

INTRODUCTION

COVID-19 continues to be a problem globally, with over 16 million incident cases and 200,000 deaths reported in November 2021.[1] Concerted global efforts have resulted in the development of vaccines, which may reduce the burden and impact of COVID-19, although suboptimal vaccine coverage and the rapid mutation of the virus continue to prolong the pandemic.[2–5] Additionally, with limited proven treatment regimens for COVID-19 to date, it is essential to continue exploring low cost and commonly available effective interventions which can be implemented as standardized therapeutic treatment regimens at large.[6] This is especially important in the context of low and middle-income countries in South Asia and Africa, which are particularly vulnerable given weak health systems and the co-existence of malnutrition and other co-morbidities. This includes India, which continues to report a substantial number of COVID-19 cases.[1]

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] including skin pigmentation and cultural practices related to clothing and sun exposure. Countrywide studies suggest VDD may affect at least 70% of the Indian population. Vitamin D shows promise as a novel, cost-effective prevention and adjunctive treatment for respiratory infections. In laboratory studies, vitamin D metabolites support innate immune responses to rhinoviruses and respiratory syncytial virus.[10–14] In participants with influenza, high-dose vitamin D supplementation shortened durations of fever, cough and wheezing, particularly among those with low vitamin D levels.[15] In a recent systematic review and meta-analysis of randomised controlled trials, vitamin D supplementation was associated with decreased risk of acute respiratory infections and shortened duration of symptoms.[16]

Zinc is an essential mineral that plays critical roles in gene expression, cell division, and immunity.[17] In India, dietary predominance of micronutrient-sparse staples, limited consumption of animal foods, and high consumption of zinc absorption inhibitors render the population at extremely high risk of inadequacy, which is exacerbated due to global climate change.[18] About 25% of the Indian population is zinc inadequate, and 4.3 million child deaths (<5 years) were attributable to zinc deficiency in 2017.[19] Multiple meta-analyses and pooled analyses of randomized controlled trials conducted in the US and low- and middle-income countries have shown that oral zinc supplementation reduces incidence of acute respiratory infections by 35%, shortens duration of symptoms, and improves recovery rate.[16,20–23] Zinc is a potential treatment in COVID-19, due to its immune modulatory effect, as well as direct antiviral effect.[24] The mechanisms by which zinc may serve as adjunct therapy in COVID-19 has been recently reviewed by Skalny et al. 2020,[25] who note that Zn²⁺ cations, especially in combination with zinc ionophore pyrithione inhibit SARS-coronavirus RNA polymerase activity by decreasing replication.[26]

Vitamin D and zinc are safe, inexpensive, and widely available therapies; therefore, experimental evidence that these nutrient supplements are effective against COVID-19 would readily support their inclusion in standard of care. Therefore, we are undertaking a randomized controlled trial to determine the effect of vitamin D and zinc supplementation on treatment outcomes among individuals with COVID-19 in India.

OBJECTIVES

The primary objectives of this trial are:

- ❖ To determine the effect of vitamin D supplementation versus placebo on time to recovery among patients with COVID-19
- ❖ To determine the effect of zinc supplementation versus placebo on time to recovery among patients with COVID-19

Secondary objectives include:

- ❖ To determine the effect of vitamin D or zinc supplementation on duration of hospital stay, all-cause mortality, necessity for assisted ventilation, and individual symptoms duration
- ❖ To examine the effect of vitamin D or zinc supplementation on key blood biomarkers, including serum vitamin D and zinc, and immunological and inflammatory markers

METHODS AND ANALYSIS

Trial design, population, and enrolment sites

This is a double-blind, placebo-controlled, randomized superiority trial with 2x2 factorial design and 1:1:1:1 allocation ratio, being conducted at two site hospitals in Mumbai and Pune, Maharashtra, India (**Figure 1**). Maharashtra has the highest cumulative number of COVID-19 cases and fatalities out of all states in India.[27] Within the state, both Pune and Mumbai have emerged as COVID-19 hotspots.[28,29]

The two study sites (King Edward Memorial Hospital and Research Centre, Pune, and Saifee Hospital, Mumbai) are established medical institutions located within the cities of Pune and Mumbai. These hospitals have been designated as COVID-19 dedicated hospitals by local municipal corporations, where people can avail COVID-19-related treatment and services. The trial is targeting a sample size of 700; participant recruitment commenced in April 2021. While we initially targeted only hospitalized inpatients at each site for the study, we broadened our target population in June 2021 to include all hospital outpatients. This was done in order to increase generalizability of results and maintain enrolment in light of decreasing COVID-19 cases.[30]

Eligibility criteria

The original inclusion criteria for this study were as follows: (1) men and women aged ≥ 18 years, (2) RT-PCR-confirmed infection with SARS-COV-2, (3) oxygen saturation level (SpO₂) ≥ 90 , and (4) written informed consent.

The exclusion criteria were as following: (1) pregnant women, (2) individuals enrolled in other clinical trials, (3) daily use of multivitamins for the past 1 month.

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.

Study procedures

An overview of trial procedures is summarised in **Figure 2**.

Recruitment and obtaining informed consent

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. These dedicated site hospital staff members determine their interest and eligibility, and provide a brief introduction including key details about the study and what participation involves. The staff members read out the participant information sheet in the appropriate conversational language (English, Hindi or Marathi), and discuss the trial components and the role of the participant in the study. Information provided includes a clear outline of potential benefits and harms, the length of the follow up period, remuneration that can be expected, future use of information and samples, and resources available to the participant such as access to study clinics. Informed consent is obtained after responding to any raised queries. As part of the process, potential participants are informed that their participation is completely voluntary and they can withdraw any time at any stage of the study without providing any reasons. The informed consent process is completed once participants provide their signature on two copies of the consent document; one copy for the trial record and another provided to participants for their reference.

Information regarding eligibility of potential participants is collected on a secure electronic tablet using Open Data Kit (ODK),^[31] with questionnaires including built-in checks and data uploaded to a secure server. No identifiable data are collected until the participant has provided informed consent.

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2 157 Baseline data and sample collection
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5 158 Following informed consent, participants undergo baseline data and sample collection, including recording of key
6
7 159 background and clinical information as follows:
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- 10 160 ➤ Screening and background: the initial screening form is extended to collect information including
11
12 161 participants' demographic background, socio-economic status, and health and prevention behaviours
13 162 (smoking and drinking)
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15 163 ➤ Baseline dietary information: a food frequency questionnaire (FFQ) is administered, collecting
16
17 164 information on dietary practices and habits in relation to 25 food groups. The FFQ is validated for use in
18 165 India and has been adapted to the Maharashtra context
19
20 166 ➤ Clinical baseline: clinical and physical measures are collected alongside information on COVID-19
21
22 167 vaccination status, COVID-19 symptoms, vital signs, blood investigations, medical conditions, treatment
23 168 and medications, complications, and medical history
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26 169 A blood sample is also collected at baseline. All information is collected securely on electronic tablets, as described
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28 170 above.
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31 171 Randomization and blinding
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33 172 Participants are assigned randomly to one of four groups: (1) vitamin D, (2) zinc, (3) vitamin D and zinc, or (4)
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35 173 placebo. Supplement bottles and envelopes are pre-labelled with codes, and active tablets and placebo are
36
37 174 indistinguishable, so that participants and investigators are blinded. For randomization, a computer-generated
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39 175 list from 1 to 1000 was prepared by the study statistician, according to a randomization sequence in blocks of 20
40 176 and stratified by follow up clinic. Extra codes were generated to account for unforeseen circumstances such as
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42 177 lost supplements, or abrasion of labels.
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44
45 178 Intervention
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47 179 Patients are randomized to one of four groups:
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- 50 180 1. Placebo-Placebo group will receive a *placebo* vitamin D3 bolus at the hospital followed by *placebo*
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52 181 daily vitamin D3 maintenance doses and *placebo* daily zinc supplements
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54 182 2. Vitamin D-Placebo group will receive an *actual* vitamin D3 bolus (180,000 IU) at the hospital followed
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56 183 by *actual* daily vitamin D3 maintenance doses (2000 IU daily) and daily *placebo* zinc supplements
57 184 3. Placebo-Zinc group will receive a *placebo* vitamin D3 bolus at the hospital followed by *placebo* daily
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59 185 vitamin D3 maintenance doses and *actual* daily zinc supplements (40 mg daily)
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3 186 4. Vitamin D3-Zinc group will receive an *actual* vitamin D3 bolus (180,000 IU) at the hospital followed by
4 187 *actual* daily vitamin D3 maintenance doses (2000 IU daily) and *actual* daily zinc supplements (40 mg
5
6 188 daily)
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9 189 A placebo was chosen as the comparator group given that there is currently no widespread consensus on the use
10
11 of any nutritional supplement as part of standard or routine treatment for COVID-19.[16]
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13 191 Participants receive a pre-labelled daily supplement bottle with 60 tablets, and an envelope which contains three
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15 192 vitamin D3/placebo bolus tablets to be consumed at baseline under supervision of site hospital staff. Following
16
17 193 the bolus dose, participants are instructed to take supplements daily for 8 weeks. Participants are contacted daily
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19 194 while in hospital or regularly via telephone after leaving the hospital to ensure compliance. Research nurses
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21 195 identify barriers to compliance, and assess compliance at 8 weeks via direct questioning and pill count.
22

23 196 Supplement and placebo tablets were manufactured by Excellamed Laboratories Private Limited (Mumbai, India)
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25 197 with an external quality check done by an independent service provider (Bee Pharmo Labs Private Limited,
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27 198 Mumbai, India).
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29 199 All participants are provided with care and treatment consistent with Indian national guidelines, and are
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31 200 encouraged to visit the study clinics seven days a week for medical attention if they feel unwell.
32

33 201 Study outcomes and follow up 34

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36 202 Following baseline assessment and provision of supplements, participants are regularly followed up as described
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38 203 below:
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41 204 ➤ Daily hospital follow up: Daily assessment of COVID-19 symptoms, vital signs, complications, medical
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43 205 conditions and study supplement compliance is recorded for hospitalised participants
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45 206 ➤ Telephone follow up: Assessment of COVID-19 symptoms, supplement compliance and adverse events is
46
47 207 conducted in a follow up call every three days after leaving the hospital for all participants
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49 208 ➤ 8-week clinical assessment: After completion of study supplements at 8 weeks, information is gathered
50
51 209 on results of a clinical and physical examination, COVID-19 symptoms, compliance with regimen (including
52
53 210 direct questioning and pill count), vital signs, blood investigations (from a collected blood sample),
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55 211 medical conditions, treatment and medications, complications, and history. This assessment is conducted
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57 212 in person at the hospital, or at a location convenient to the participant where privacy can be ensured
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59 213 (including an option to collect some information via telephone if an in-person visit is not possible)
60
61 214 ➤ 12-week telephone follow up: A final assessment is conducted of long-term COVID-19 symptoms
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63 215 All data are collected using standardized questionnaire forms on electronic tablets,[31] as described above.

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3 216 The primary outcome of the study is time to resolution of all of the following symptoms: (1) fever, (2) cough and
4 217 (3) shortness of breath. These and additional symptoms are captured on multiple time points, including baseline,
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6 218 daily hospital follow ups for admitted patients, telephone follow ups every three days after leaving the hospital
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8 219 until 8 weeks post-enrolment, the 8-week clinical assessment, and finally at a 12-week assessment call. Metrics of
9 220 individual symptoms and combination of symptoms are used to identify the time point of resolution symptoms
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11 221 from baseline.

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14 222 Secondary outcomes include duration of hospital stay, need for assisted ventilation, individual symptoms duration,
15 223 all-cause mortality, occurrence of other severe adverse events and change in blood biomarker levels, including 25-
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17 224 hydroxy vitamin D, zinc and calcium, and other immunological and inflammatory biomarkers. Biomarker levels are
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19 225 assessed using blood samples collected at baseline and at the 8-week clinical assessment. Occurrence of any other
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21 226 secondary endpoints between baseline to 8-week clinical assessment is recorded during follow up calls or visits as
22 227 described above.

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25 228 A list of collected data and blood investigations with time points at baseline, during follow up visits or calls, and
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27 229 at 8 and 12 weeks is summarized in **Table 1**.

Table 1. Collection of data points in the trial.

Data category	Baseline (enrolment)	Follow up	8 weeks	12 weeks
Demographic and background information	Age, gender, education, marital status, occupation, socio-economic status, health and prevention behaviours, COVID-19 vaccination			
Dietary information	Food frequency questionnaire: consumption frequency of 25 diverse food groups in last three months			
Clinical examination	Medical history, comorbidities, preadmission medications, clinical symptoms	Hospital and telephone follow up: Clinical symptoms	Medical history, comorbidities, pre-assessment medications, clinical symptoms	Clinical symptoms
Clinical measurements	Respiratory rate, pulse, auxiliary temperature, SpO ₂ , systolic and diastolic blood pressure, weight and height	Hospital follow up only: Respiratory rate, pulse, auxiliary temperature, SpO ₂ , systolic and diastolic blood pressure, weight, requirement for non-invasive ventilation or intubation/ventilator support, need for dialysis, lab investigations	Respiratory rate, pulse, auxiliary temperature, SpO ₂ , systolic and diastolic blood pressure, weight and height	
Blood and other investigations and biomarkers	SARS-COV-2 RT-PCR, chest X-Ray, complete blood count, blood glucose, serum creatinine, CRP, LDH, serum ferritin, D-dimer, vitamin D, zinc, calcium, IgG, IgM, Ang2, IL-6 and sTREM-1		CRP, LDH, serum ferritin, D-dimer, vitamin D, zinc, calcium, IgG, IgM, Ang2, IL-6 and sTREM-1	
Other information		Hospital and telephone follow up: Compliance, adverse events		

SpO₂: Oxygen saturation, CRP: C-reactive protein, LDH: lactate dehydrogenase, IgG: Immunoglobulin G, IgM: immunoglobulin M, Ang2: angiotensin-2, IL-6: interleukin 6, sTREM-1: soluble triggering receptor expressed on myeloid cells-1.

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2 Adverse events and reporting

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5 3 Any undesirable circumstance or experiences reported by study participants during the study are categorised as
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7 4 adverse events. All adverse events which are possibly, probably or very likely related to administration of any
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9 5 supplement are monitored and reported to site institutional review boards (IRBs) within 72 hours (serious adverse
10
11 6 events) or 1 month (all other adverse events), using a standardized reporting format. The trial data and safety
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13 7 monitoring board (DSMB) is also notified. Site principal investigators and independent physicians are responsible
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15 8 for assessing the causal relationship and making the conclusive decision about continuation of the trial for a
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17 9 particular participant. Additionally, medical insurance is provided to all study participants to take care of any
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19 10 progression of severe adverse events.

20 11 Data and sample management

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23 12 All data collected as part of this trial are entered into password-protected android electronic tablets, with pre-
24
25 13 programmed questionnaires using ODK.[31] All data are automatically and directly uploaded from the tablets onto
26
27 14 a secure electronic server, and entered into a password-protected database accessible only to authorised study
28
29 15 team members. Data are stored in linked-anonymised form, with identifiable information and the linking key
30
31 16 stored separately. All analyses and data checks are conducted on anonymised data only.

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33 17 Blood samples collected as part of this trial are processed at the Foundation for Medical Research, Mumbai, and
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35 18 accredited laboratories in India including at the site hospitals. Specimens are linked-anonymised and are stored
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37 19 securely at the Foundation for Medical Research for a maximum of three years.

38 20 Data analysis

39 21 Planned analyses

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44 22 An intent-to-treat analysis will be used as the primary analytic strategy. Time to primary outcome will be compared
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46 23 between participants randomized to vitamin D vs. placebo and zinc vs. placebo using Cox regression. We will
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48 24 investigate effect modification of either treatment effect by the other, and by third variables collected at baseline
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50 25 (including, anthropometric status, and vitamin D status). Effect modification will be assessed by including
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52 26 interaction terms in Cox regression models, and statistical significance assessed via likelihood ratio tests. There are
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54 27 no *a priori* effect modifiers hypothesized, and unless there is strong modification of a treatment effect, our power
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56 28 to detect these may be low. We will assess the success of randomization by comparing baseline variables by
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58 29 treatment group using χ^2 and t-tests and use multivariate modelling to adjust for imbalances if needed.

59 30 The effect of vitamin D or zinc on dichotomous secondary outcomes will be analyzed in a similar approach. The
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61 31 proportion of individuals experiencing hypercalcemia will be compared between treatment groups using χ^2 tests,

and effects of the supplements on blood biomarkers will be compared via Wilcoxon and t-tests. This study will measure numerous risk factors for COVID-19 progression and severe treatment outcomes including hemoglobin; co-morbidities; medications including chloroquine, hydroxychloroquine, and ACE inhibitors; and socio-demographic, clinical, nutritional, and lifestyle-related risk factors. We will examine relationships of these factors in the placebo group first, to avoid complex questions concerning interactions between risk factors and treatments. Once we find a satisfactory parsimonious model using principles of model selection as detailed by Greenland,[32] we will test and modify it if needed in the whole study population, adjusting for treatment effects.

Analyses will consider sex and gender throughout, by disaggregating findings, and attempting to elucidate the roles of sex and gender in the clinical course and immune response by controlling for potential sociodemographic, nutritional, and immunological confounders.

Statistical power calculations

With a single endpoint for both interventions, the factorial design does not provide a “two-for-one” power advantage.[33] Power will decrease if each treatment has a moderate effect; we accounted for this in calculating the sample size. Assumptions related to treatment effects may be reasonably inferred from meta-analyses of well-designed randomized controlled trials studying these supplements in other acute respiratory illnesses.[15,16,20–23] We based power analysis on the primary outcome of time from onset of disease to clinical recovery, using methodology for survival times.[34] 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,[35] 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.

Table 2. Statistical power estimation.

Effect of Treatment A	True effect of Treatment B						
	0%	5%	10%	15%	20%	25%	30%
30%	99%	99%	99%	99%	98%	98%	97%
25%	95%	94%	93%	92%	90%	88%	86%
20%	81%	79%	76%	74%	71%	69%	66%

Patient and public involvement

Patients and the public were not involved in the design of this study.

56 DATA AND SAFETY MONITORING BOARD

57 The Data and Safety Monitoring Board (DSMB) was established prior to commencement of the trial. It consists of
58 independent experts in respiratory infection and communicable diseases, public health and nutrition, clinical
59 research, and biostatistics. The role of the board is to provide their inputs, recommendations, review the trial
60 protocols and progress by ensuring the rights and safety of involving participants in the study through periodic
61 trial review meetings.

62 The trial DSMB will examine efficacy endpoints by study arms when half of individuals are enrolled. In accordance
63 with the Haybittle-Peto rule, if the difference in the primary outcome between study arms is <0.001 , unblinding of
64 the DSMB and stopping will be considered.[36]

65 ETHICS AND DISSEMINATION

66 This study is being conducted in the accordance with the Declaration of Helsinki 2013. The study was approved
67 by the Institutional Review Board of the Harvard T.H. Chan School of Public Health (Protocol No. IRB20-1425), the
68 University Health Network Research Ethics Board (20-5775), the Institutional Research Ethics Committee of the
69 Foundation for Medical Research (IREC No. FMR/IREC/C19/02/2020), the Institutional Review Board of Saifee
70 Hospital (Project No. EC/008/2020) and the KEM Hospital Research Centre Ethics Committee (KEMHRC ID No.
71 2027). The trial is registered on ClincialTrials.gov (NCT04641195). Permission for the study was also obtained from
72 the Health Management and Screening Comments (HMSC), Government of India (HMSC (GOI)-2021-0060), and
73 the study was registered prospectively in the Clinical Trials Registry India (CTRI/2021/04/032593). Since the study
74 intervention is related to micronutrient supplementation, endorsement from the Drugs Controller General of
75 India was non-obligatory. The study findings will be presented in peer-reviewed medical journals.

DISCUSSION

With continued high incidence of global cases, COVID-19 remains a global health challenge. Alongside vaccination and other preventative measures, low-cost and efficient interventions which may help minimize the occurrence of serious disease are needed. These would be particularly valuable in low- and middle-income countries, where health systems are more overburdened and resources much fewer. In this context, and given previous evidence regarding the role of vitamin D and zinc in the development of and recovery from respiratory infections,[15,16,20–23] there is a need to explore their potential value as part of therapeutic regimens for COVID-19.

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. The frequent follow up of participants and collection of a range of sociodemographic, clinical and biomarker measures alongside blood samples will enable a detailed investigation of the effect of supplementation on disease progression, including potentially important immunological and inflammatory pathways. Importantly, in comparison with other vitamin D or zinc COVID-19 intervention studies currently registered on ClinicalTrials.gov, this would be the first conducted outside of the U.S. or Europe and other similar high-income countries. The location of this study in two large cities, alongside the broad eligibility criteria, increases the generalizability of study results. Given the current unpredictability of COVID-19 waves, one challenge to the study is to maintain recruitment during periods where cases may be on the decline. 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. Regardless, the findings of this study will have direct relevance to many settings in South Asia and sub-Saharan Africa with weak health systems and prevalent malnutrition. Ultimately, the evidence generated as part of this trial will enhance our understanding of the role of vitamin D and zinc in COVID-19 disease, and contribute high quality evidence on the potential value of supplementation of these micronutrients for the same.

FUNDING

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AUTHOR CONTRIBUTIONS

WWF, KCK, YD, and NM conceptualised the project, and designed the study along with SB, KKS, ECH, YM and UP. YD, NM, PDC, GG, KKS, YM, and SS are involved in data acquisition, and in study monitoring along with KCK, WWF and UP. MW provides statistical expertise. KKS and UP drafted the manuscript, and all authors reviewed and critically revised the draft and approved the final manuscript.

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COMPETING INTERESTS

All authors declare no conflicts of interest.

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For peer review only

REFERENCES

- 1 Dong E, Du H, Gardner L. An interactive web-based dashboard to track COVID-19 in real time. *The Lancet Infectious Diseases* 2020;**20**:533–4. doi:10.1016/S1473-3099(20)30120-1
- 2 Hogan A, Winksill P, Watson O, *et al.* Modelling the allocation and impact of a COVID-19 vaccine. Published Online First: 25 September 2020. doi:https://doi.org/10.25561/82822
- 3 Phillips N. The coronavirus is here to stay — here's what that means. *Nature* 2021;**590**:382–4. doi:10.1038/d41586-021-00396-2
- 4 Torjesen I. Covid-19 will become endemic but with decreased potency over time, scientists believe. *BMJ* 2021;**372**:n494. doi:10.1136/bmj.n494
- 5 Hockham C, Kotwal S, Wilcox A, *et al.* Protocol for the Controlled evaluation of Angiotensin Receptor blockers for COVID-19 respiratory disease (CLARITY): a randomised controlled trial. *Trials* 2021;**22**:573. doi:10.1186/s13063-021-05521-0
- 6 Teshome A, Adane A, Girma B, *et al.* The Impact of Vitamin D Level on COVID-19 Infection: Systematic Review and Meta-Analysis. *Front Public Health* 2021;**9**:624559. doi:10.3389/fpubh.2021.624559
- 7 Bouillon R, Marcocci C, Carmeliet G, *et al.* Skeletal and Extraskeletal Actions of Vitamin D: Current Evidence and Outstanding Questions. *Endocr Rev* 2019;**40**:1109–51. doi:10.1210/er.2018-00126
- 8 Pearce SH, Cheetham TD. Diagnosis and management of vitamin D deficiency. *BMJ* 2010;**340**:b5664. doi:10.1136/bmj.b5664
- 9 G R, Gupta A. Vitamin D deficiency in India: prevalence, causalities and interventions. *Nutrients* 2014;**6**:729–75. doi:10.3390/nu6020729
- 10 Greiller CL, Martineau AR. Modulation of the immune response to respiratory viruses by vitamin D. *Nutrients* 2015;**7**:4240–70. doi:10.3390/nu7064240
- 11 Greiller CL, Suri R, Jolliffe DA, *et al.* Vitamin D attenuates rhinovirus-induced expression of intercellular adhesion molecule-1 (ICAM-1) and platelet-activating factor receptor (PAFR) in respiratory epithelial cells. *J Steroid Biochem Mol Biol* 2019;**187**:152–9. doi:10.1016/j.jsbmb.2018.11.013
- 12 Telcian AG, Zdrengeha MT, Edwards MR, *et al.* Vitamin D increases the antiviral activity of bronchial epithelial cells in vitro. *Antiviral Res* 2017;**137**:93–101. doi:10.1016/j.antiviral.2016.11.004
- 13 Hansdottir S, Monick MM, Lovan N, *et al.* Vitamin D decreases respiratory syncytial virus induction of NF-kappaB-linked chemokines and cytokines in airway epithelium while maintaining the antiviral state. *J Immunol* 2010;**184**:965–74. doi:10.4049/jimmunol.0902840
- 14 Hansdottir S, Monick MM, Hinde SL, *et al.* Respiratory epithelial cells convert inactive vitamin D to its active form: potential effects on host defense. *J Immunol* 2008;**181**:7090–9. doi:10.4049/jimmunol.181.10.7090
- 15 Martineau AR, Jolliffe DA, Hooper RL, *et al.* Vitamin D supplementation to prevent acute respiratory tract infections: systematic review and meta-analysis of individual participant data. *BMJ* 2017;**356**:i6583. doi:10.1136/bmj.i6583
- 16 Abioye AI, Bromage S, Fawzi W. Effect of micronutrient supplements on influenza and other respiratory tract infections among adults: a systematic review and meta-analysis. *BMJ Glob Health* 2021;**6**:e003176. doi:10.1136/bmjgh-2020-003176

- 1
2 156 17 International Zinc Nutrition Consultative Group (IZiNCG), Brown KH, Rivera JA, *et al.* International Zinc
3 157 Nutrition Consultative Group (IZiNCG) technical document #1. Assessment of the risk of zinc deficiency in
4 158 populations and options for its control. *Food Nutr Bull* 2004;**25**:S99-203.
- 6
7 159 18 Smith MR, DeFries R, Chhatre A, *et al.* Inadequate Zinc Intake in India: Past, Present, and Future. *Food Nutr*
8 160 *Bull* 2019;**40**:26–40. doi:10.1177/0379572118825176
- 9
10 161 19 GBD Compare. Institute for Health Metrics and Evaluation. 2014. [https://www.healthdata.org/data-](https://www.healthdata.org/data-visualization/gbd-compare)
11 162 [visualization/gbd-compare](https://www.healthdata.org/data-visualization/gbd-compare) (accessed 17 Dec 2021).
- 12
13 163 20 Hemilä H, Fitzgerald JT, Petrus EJ, *et al.* Zinc Acetate Lozenges May Improve the Recovery Rate of Common
14 164 Cold Patients: An Individual Patient Data Meta-Analysis. *Open Forum Infect Dis* 2017;**4**:ofx059.
15 165 doi:10.1093/ofid/ofx059
- 16
17 166 21 Roth DE, Richard SA, Black RE. Zinc supplementation for the prevention of acute lower respiratory infection
18 167 in children in developing countries: meta-analysis and meta-regression of randomized trials. *Int J Epidemiol*
19 168 2010;**39**:795–808. doi:10.1093/ije/dyp391
- 21
22 169 22 Bhatnagar S, Wadhwa N, Aneja S, *et al.* Zinc as adjunct treatment in infants aged between 7 and 120 days
23 170 with probable serious bacterial infection: a randomised, double-blind, placebo-controlled trial. *Lancet*
24 171 2012;**379**:2072–8. doi:10.1016/S0140-6736(12)60477-2
- 25
26 172 23 Banupriya N, Bhat BV, Benet BD, *et al.* Short Term Oral Zinc Supplementation among Babies with Neonatal
27 173 Sepsis for Reducing Mortality and Improving Outcome - A Double-Blind Randomized Controlled Trial. *Indian*
28 174 *J Pediatr* 2018;**85**:5–9. doi:10.1007/s12098-017-2444-8
- 30
31 175 24 Zhang L, Liu Y. Potential interventions for novel coronavirus in China: A systematic review. *J Med Virol*
32 176 2020;**92**:479–90. doi:10.1002/jmv.25707
- 33
34 177 25 Skalny AV, Rink L, Ajsuvakova OP, *et al.* Zinc and respiratory tract infections: Perspectives for COVID-19
35 178 (Review). *Int J Mol Med* 2020;**46**:17–26. doi:10.3892/ijmm.2020.4575
- 36
37 179 26 te Velthuis AJW, van den Worm SHE, Sims AC, *et al.* Zn(2+) inhibits coronavirus and arterivirus RNA
38 180 polymerase activity in vitro and zinc ionophores block the replication of these viruses in cell culture. *PLoS*
39 181 *Pathog* 2010;**6**:e1001176. doi:10.1371/journal.ppat.1001176
- 40
41 182 27 COVID19 STATEWISE STATUS. MyGov.in. 2020. <https://mygov.in/corona-data/covid19-statewise-status/>
42 183 (accessed 17 Dec 2021).
- 44
45 184 28 Tambe MP, Parande MA, Tapare VS, *et al.* An epidemiological study of laboratory confirmed COVID-19 cases
46 185 admitted in a tertiary care hospital of Pune, Maharashtra. *Indian J Public Health* 2020;**64**:S183–7.
47 186 doi:10.4103/ijph.IJPH_522_20
- 48
49 187 29 Kodge BG. A review on current status of COVID19 cases in Maharashtra state of India using GIS: a case
50 188 study. *Spatial Information Research* 2020;**1**–7. doi:10.1007/s41324-020-00349-3
- 51
52 189 30 India: WHO Coronavirus Disease (COVID-19) Dashboard With Vaccination Data. <https://covid19.who.int>
53 190 (accessed 17 Dec 2021).
- 54
55 191 31 ODK - Collect data anywhere. <https://getodk.org> (accessed 17 Dec 2021).
- 56
57 192 32 Greenland S. Modeling and variable selection in epidemiologic analysis. *Am J Public Health* 1989;**79**:340–9.
58 193 doi:10.2105/ajph.79.3.340
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2 194 33 Ellenberg SS, Finkelstein DM, Schoenfeld DA. Statistical Issues Arising in AIDS Clinical Trials. *Journal of the*
3 *American Statistical Association* 1992;**87**:562–9. doi:10.1080/01621459.1992.10475240
4 195
- 5
6 196 34 Schoenfeld DA, Richter JR. Nomograms for calculating the number of patients needed for a clinical trial with
7 197 survival as an endpoint. *Biometrics* 1982;**38**:163–70.
- 8
9 198 35 Dorigatti I, Okell L, Cori A, *et al.* Report 4: Severity of 2019-novel coronavirus (nCoV). Imperial College
10 199 London 2020. doi:10.25561/77154
11
- 12 200 36 Blenkinsop A, Parmar MK, Choodari-Oskooei B. Assessing the impact of efficacy stopping rules on the error
13 201 rates under the multi-arm multi-stage framework. *Clin Trials* 2019;**16**:132–41.
14 202 doi:10.1177/1740774518823551
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FIGURE LEGENDS

Figure 1: Map of India (grey) with Maharashtra highlighted in red, and Mumbai (black dot) and Pune (blue dot) identified.

Map created with mapchart.net.

Figure 2: Overview of trial procedures. RAT: Rapid Antigen Test.

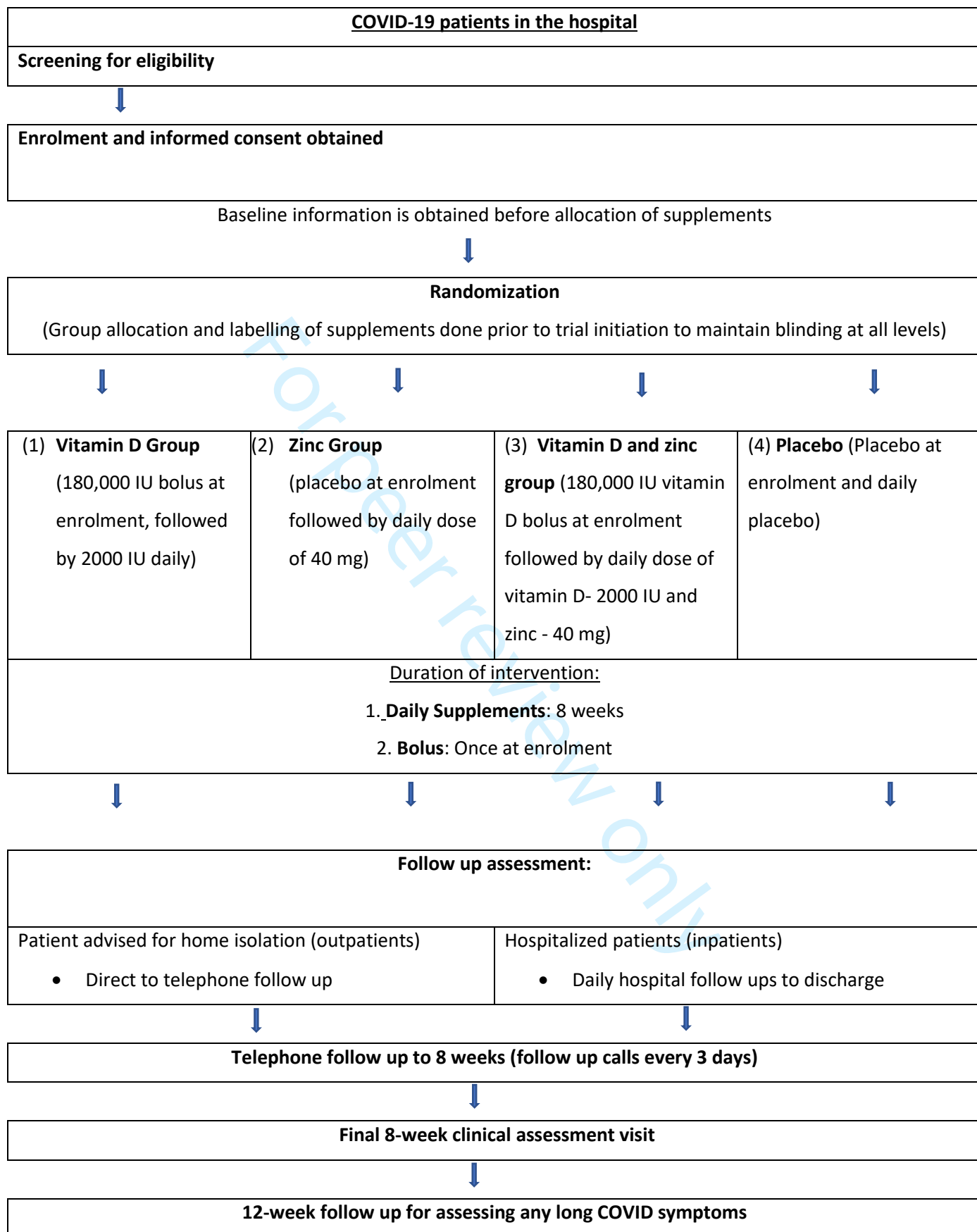
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Figure 1: Map of India (grey) with Maharashtra highlighted in red, and Mumbai (black dot) and Pune (blue dot) identified.

Map created with mapchart.net.

156x188mm (300 x 300 DPI)





SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description
Administrative information		
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym Title page (p1): “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”
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry Abstract (p2): “Trial Registration number: NCT04641195, CTRI/2021/04/032593, HMSC (GOI)-2021-0060”
	2b	All items from the World Health Organization Trial Registration Data Set Manuscript: Items from the WHO Trial Registration Data Set (including elements such as trial registration, financial support, study contacts, study title, countries of recruitment and details on design and recruitment status) are noted throughout the manuscript.
Protocol version	3	Date and version identifier NA: This is a manuscript of a study protocol.
Funding	4	Sources and types of financial, material, and other support Funding (p14): “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). The funding body has no role in study design and procedures, or the decision to submit manuscripts for publication.”
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors Author contributions (p14): “KCK, YD, WWF and NM conceptualised the project, and designed the study along with SB, KKS, ECH, YM and UP.”

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- 5b Name and contact information for the trial sponsor
The name of the study sponsor is included in the clinical trial registration records (NCT04641195, CTRI/2021/04/032593).
- 5c Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities
Funding (p14):
“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). The funding body has no role in study design and procedures, or the decision to submit manuscripts for publication.”
- 5d Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)
NA

28 **Introduction**

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2 Background and 6a Description of research question and justification for undertaking the
3 rationale trial, including summary of relevant studies (published and
4 unpublished) examining benefits and harms for each intervention
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6 Introduction (p4-5):
7 “[...] Additionally, with limited proven treatment regimens for COVID-
8 19 to date, it is essential to continue exploring low cost and commonly
9 available effective interventions which can be implemented as
10 standardized therapeutic treatment regimens at large.[6] This is
11 especially important in the context of low and middle-income countries
12 in South Asia and Africa, which are particularly vulnerable given weak
13 health systems and the co-existence of malnutrition and other co-
14 morbidities. This includes India, which continues to report a
15 substantial number of COVID-19 cases.[1]
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17 [...]
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19 Vitamin D shows promise as a novel, cost-effective prevention and
20 adjunctive treatment for respiratory infections. [...] In laboratory
21 studies, vitamin D metabolites support innate immune responses to
22 rhinoviruses and respiratory syncytial virus.[10–14] In participants with
23 influenza, high-dose vitamin D supplementation shortened durations
24 of fever, cough and wheezing, particularly among those with low
25 vitamin D levels.[15] In a recent systematic review and meta-analysis
26 of randomised controlled trials, vitamin D supplementation was
27 associated with decreased risk of acute respiratory infections and
28 shortened duration of symptoms.[16]
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30 [...]
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32 Multiple meta-analyses and pooled analyses of randomized controlled
33 trials conducted in the US and low- and middle-income countries have
34 shown that oral zinc supplementation reduces incidence of acute
35 respiratory infections by 35%, shortens duration of symptoms, and
36 improves recovery rate.[16,20–23] Zinc is a potential treatment in
37 COVID-19, due to its immune modulatory effect, as well as direct
38 antiviral effect.[24] The mechanisms by which zinc may serve as
39 adjunct therapy in COVID-19 has been recently reviewed by Skalny et
40 al. 2020,[25] who note that Zn²⁺ cations, especially in combination
41 with zinc ionophore pyrithione inhibit SARS-coronavirus RNA
42 polymerase activity by decreasing replication.[26]
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Vitamin D and zinc are safe, inexpensive, and widely available therapies; therefore, experimental evidence that these nutrient supplements are effective against COVID-19 would readily support their inclusion in standard of care. Therefore, we are undertaking a randomized controlled trial to determine the effect of vitamin D and zinc supplementation on treatment outcomes among individuals with COVID-19 in India.”

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2		6b	Explanation for choice of comparators
3			Methods and analysis // Study procedures // Intervention (p8):
4			“A placebo was chosen as the comparator group given that there is
5			currently no widespread consensus on the use of any nutritional
6			supplement as part of standard or routine treatment for COVID-
7			19.[16]”
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9			
10	Objectives	7	Specific objectives or hypotheses
11			Objectives (p5):
12			“The primary objectives of this trial are:
13			
14			<input type="checkbox"/> To determine the effect of vitamin D supplementation versus
15			placebo on time to recovery among patients with COVID-19
16			<input type="checkbox"/> To determine the effect of zinc supplementation versus
17			placebo on time to recovery among patients with COVID-19
18			Secondary objectives include:
19			
20			<input type="checkbox"/> To determine the effect of vitamin D or zinc supplementation
21			on duration of hospital stay, all-cause mortality, necessity for assisted
22			ventilation, and individual symptoms duration
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24			<input type="checkbox"/> To examine the effect of vitamin D or zinc supplementation on
25			key blood biomarkers, including serum vitamin D and zinc, and
26			immunological and inflammatory markers”
27			
28	Trial design	8	Description of trial design including type of trial (eg, parallel group,
29			crossover, factorial, single group), allocation ratio, and framework (eg,
30			superiority, equivalence, noninferiority, exploratory)
31			Methods and analysis // Trial design, population and enrolment sites
32			(p5):
33			“This is a double-blind, placebo-controlled, randomized superiority trial
34			with 2x2 factorial design and 1:1:1:1 allocation ratio, being conducted
35			at two site hospitals in Mumbai and Pune, Maharashtra, India (Figure
36			1).”
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Methods: Participants, interventions, and outcomes

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24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60	Eligibility criteria 10	<p>Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)</p> <p>Methods and analysis // Eligibility criteria (p6):</p> <p>“The original inclusion criteria for this study were as follows: (1) men and women aged ≥ 18 years, (2) RT-PCR-confirmed infection with SARS-COV-2, (3) oxygen saturation level (SpO₂) ≥ 90, and (4) written informed consent.</p> <p>The exclusion criteria were as following: (1) pregnant women, (2) individuals enrolled in other clinical trials, (3) daily use of multivitamins for the past 1 month.</p> <p>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.</p>

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2 Interventions 11a Interventions for each group with sufficient detail to allow replication,
3 including how and when they will be administered
4 Methods and analysis // Study procedures // Intervention (p7-8):
5 Patients are randomized to one of four groups:
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7 1. Placebo-Placebo group will receive a placebo vitamin D3 bolus
8 at the hospital followed by placebo daily vitamin D3 maintenance
9 doses and placebo daily zinc supplements
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11 2. Vitamin D-Placebo group will receive an actual vitamin D3
12 bolus (180,000 IU) at the hospital followed by actual daily vitamin D3
13 maintenance doses (2000 IU daily) and daily placebo zinc
14 supplements
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16 3. Placebo-Zinc group will receive a placebo vitamin D3 bolus at
17 the hospital followed by placebo daily vitamin D3 maintenance doses
18 and actual daily zinc supplements (40 mg daily)
19
20 4. Vitamin D3-Zinc group will receive an actual vitamin D3 bolus
21 (180,000 IU) at the hospital followed by actual daily vitamin D3
22 maintenance doses (2000 IU daily) and actual daily zinc supplements
23 (40 mg daily)
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26 A placebo was chosen as the comparator group given that there is
27 currently no widespread consensus on the use of any nutritional
28 supplement as part of standard or routine treatment for COVID-19.[16]
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31 Participants receive a pre-labelled daily supplement bottle with 60
32 tablets, and an envelope which contains three vitamin D3/placebo
33 bolus tablets to be consumed at baseline under supervision of site
34 hospital staff. Following the bolus dose, participants are instructed to
35 take supplements daily for 8 weeks. Participants are contacted daily
36 while in hospital or regularly via telephone after leaving the hospital to
37 ensure compliance. Research nurses identify barriers to compliance,
38 and assess compliance at 8 weeks via direct questioning and pill
39 count.
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43 Supplement and placebo tablets were manufactured by Excellamed
44 Laboratories Private Limited (Mumbai, India) with an external quality
45 check done by an independent service provider (Bee Pharmo Labs
46 Private Limited, Mumbai, India).
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49 All participants are provided with care and treatment consistent with
50 Indian national guidelines, and are encouraged to visit the study
51 clinics seven days a week for medical attention if they feel unwell.
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- 11b Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)
Methods and analysis // Adverse events and reporting (p11):
“All adverse events which are possibly, probably or very likely related to administration of any supplement are monitored and reported to site institutional review boards (IRBs) within 72 hours (serious adverse events) or 1 month (all other adverse events), using a standardized reporting format. The trial data and safety monitoring board (DSMB) is also notified. Site principal investigators and independent physicians are responsible for assessing the causal relationship and making the conclusive decision about continuation of the trial for a particular participant.”
- 11c Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)
Methods and analysis // Study procedures // Intervention (p8):
“Participants are contacted daily while in hospital or regularly via telephone after leaving the hospital to ensure compliance. Research nurses identify barriers to compliance, and assess compliance at 8 weeks via direct questioning and pill count.”
- 11d Relevant concomitant care and interventions that are permitted or prohibited during the trial
Methods and analysis // Study procedures // Intervention (p8):
“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.”

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- Outcomes** 12 Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended
- [Methods and analysis // Study procedures // 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 and additional symptoms 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. Metrics of individual symptoms and combination of symptoms are used to identify the time point of resolution symptoms from baseline.
- Secondary outcomes include duration of hospital stay, need for assisted ventilation, individual symptoms duration, all-cause mortality, occurrence of other severe adverse events and change in blood biomarker levels, including 25-hydroxy vitamin D, zinc and calcium, and other immunological and inflammatory biomarkers. Biomarker levels are assessed using blood samples collected at baseline and at the 8-week clinical assessment. Occurrence of any other secondary endpoints between baseline to 8-week clinical assessment is recorded during follow up calls or visits as described above.”
- Participant timeline** 13 Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)
- [See Methods and analysis // Study procedures section \(p6\) for details on enrolment, intervention, and follow up. Table 1 and Figure 2, referred to in this section, also outline the sequence and schedule of enrolment and follow up.](#)

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24	Sample size	14	<p>Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations</p> <p>Methods and analysis // Data analysis // Statistical power calculations (p12) and Table 2:</p> <p>“Assumptions related to treatment effects may be reasonably inferred from meta-analyses of well-designed randomized controlled trials studying these supplements in other acute respiratory illnesses.[15,16,20–23] We based power analysis on the primary outcome of time from onset of disease to clinical recovery, using methodology for survival times.[34] 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,[35] 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.”</p>
25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60	Recruitment	15	<p>Strategies for achieving adequate participant enrolment to reach target sample size</p> <p>Methods and analysis // Trial design, population and enrolment sites (p5):</p> <p>“While we initially targeted only hospitalized inpatients at each site for the study, we broadened our target population in June 2021 to include all hospital outpatients. This was done in order to increase generalizability of results and maintain enrolment in light of decreasing COVID-19 cases.[30]”</p> <p>Methods and analysis // Eligibility criteria (p6):</p> <p>“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.”</p> <p>Methods and analysis // Study procedures // Recruitment and obtaining informed consent (p6):</p> <p>“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. [...]”</p>

Methods: Assignment of interventions (for controlled trials)

Allocation:

Sequence generation	16a	<p>Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions</p> <p>Methods and analysis // Study procedures // Randomization and blinding (p7): “For randomization, a computer-generated list from 1 to 1000 was prepared by the study statistician, according to a randomization sequence in blocks of 20 and stratified by follow up clinic.”</p>
Allocation concealment mechanism	16b	<p>Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned</p> <p>Methods and analysis // Study procedures // Randomization and blinding (p7): “Supplement bottles and envelopes are pre-labelled with codes, and active tablets and placebo are indistinguishable, so that participants and investigators are blinded. For randomization, a computer-generated list from 1 to 1000 was prepared by the study statistician, according to a randomization sequence in blocks of 20 and stratified by follow up clinic.”</p>
Implementation	16c	<p>Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions</p> <p>Methods and analysis // Study procedures // Recruitment and obtaining informed consent (p6): “Potential participants are approached by trained site hospital staff members when they present to site hospitals. [...] Informed consent is obtained after responding to any raised queries.”</p> <p>Methods and analysis // Study procedures // Randomization and blinding (p7): “For randomization, a computer-generated list from 1 to 1000 was prepared by the study statistician, according to a randomization sequence in blocks of 20 and stratified by follow up clinic.”</p>

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2 Blinding
3 (masking)
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- 17a Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how
Methods and analysis // Study procedures // Randomization and blinding (p7):
“Supplement bottles and envelopes are pre-labelled with codes, and active tablets and placebo are indistinguishable, so that participants and investigators are blinded. For randomization, a computer-generated list from 1 to 1000 was prepared by the study statistician, according to a randomization sequence in blocks of 20 and stratified by follow up clinic.”
- 17b If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant’s allocated intervention during the trial
Data and Safety Monitoring Board (p13):
“The trial DSMB will examine efficacy endpoints by study arms when half of individuals are enrolled. In accordance with the Haybittle-Peto rule, if the difference in the primary outcome between study arms is <0.001 , unblinding of the DSMB and stopping will be considered.[36]”

27 **Methods: Data collection, management, and analysis**
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2 Data collection 18a Plans for assessment and collection of outcome, baseline, and other
3 methods trial data, including any related processes to promote data quality (eg,
4 duplicate measurements, training of assessors) and a description of
5 study instruments (eg, questionnaires, laboratory tests) along with
6 their reliability and validity, if known. Reference to where data
7 collection forms can be found, if not in the protocol
8
9 [Methods and analysis // Study procedures // Baseline data and](#)
10 [sample collection \(p7\):](#)
11 “Following informed consent, participants undergo baseline data and
12 [sample collection, including recording of key background and clinical](#)
13 [information as follows:](#)
14
15 [Screening and background: the initial screening form is](#)
16 [extended to collect information including participants’ demographic](#)
17 [background, socio-economic status, and health and prevention](#)
18 [behaviours \(smoking and drinking\)](#)
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20 [Baseline dietary information: a food frequency questionnaire](#)
21 [\(FFQ\) is administered, collecting information on dietary practices and](#)
22 [habits in relation to 25 food groups. The FFQ is validated for use in](#)
23 [India and has been adapted to the Maharashtra context](#)
24
25 [Clinical baseline: clinical and physical measures are collected](#)
26 [alongside information on COVID-19 vaccination status, COVID-19](#)
27 [symptoms, vital signs, blood investigations, medical conditions,](#)
28 [treatment and medications, complications, and medical history](#)
29 [A blood sample is also collected at baseline. All information is](#)
30 [collected securely on electronic tablets, as described above.”](#)
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34 [Methods and analysis // Study procedures // Study outcomes and](#)
35 [follow up \(p8 – 9\):](#)
36 “Following baseline assessment and provision of supplements,
37 participants are regularly followed up as described below:
38
39 [Daily hospital follow up: Daily assessment of COVID-19](#)
40 [symptoms, vital signs, complications, medical conditions and study](#)
41 [supplement compliance is recorded for hospitalised participants](#)
42
43 [Telephone follow up: Assessment of COVID-19 symptoms,](#)
44 [supplement compliance and adverse events is conducted in a follow](#)
45 [up call every three days after leaving the hospital for all participants](#)
46
47 [8-week clinical assessment: After completion of study](#)
48 [supplements at 8 weeks, information is gathered on results of a](#)
49 [clinical and physical examination, COVID-19 symptoms, compliance](#)
50 [with regimen \(including direct questioning and pill count\), vital signs,](#)
51 [blood investigations \(from a collected blood sample\), medical](#)
52 [conditions, treatment and medications, complications, and history.](#)
53 [This assessment is conducted in person at the hospital, or at a](#)
54 [location convenient to the participant where privacy can be ensured](#)
55 [\(including an option to collect some information via telephone if an in-](#)
56 [person visit is not possible\)](#)
57
58 [12-week telephone follow up: A final assessment is conducted](#)
59 [of long-term COVID-19 symptoms \[...\]](#)
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61 [A list of collected data and blood investigations with time points at](#)
62 [baseline, during follow up visits or calls, and at 8 and 12 weeks is](#)
63 [summarized in Table 1. \(Please also refer to Table 1\)](#)

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- Data management
- 18b Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols
Methods and analysis // Study procedures // Intervention (p8):
“Participants are contacted daily while in hospital or regularly via telephone after leaving the hospital to ensure compliance.”
- 19 Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol
Methods and analysis // Study procedures // Recruitment and obtaining informed consent (p6):
“Information regarding eligibility of potential participants is collected on a secure electronic tablet using Open Data Kit (ODK),[31] with questionnaires including built-in checks and data uploaded to a secure server. No identifiable data are collected until the participant has provided informed consent.”

Methods and analysis // Study procedures // Baseline data and sample collection (p7):
“All information is collected securely on electronic tablets, as described above.”

Methods and analysis // Study procedures // Study outcomes and follow up (p8):
“All data are collected using standardized questionnaire forms on electronic tablets,[31] as described above.”

Methods and analysis // Data and sample management (p11):
“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. Data are stored in linked-anonymised form, with identifiable information and the linking key stored separately. All analyses and data checks are conducted on anonymised data only.”

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Statistical methods

- 20a Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol
- Methods and analysis // Data analysis // Planned analyses (p11):
 “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. placebo and zinc vs. placebo using Cox regression. [...] We will assess the success of randomization by comparing baseline variables by treatment group using χ^2 and t-tests and use multivariate modelling to adjust for imbalances if needed.
 [...] The effect of vitamin D or zinc on dichotomous secondary outcomes will be analyzed in a similar approach.”
- 20b Methods for any additional analyses (eg, subgroup and adjusted analyses)
- Methods and analysis // Data analysis // Planned analyses (p11-12):
 “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.
 [...] The proportion of individuals experiencing hypercalcemia will be compared between treatment groups using χ^2 tests, and effects of the supplements on blood biomarkers will be compared via Wilcoxon and t-tests. This study will measure numerous risk factors for COVID-19 progression and severe treatment outcomes including hemoglobin; co-morbidities; medications including chloroquine, hydroxychloroquine, and ACE inhibitors; and socio-demographic, clinical, nutritional, and lifestyle-related risk factors. We will examine relationships of these factors in the placebo group first, to avoid complex questions concerning interactions between risk factors and treatments. Once we find a satisfactory parsimonious model using principles of model selection as detailed by Greenland,[32] we will test and modify it if needed in the whole study population, adjusting for treatment effects.
- Analyses will consider sex and gender throughout, by disaggregating findings, and attempting to elucidate the roles of sex and gender in the clinical course and immune response by controlling for potential sociodemographic, nutritional, and immunological confounders.”

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2 20c Definition of analysis population relating to protocol non-adherence
3 (eg, as randomised analysis), and any statistical methods to handle
4 missing data (eg, multiple imputation)

5 [Methods and analysis // Data analysis // Planned analyses \(p11\):](#)

6 “An intent-to-treat analysis will be used as the primary analytic
7 strategy.”
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10 **Methods: Monitoring**

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12 Data monitoring 21a Composition of data monitoring committee (DMC); summary of its role
13 and reporting structure; statement of whether it is independent from
14 the sponsor and competing interests; and reference to where further
15 details about its charter can be found, if not in the protocol.

16 Alternatively, an explanation of why a DMC is not needed

17 [Data and Safety Monitoring Board \(p12-13\):](#)

18 “The Data and Safety Monitoring Board (DSMB) was established prior
19 to commencement of the trial. It consists of independent experts in
20 respiratory infection and communicable diseases, public health and
21 nutrition, clinical research, and biostatistics. The role of the board is to
22 provide their inputs, recommendations, review the trial protocols and
23 progress by ensuring the rights and safety of involving participants in
24 the study through periodic trial review meetings.
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30 The trial DSMB will examine efficacy endpoints by study arms when
31 half of individuals are enrolled. In accordance with the Haybittle-Peto
32 rule, if the difference in the primary outcome between study arms is
33 <0.001 , unblinding of the DSMB and stopping will be considered.[36]”
34

35 21b Description of any interim analyses and stopping guidelines, including
36 who will have access to these interim results and make the final
37 decision to terminate the trial

38 [Data and Safety Monitoring Board \(p13\):](#)

39 “The trial DSMB will examine efficacy endpoints by study arms when
40 half of individuals are enrolled. In accordance with the Haybittle-Peto
41 rule, if the difference in the primary outcome between study arms is
42 <0.001 , unblinding of the DSMB and stopping will be considered.[36]”
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2 Harms 22 Plans for collecting, assessing, reporting, and managing solicited and
3 spontaneously reported adverse events and other unintended effects
4 of trial interventions or trial conduct
5 [Methods and analysis // Adverse events and reporting \(p11\):](#)
6 “Any undesirable circumstance or experiences reported by study
7 participants during the study are categorised as adverse events. All
8 adverse events which are possibly, probably or very likely related to
9 administration of any supplement are monitored and reported to site
10 institutional review boards (IRBs) within 72 hours (serious adverse
11 events) or 1 month (all other adverse events), using a standardized
12 reporting format. The trial data and safety monitoring board (DSMB) is
13 also notified. Site principal investigators and independent physicians
14 are responsible for assessing the causal relationship and making the
15 conclusive decision about continuation of the trial for a particular
16 participant. Additionally, medical insurance is provided to all study
17 participants to take care of any progression of severe adverse
18 events.”

23 Auditing 23 Frequency and procedures for auditing trial conduct, if any, and
24 whether the process will be independent from investigators and the
25 sponsor
26 NA
27
28

29 Ethics and dissemination

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31 Research ethics 24 Plans for seeking research ethics committee/institutional review board
32 approval (REC/IRB) approval
33 [Abstract // Ethics and dissemination \(p2\):](#)
34 “Ethical approval was obtained from institutional ethical committees of
35 all participating institutions.”
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39 [Ethics and dissemination \(p13\):](#)
40 “This study is being conducted in the accordance with the Declaration
41 of Helsinki 2013. The study was approved by the Institutional Review
42 Board of the Harvard T.H. Chan School of Public Health (Protocol No.
43 IRB20-1425), the University Health Network Research Ethics Board
44 (20-5775), the Institutional Research Ethics Committee of the
45 Foundation for Medical Research (IREC No.
46 FMR/IREC/C19/02/2020), the Institutional Review Board of Saifee
47 Hospital (Project No. EC/008/2020) and the KEM Hospital Research
48 Centre Ethics Committee (KEMHRC ID No. 2027).”
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- Protocol amendments 25 Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)
NA:
As this is a manuscript of a study protocol, such detail has not been included in this specific document.
- Consent or assent 26a Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)
Methods and analysis // Recruitment and obtaining informed consent (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. These dedicated site hospital staff members determine their interest and eligibility, and provide a brief introduction including key details about the study and what participation involves. The staff members read out the participant information sheet in the appropriate conversational language (English, Hindi or Marathi), and discuss the trial components and the role of the participant in the study. Information provided includes a clear outline of potential benefits and harms, the length of the follow up period, remuneration that can be expected, future use of information and samples, and resources available to the participant such as access to study clinics. Informed consent is obtained after responding to any raised queries. [...] The informed consent process is completed once participants provide their signature on two copies of the consent document; one copy for the trial record and another provided to participants for their reference.”
- 26b Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable
Methods and analysis // Recruitment and obtaining informed consent (p6):
“The staff members read out the participant information sheet in the appropriate conversational language (English, Hindi or Marathi), and discuss the trial components and the role of the participant in the study. Information provided includes a clear outline of potential benefits and harms, the length of the follow up period, remuneration that can be expected, future use of information and samples, and resources available to the participant such as access to study clinics. Informed consent is obtained after responding to any raised queries.”

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- Confidentiality** 27 How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial
- Methods and analysis // Study procedures // Recruitment and obtaining informed consent (p6):
 “Information regarding eligibility of potential participants is collected on a secure electronic tablet using Open Data Kit (ODK),[31] with questionnaires including built-in checks and data uploaded to a secure server. No identifiable data are collected until the participant has provided informed consent.”
- Methods and analysis // Study procedures // Baseline data and sample collection (p7):
 “All information is collected securely on electronic tablets, as described above.”
- Methods and analysis // Study procedures // Study outcomes and follow up (p8):
 “All data are collected using standardized questionnaire forms on electronic tablets,[31] as described above.”
- Methods and analysis // Data and sample management (p11):
 “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. Data are stored in linked-anonymised form, with identifiable information and the linking key stored separately. All analyses and data checks are conducted on anonymised data only.”
- Declaration of interests** 28 Financial and other competing interests for principal investigators for the overall trial and each study site
- Competing interests (p15):
 “All authors declare no conflicts of interest.”
- Access to data** 29 Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators
- Methods and analysis // Data and sample management (p11):
 “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. Data are stored in linked-anonymised form, with identifiable information and the linking key stored separately.”

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2	Ancillary and	30	Provisions, if any, for ancillary and post-trial care, and for
3	post-trial care		compensation to those who suffer harm from trial participation
4			Methods and analysis // Adverse events and reporting (p11):
5			“Additionally, medical insurance is provided to all study participants to
6			take care of any progression of severe adverse events.”
7			
8	Dissemination	31a	Plans for investigators and sponsor to communicate trial results to
9	policy		participants, healthcare professionals, the public, and other relevant
10			groups (eg, via publication, reporting in results databases, or other
11			data sharing arrangements), including any publication restrictions
12			Ethics and dissemination (p13):
13			“The study findings will be presented in peer-reviewed medical
14			journals.”
15		31b	Authorship eligibility guidelines and any intended use of professional
16			writers
17			NA
18		31c	Plans, if any, for granting public access to the full protocol, participant-
19			level dataset, and statistical code
20			NA
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28	Appendices		
29	Informed consent	32	Model consent form and other related documentation given to
30	materials		participants and authorised surrogates
31			NA:
32			As this is a manuscript of a study protocol, such detail has not been
33			included in this specific document.
34			
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36	Biological	33	Plans for collection, laboratory evaluation, and storage of biological
37	specimens		specimens for genetic or molecular analysis in the current trial and for
38			future use in ancillary studies, if applicable
39			Methods and analysis // Data and sample management (p11):
40			“Blood samples collected as part of this trial are processed at the
41			Foundation for Medical Research, Mumbai, and accredited
42			laboratories in India including at the site hospitals. Specimens are
43			linked-anonymised and are stored securely at the Foundation for
44			Medical Research for a maximum of three years.”
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*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons [“Attribution-NonCommercial-NoDerivs 3.0 Unported”](#) license.

BMJ Open

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

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2022-061301.R1
Article Type:	Protocol
Date Submitted by the Author:	20-Mar-2022
Complete List of Authors:	Sharma, Kamal Kant; Foundation for Medical Research Partap, Uttara; Harvard University T H Chan School of Public Health, Department of Global Health and Population Mistry, Nerges; Foundation for Medical Research Marathe, Yogesh; Foundation for Medical Research Wang, Molin; Harvard University T H Chan School of Public Health, Departments of Epidemiology and Biostatistics Shaikh, Sanaa; Foundation for Medical Research D'Costa, Pradeep; King Edward Memorial Hospital Pune Gupta, Gaurav; Saifee Hospital Bromage, Sabri; Harvard University T H Chan School of Public Health, Department of Nutrition Hemler, Elena ; Harvard University T H Chan School of Public Health, Department of Global Health and Population Kain, Kevin; University Health Network, Department of Medicine; University of Toronto, Department of Medicine Dholakia, Yatin; Foundation for Medical Research Fawzi, Wafaie.; Harvard University T H Chan School of Public Health, Departments of Global Health and Population; Epidemiology; and Nutrition
Primary Subject Heading:	Infectious diseases
Secondary Subject Heading:	Nutrition and metabolism
Keywords:	COVID-19, Nutrition < TROPICAL MEDICINE, Clinical trials < THERAPEUTICS

SCHOLARONE™
Manuscripts

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3 1 **A randomized trial to determine the effect of vitamin D and zinc**
4
5 2 **supplementation for improving treatment outcomes among COVID-19**
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7 3 **patients in India: trial protocol**
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10 4
11 5 Kamal Kant Sharma^{1*}, Uttara Partap^{2*}, Nerges Mistry¹, Yogesh Marathe¹, Molin Wang^{3,4}, Sanaa Shaikh¹, Pradeep
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13 7 Fawzi^{2,3,7†}
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42 28
43 29 Tables: 2, Figures: 2

44 30 Word count: **4122**, Abstract: **233**
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ABSTRACT

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.

Methods and analysis: We have designed a 2x2 factorial, randomized, double-blind, multi-centre placebo-controlled trial to evaluate the effect of vitamin D and zinc on COVID-19 outcomes in Maharashtra, India. COVID-19 positive individuals are recruited from hospitals in Mumbai and Pune. Participants are provided (1) vitamin D3 bolus (180,000 IU) maintained by daily dose of 2000 IU, and/or (2) zinc gluconate (40 mg daily), versus placebo for 8 weeks. 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. A final follow up telephone call occurs 12 weeks post-enrolment to assess long-term outcomes. The primary outcome of the study is to time to recovery, defined as time to resolution of all of fever, cough and shortness of breath. Secondary outcomes include: duration of hospital stay, all-cause mortality, necessity of assisted ventilation, change in blood biomarker levels, and individual symptoms duration. Participant recruitment commenced on April 2021.

Ethics and dissemination: Ethical approval was obtained from institutional ethical committees of all participating institutions. The study findings will be presented in peer-reviewed medical journals.

Trial Registration number: NCT04641195, CTRI/2021/04/032593, HMSC (GOI)-2021-0060

STRENGTHS AND LIMITATIONS OF THIS STUDY

- 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.
- As a double-blind factorial randomized controlled trial, this study enables an efficient assessment of the effect of vitamin D and zinc on COVID-19 symptoms that is less prone to confounding and bias than other observational studies on this topic.
- With frequent follow up of participants, this study collects information across a range of domains including sociodemographic and clinical measures, and biomarker data, which will allow for a detailed investigation of the effect of supplementation on disease progression.
- 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.

INTRODUCTION

COVID-19 continues to be a problem globally, with over 16 million incident cases and 200,000 deaths reported in November 2021.[1] Concerted global efforts have resulted in the development of vaccines, which may reduce the burden and impact of COVID-19, although suboptimal vaccine coverage and the rapid mutation of the virus continue to prolong the pandemic.[2–5] Additionally, with limited proven treatment regimens for COVID-19 to date, it is essential to continue exploring low cost and commonly available effective interventions which can be implemented as standardized therapeutic treatment regimens at large.[6] This is especially important in the context of low and middle-income countries in South Asia and Africa, which are particularly vulnerable given weak health systems and the co-existence of malnutrition and other co-morbidities. This includes India, which continues to report a substantial number of COVID-19 cases.[1]

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. Countrywide studies suggest VDD may affect at least 70% of the Indian population. Vitamin D shows promise as a novel, cost-effective prevention and adjunctive treatment for respiratory infections. In laboratory studies, vitamin D metabolites support innate immune responses to rhinoviruses and respiratory syncytial virus.[11–15] In participants with influenza, high-dose vitamin D supplementation shortened durations of fever, cough and wheezing, particularly among those with low vitamin D levels.[16] In a recent systematic review and meta-analysis of randomised controlled trials, vitamin D supplementation was associated with decreased risk of acute respiratory infections and shortened duration of symptoms.[17]

Zinc is an essential mineral that plays critical roles in gene expression, cell division, and immunity.[18] In India, dietary predominance of micronutrient-sparse staples, limited consumption of animal foods, and high consumption of zinc absorption inhibitors render the population at extremely high risk of inadequacy, which is exacerbated due to global climate change.[19] About 25% of the Indian population is zinc inadequate, and 4.3 million child deaths (<5 years) were attributable to zinc deficiency in 2017.[20] Multiple meta-analyses and pooled analyses of randomized controlled trials conducted in the US and low- and middle-income countries have shown that oral zinc supplementation reduces incidence of acute respiratory infections by 35%, shortens duration of symptoms, and improves recovery rate.[17,21–24] Zinc is a potential treatment in COVID-19, due to its immune modulatory effect, as well as direct antiviral effect.[25] The mechanisms by which zinc may serve as adjunct therapy in COVID-19 has been recently reviewed by Skalny et al. 2020,[26] who note that Zn^{2+} cations, especially in combination with zinc ionophore pyrithione inhibit SARS-coronavirus RNA polymerase activity by decreasing replication.[27]

1
2 98 Vitamin D and zinc are safe, inexpensive, and widely available therapies; therefore, experimental evidence that
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4 99 these nutrient supplements are effective against COVID-19 would readily support their inclusion in standard of
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6 100 care. Therefore, we are undertaking a randomized controlled trial to determine the effect of vitamin D and zinc
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8 101 supplementation on treatment outcomes among individuals with COVID-19 in India.
9

10 OBJECTIVES

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13 103 The primary objectives of this trial are:

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16 104 ❖ To determine the effect of vitamin D supplementation versus placebo on time to recovery among patients
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18 105 with COVID-19
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20 106 ❖ To determine the effect of zinc supplementation versus placebo on time to recovery among patients with
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22 107 COVID-19

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24 108 Secondary objectives include:

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27 109 ❖ To determine the effect of vitamin D or zinc supplementation on duration of hospital stay, all-cause mortality,
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29 110 necessity for assisted ventilation, and individual symptoms duration
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32 112 ❖ To examine the effect of vitamin D or zinc supplementation on key blood biomarkers, including serum vitamin
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34 113 D and zinc, and immunological and inflammatory markers

35 METHODS AND ANALYSIS

36 37 114 Trial design, population, enrolment sites, and time frame

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40 115 This is a double-blind, placebo-controlled, randomized superiority trial with 2x2 factorial design and 1:1:1:1
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42 116 allocation ratio, being conducted at two site hospitals in Mumbai and Pune, Maharashtra, India (**Figure 1**).
43
44 117 Maharashtra has the highest cumulative number of COVID-19 cases and fatalities out of all states in India.[28]
45
46 118 Within the state, both Pune and Mumbai have emerged as COVID-19 hotspots.[29,30]

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48 119 The two study sites (King Edward Memorial Hospital and Research Centre, Pune, and Saifee Hospital, Mumbai)
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50 120 are established medical institutions located within the cities of Pune and Mumbai. These hospitals have been
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52 121 designated as COVID-19 dedicated hospitals by local municipal corporations, where people can avail COVID-19-
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54 122 related treatment and services. The trial is targeting a sample size of 700. The study commenced in April 2021 and
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56 123 study activities are expected to continue until July 2022. While we initially targeted only hospitalized inpatients
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58 124 at each site for the study, we broadened our target population in June 2021 to include all hospital outpatients.
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60 125 This was done in order to increase generalizability of results and maintain enrolment in light of decreasing COVID-
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62 126 19 cases.[31]

Eligibility criteria

The original inclusion criteria for this study were as follows: (1) men and women aged ≥ 18 years, (2) RT-PCR-confirmed infection with SARS-COV-2, (3) oxygen saturation level (SpO₂) ≥ 90 , and (4) written informed consent.

The exclusion criteria were as following: (1) pregnant women, (2) individuals enrolled in other clinical trials, (3) daily use of multivitamins for the past 1 month.

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.

Study procedures

An overview of trial procedures is summarised in **Figure 2**.

Recruitment and obtaining informed consent

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. These dedicated site hospital staff members determine their interest and eligibility, and provide a brief introduction including key details about the study and what participation involves. The staff members read out the participant information sheet in the appropriate conversational language (English, Hindi or Marathi), and discuss the trial components and the role of the participant in the study. Information provided includes a clear outline of potential benefits and harms, the length of the follow up period, remuneration that can be expected, future use of information and samples, and resources available to the participant such as access to study clinics. Informed consent is obtained after responding to any raised queries. As part of the process, potential participants are informed that their participation is completely voluntary and they can withdraw any time at any stage of the study without providing any reasons. The informed consent process is completed once participants provide their signature on two copies of the consent document; one copy for the trial record and another provided to participants for their reference.

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2
3 155 Information regarding eligibility of potential participants is collected on a secure electronic tablet using Open Data
4 156 Kit (ODK),[32] with questionnaires including built-in checks and data uploaded to a secure server. No identifiable
5
6 157 data are collected until the participant has provided informed consent.
7

8 9 158 Baseline data and sample collection

10
11 159 Following informed consent, participants undergo baseline data and sample collection, including recording of key
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13 160 background and clinical information as follows:

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16 161 ➤ Screening and background: the initial screening form is extended to collect information including
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18 162 participants' demographic background, socio-economic status, and health and prevention behaviours
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20 163 (smoking and drinking), and COVID-19 vaccination status
- 21 164 ➤ Baseline dietary information: a food frequency questionnaire (FFQ) is administered, collecting
22
23 165 information on dietary practices and habits in relation to 25 food groups. The FFQ is validated for use in
24
25 166 India and has been adapted to the Maharashtra context
- 26 167 ➤ Clinical baseline: clinical and physical measures are collected alongside information on COVID-19
27
28 168 symptoms, vital signs, blood investigations, medical conditions, treatment and medications including
29
30 169 those prescribed for COVID-19, nutritional supplement use, complications, and medical history

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32 170 A blood sample is also collected at baseline. All information is collected securely on electronic tablets, as described
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34 171 above.

35 36 37 172 Randomization and blinding

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40 173 Participants are assigned randomly to one of four groups: (1) vitamin D, (2) zinc, (3) vitamin D and zinc, or (4)
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42 174 placebo. For randomization, a computer-generated list was prepared by the study statistician, according to a
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44 175 randomization sequence in blocks of 20 and stratified by follow up clinic. The randomization list assigns each
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46 176 participant randomization identifier (ID) to a regimen code, with the actual regimen known only to the
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48 177 manufacturer and accessible to the statistician in a currently unopened, sealed envelope. Supplement bottles and
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50 178 envelopes are pre-labelled with codes, and active tablets and placebo are indistinguishable, so that participants
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52 180 and all research staff including investigators remain blinded. At each site, each participant entering the trial is
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54 181 given the next available randomization ID, and is provided their corresponding regimen based on the assigned
55
56 182 regimen code.

57 58 59 182 Intervention

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61 183 Patients are randomized to one of four groups:

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2
3 184 1. Placebo-Placebo group will receive a *placebo* vitamin D3 bolus at the hospital followed by *placebo*
4 185 daily vitamin D3 maintenance doses and *placebo* daily zinc supplements
- 5
6 186 2. Vitamin D-Placebo group will receive an *actual* vitamin D3 bolus (180,000 IU) at the hospital followed
7 187 by *actual* daily vitamin D3 maintenance doses (2000 IU daily) and daily *placebo* zinc supplements
- 8
9 188 3. Placebo-Zinc group will receive a *placebo* vitamin D3 bolus at the hospital followed by *placebo* daily
10 vitamin D3 maintenance doses and *actual* daily zinc supplements (40 mg daily)
- 11 189
12 190 4. Vitamin D3-Zinc group will receive an *actual* vitamin D3 bolus (180,000 IU) at the hospital followed by
13
14 191 *actual* daily vitamin D3 maintenance doses (2000 IU daily) and *actual* daily zinc supplements (40 mg
15
16 192 daily)

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19 193 We selected vitamin D3 as it has been shown to be more effective in raising and maintaining high levels of
20 circulating 25(OH)D than vitamin D2 [33,34]. A bolus dose followed by daily doses was chosen to boost vitamin D
21 194 levels quickly and safely within the first few days and maintain levels thereafter. Previous studies have indicated
22 195 the efficacy of large oral doses (>200 000 IU bolus, and 1,700-2,000 IU per day) in increasing and sustaining blood
23 196 25(OH)D concentrations, with very low risk of side effects [35–41]. The 40 mg dosage of zinc is understood to be
24 197 sufficiently high to assess efficacy, while remaining within the Institute of Medicine’s tolerable upper intake level
25 198 for adults [42]. A placebo was chosen as the comparator group given that there is currently no widespread
26 199 consensus on the use of any nutritional supplement as part of standard or routine treatment for COVID-19.[17]

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28
29 200 Participants receive a pre-labelled daily supplement bottle with 60 tablets, and an envelope which contains three
30 201 vitamin D3/placebo bolus tablets to be consumed at baseline under supervision of site hospital staff. Following
31 202 the bolus dose, participants are instructed to take supplements daily for 8 weeks. Participants are observed taking
32 203 supplements daily while in hospital or contacted regularly via telephone after leaving the hospital to ensure
33 204 compliance. Research staff identify barriers to compliance and aim to address these via appropriate counselling,
34 205 and assess compliance at 8 weeks via direct questioning and pill count.

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36
37 206 Supplement and placebo tablets were manufactured by Excellamed Laboratories Private Limited (Mumbai, India)
38 207 with an external quality check done by an independent service provider (Bee Pharmo Labs Private Limited,
39 208 Mumbai, India).

40
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42 209 All participants are provided with care and treatment consistent with Indian national guidelines, and are
43 210 encouraged to visit the study clinics seven days a week for medical attention if they feel unwell. Indian national
44 211 guidelines have evolved during the pandemic, and currently consist of appropriate treatment (which may include
45 212 oxygen support, respiratory support, anti-inflammatory or immunomodulatory therapy, and anticoagulation
46 213 therapy) according to disease severity; discharge of admitted patients from the hospital upon resolution of
47 214 symptoms and sufficient oxygen saturation (SpO₂ > 93%) for three days; and self-monitoring during home isolation
48 215 [43–45].
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2 217 Study outcomes and follow up

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5 218 Following baseline assessment and provision of supplements, participants are regularly followed up as described
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7 219 below and in **Table 1**:

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10 220 ➤ Daily hospital follow up: Daily assessment of COVID-19 symptoms, vital signs, complications, medical
11 conditions and study supplement compliance is recorded for hospitalised participants. Any new
12 prescribed medications and supplements are also recorded alongside other interventions such as need
13 for non-invasive ventilation or dialysis. Symptoms are specifically asked to participants; other measures
14 are asked, observed, assessed, or abstracted from the participants' records.

15 221
16 222
17 223
18 224 ➤ Telephone follow up: Assessment of COVID-19 symptoms, supplement compliance and adverse events is
19 conducted in a follow up call every three days after leaving the hospital for all participants. All information
20 is self-reported by participants.

21 225
22 226
23 227
24 228 ➤ 8-week clinical assessment: After completion of study supplements at 8 weeks, information is gathered
25 on results of a clinical and physical examination, COVID-19 symptoms, compliance with regimen (including
26 direct questioning and pill count), vital signs, blood investigations (from a collected blood sample),
27 medical conditions, treatment and medications, use of any other nutritional supplements, updates to
28 COVID-19 vaccination status, complications, and history. This assessment is conducted in person at the
29 hospital, or at a location convenient to the participant where privacy can be ensured (including an option
30 to collect some information via telephone if an in-person visit is not possible). Symptoms are specifically
31 asked to participants; other measures are asked, observed, assessed, or abstracted from the participants'
32 records.

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37 237 ➤ 12-week telephone follow up: A final assessment is conducted of long-term COVID-19 symptoms, and any
38 updates to COVID-19 vaccination status. All information is self-reported by participants.
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42 239 All data are collected using standardized questionnaire forms on electronic tablets,[32] as described above.

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45 240 The primary outcome of the study is time to resolution of all of the following symptoms: (1) fever, (2) cough and
46 (3) shortness of breath. These symptoms are most commonly reported among COVID-19 patients, including in
47 Indian populations,[46,47] and have also been assessed as part of studies examining vitamin D and zinc in
48 respiratory illnesses [17]. These and additional symptoms (including fatigue, headache, loss of smell and taste and
49 sore throat) are captured on multiple time points, including baseline, daily hospital follow ups for admitted patients,
50 telephone follow ups every three days after leaving the hospital until 8 weeks post-enrolment, the 8-week clinical
51 assessment, and finally at a 12-week assessment call. Data on symptoms are collected using the same structured
52 questions at each time point: (1) whether the participant has experienced X symptom today, and if so, (2) how
53 many days in total including today the participant has experienced X symptom. Staff conducting in-person and
54 telephone follow ups are trained uniformly using a standardised telephone script with regards to collecting this

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3 250 information. Metrics of individual symptoms and combination of symptoms are used to identify the time point of
4 251 resolution symptoms from baseline.
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7 252 Secondary outcomes include duration of hospital stay, need for assisted ventilation, individual symptoms
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9 253 duration, all-cause mortality, and blood biomarker levels, including 25-hydroxy vitamin D, zinc, and other
10 254 immunological and inflammatory biomarkers (including interleukin 6, angiotensin-converting enzyme 2, soluble triggering receptor
11
12 255 expressed on myeloid cells-1, immunoglobulin G and immunoglobulin M). Biomarker levels are assessed using
13
14 256 blood samples collected at baseline and at the 8-week clinical assessment. Occurrence of any other secondary
15 257 endpoints between baseline to 8-week clinical assessment is recorded during follow up calls or visits as described
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17 258 above. A list of collected data and blood investigations with time points at baseline, during follow up visits or calls,
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19 259 and at 8 and 12 weeks is summarized in **Table 1**.
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Table 1. Collection of data points in the trial.

Data category	Baseline (enrolment)	Follow up	8 weeks	12 weeks
Demographic and background information	Age, gender, education, marital status, occupation, socio-economic status, health and prevention behaviours, COVID-19 vaccination <i>(Self-reported by participant, assessed by staff or abstracted from participant record)</i>		COVID-19 vaccination <i>(Self-reported by participant)</i>	COVID-19 vaccination <i>(Self-reported by participant)</i>
Dietary information	Food frequency questionnaire: consumption frequency of 25 diverse food groups in last three months <i>(Self-reported by participant)</i>			
Clinical examination	Medical history, comorbidities, preadmission medications, non-intervention nutritional supplement use <i>(Self-reported by participant, assessed by staff or abstracted from participant record)</i> Clinical symptoms ¹ <i>(Self-reported by participant)</i>	Hospital and telephone follow up: Clinical symptoms ¹ <i>(Self-reported by participant)</i> Hospital follow up only: Changes in medications, changes in non-intervention nutritional supplement use <i>(Assessed by staff or abstracted from participant record)</i>	Medical history, comorbidities, pre-assessment medications, non-intervention nutritional supplement use <i>(Self-reported by participant, assessed by staff or abstracted from participant record)</i> Clinical symptoms ¹ <i>(Self-reported by participant)</i>	Clinical symptoms ¹ <i>(Self-reported by participant)</i>
Clinical measurements	Respiratory rate, pulse, auxiliary temperature, SpO ₂ , systolic and diastolic blood pressure, weight and height <i>(Assessed by staff or abstracted from participant record)</i>	Hospital follow up only: Respiratory rate, pulse, auxiliary temperature, SpO ₂ , systolic and diastolic blood pressure, weight, requirement for non-invasive ventilation or intubation/ventilator support, need for dialysis, lab investigations <i>(Assessed by staff or abstracted from participant record)</i>	Respiratory rate, pulse, auxiliary temperature, SpO ₂ , systolic and diastolic blood pressure, weight and height <i>(Assessed by staff or abstracted from participant record)</i>	
Blood and other investigations and biomarkers	SARS-COV-2 RT-PCR, chest X-Ray, complete blood count, blood glucose, serum creatinine, CRP, LDH, serum ferritin, D-dimer, vitamin D, zinc, calcium, IgG, IgM, Ang2, IL-6 and sTREM-1		CRP, LDH, serum ferritin, D-dimer, vitamin D, zinc, calcium, IgG, IgM, Ang2, IL-6 and sTREM-1 <i>(Assessed by laboratory or abstracted from participant record)</i>	

(Assessed by laboratory or abstracted from participant record)

<p>Other information</p>	<p>Hospital and telephone follow up: Compliance, adverse events (Self-reported by participant, assessed by staff or abstracted from participant record)</p>	<p>Compliance (count of remaining pills) (Assessed by staff)</p>
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SpO2: Oxygen saturation, CRP: C-reactive protein, LDH: lactate dehydrogenase, IgG: Immunoglobulin G, IgM: immunoglobulin M, Ang2: angiotensin II, IL-6: interleukin 6, sTREM-1: soluble triggering receptor expressed on myeloid cells-1.

¹Clinical symptoms include: fever, cough, shortness of breath, fatigue, headache, loss of smell, loss of taste, diarrhea, anorexia, sore throat, nasal congestion, nausea and vomiting, and any other reported by the participant.

1

2 Adverse events and reporting

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5 3 Any undesirable circumstance or experiences reported by study participants during the study are categorised as
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7 4 adverse events. All adverse events which are possibly, probably or very likely related to administration of any
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9 5 supplement are monitored and reported to site institutional review boards (IRBs) within 72 hours (serious adverse
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11 6 events) or 1 month (all other adverse events), using a standardized reporting format. The trial data and safety
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13 7 monitoring board (DSMB) is also notified. Site principal investigators and independent physicians are responsible
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15 8 for assessing the causal relationship and making the conclusive decision about continuation of the trial for a
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17 9 particular participant. Additionally, medical insurance is provided to all study participants to take care of any
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19 10 progression of severe adverse events.

20 11 Data and sample management

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23 12 All data collected as part of this trial are entered into password-protected android electronic tablets, with pre-
24
25 13 programmed questionnaires using ODK.[32] All data are automatically and directly uploaded from the tablets onto
26
27 14 a secure electronic server, and entered into a password-protected database accessible only to authorised study
28
29 15 team members. Data are stored in linked-anonymised form, with identifiable information and the linking key
30
31 16 stored separately. All analyses and data checks are conducted on anonymised data only.

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33 17 Blood samples collected as part of this trial are processed at the Foundation for Medical Research, Mumbai, and
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35 18 accredited laboratories in India including at the site hospitals. Specimens are linked-anonymised and are stored
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37 19 securely at the Foundation for Medical Research for a maximum of three years.

38 20 Data analysis

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41 21 Planned analyses will initially be undertaken in blinded fashion (comparing coded treatment groups); unblinding of
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43 22 investigators and research staff with respect to treatment allocation will only occur once analyses are completed.

46 23 Planned analyses

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49 24 An intent-to-treat analysis will be used as the primary analytic strategy. Time to primary outcome will be compared
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51 25 between participants randomized to vitamin D vs. no vitamin D and zinc vs. no zinc using Cox regression. We will
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53 26 investigate effect modification of either treatment effect by the other, and by third variables collected at baseline
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55 27 (including, anthropometric status, and vitamin D status). Effect modification will be assessed by including
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57 28 interaction terms in Cox regression models, and statistical significance assessed via likelihood ratio tests. There are
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59 29 no *a priori* effect modifiers hypothesized, and unless there is strong modification of a treatment effect, our power
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31 to detect these may be low. We will assess the success of randomization by comparing baseline variables by
treatment group using χ^2 and t-tests and use multivariate modelling to adjust for imbalances if needed. 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.

The effect of vitamin D or zinc on dichotomous secondary outcomes will be analyzed in a similar approach. The proportion of individuals experiencing hypercalcemia will be compared between treatment groups using χ^2 tests, and effects of the supplements on blood biomarkers will be compared via Wilcoxon and t-tests. This study will measure numerous risk factors for COVID-19 progression and severe treatment outcomes including hemoglobin; co-morbidities; medications including chloroquine, hydroxychloroquine, and ACE inhibitors; and socio-demographic, clinical, nutritional, and lifestyle-related risk factors. We will examine relationships of these factors in the placebo group first, to avoid complex questions concerning interactions between risk factors and treatments. Once we find a satisfactory parsimonious model using principles of model selection as detailed by Greenland,[48] we will test and modify it if needed in the whole study population, adjusting for treatment effects.

Analyses will consider sex and gender throughout, by disaggregating findings, and attempting to elucidate the roles of sex and gender in the clinical course and immune response by controlling for potential sociodemographic, nutritional, and immunological confounders.

Statistical power calculations

With a single endpoint for both interventions, the factorial design does not provide a “two-for-one” power advantage.[49] Power will decrease if each treatment has a moderate effect; we accounted for this in calculating the sample size. Assumptions related to treatment effects may be reasonably inferred from meta-analyses of well-designed randomized controlled trials studying these supplements in other acute respiratory illnesses.[17,21–24,50] 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] 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,[52] 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.

Table 2. Statistical power estimation.

Effect of Treatment A	True effect of Treatment B						
	0%	5%	10%	15%	20%	25%	30%
30%	99%	99%	99%	99%	98%	98%	97%
25%	95%	94%	93%	92%	90%	88%	86%
20%	81%	79%	76%	74%	71%	69%	66%

Patient and public involvement

Patients and the public were not involved in the design of this study.

DATA AND SAFETY MONITORING BOARD

The Data and Safety Monitoring Board (DSMB) was established prior to commencement of the trial. It consists of independent experts in respiratory infection and communicable diseases, public health and nutrition, clinical research, and biostatistics. The role of the board is to provide their inputs, recommendations, review the trial protocols and progress by ensuring the rights and safety of involving participants in the study through periodic trial review meetings.

The trial DSMB will examine efficacy endpoints by study arms when half of individuals are enrolled. In accordance with the Haybittle-Peto rule, if the difference in the primary outcome between study arms is <0.001 , unblinding of the DSMB and stopping will be considered.[53]

ETHICS AND DISSEMINATION

This study is being conducted in the accordance with the Declaration of Helsinki 2013. The study was approved by the Institutional Review Board of the Harvard T.H. Chan School of Public Health (Protocol No. IRB20-1425), the University Health Network Research Ethics Board (20-5775), the Institutional Research Ethics Committee of the Foundation for Medical Research (IREC No. FMR/IREC/C19/02/2020), the Institutional Review Board of Saifee Hospital (Project No. EC/008/2020) and the KEM Hospital Research Centre Ethics Committee (KEMHRC ID No. 2027). The trial is registered on ClinicalTrials.gov (NCT04641195). Permission for the study was also obtained from the Health Management and Screening Comments (HMSC), Government of India (HMSC (GOI)-2021-0060), and the study was registered prospectively in the Clinical Trials Registry India (CTRI/2021/04/032593). Since the study intervention is related to micronutrient supplementation, endorsement from the Drugs Controller General of India was non-obligatory. The study findings will be presented in peer-reviewed medical journals.

DISCUSSION

With continued high incidence of global cases, COVID-19 remains a global health challenge. Alongside vaccination and other preventative measures, low-cost and efficient interventions which may help minimize the occurrence of serious disease are needed. These would be particularly valuable in low- and middle-income countries, where health systems are more overburdened and resources much fewer. In this context, and given previous evidence regarding the role of vitamin D and zinc in the development of and recovery from respiratory infections,[17,21–24,50] there is a need to explore their potential value as part of therapeutic regimens for COVID-19.

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. The frequent follow up of participants and collection of a range of sociodemographic, clinical and biomarker measures alongside blood samples will enable a detailed investigation of the effect of supplementation on disease progression, including potentially important immunological and inflammatory pathways. Importantly, in comparison with other vitamin D or zinc COVID-19 intervention studies currently registered on ClinicalTrials.gov, this would be the first conducted outside of the U.S. or Europe and other similar high-income countries. The location of this study in two large cities, alongside the broad eligibility criteria, increases the generalizability of study results. Given the current unpredictability of COVID-19 waves, one challenge to the study is to maintain recruitment during periods where cases may be on the decline. 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. Regardless, the findings of this study will have direct relevance to many settings in South Asia and sub-Saharan Africa with weak health systems and prevalent malnutrition. Ultimately, the evidence generated as part of this trial will enhance our understanding of the role of vitamin D and zinc in COVID-19 disease, and contribute high quality evidence on the potential value of supplementation of these micronutrients for the same.

FUNDING

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AUTHOR CONTRIBUTIONS

WWF, KCK, YD, and NM conceptualised the project, and designed the study along with SB, KKS, ECH, YM and UP. YD, NM, PDC, GG, KKS, YM, and SS are involved in data acquisition, and in study monitoring along with KCK, WWF and UP. MW provides statistical expertise. KKS and UP drafted the manuscript, and all authors reviewed and critically revised the draft and approved the final manuscript.

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2 116 **COMPETING INTERESTS**
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5 117 All authors declare no conflicts of interest.
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8 118 **ACKNOWLEDGEMENTS**
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10
11 119 We would like to thank all participants, doctors, nurses, and site hospital staff at participating sites for their
12
13 120 contribution in the trial implementation. We also thank all members of the DSMB and respective IRBs for their
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15 121 guidance and valuable inputs in the trial.
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For peer review only

REFERENCES

- 1 Dong E, Du H, Gardner L. An interactive web-based dashboard to track COVID-19 in real time. *The Lancet Infectious Diseases* 2020;**20**:533–4. doi:10.1016/S1473-3099(20)30120-1
- 2 Hogan A, Winksill P, Watson O, *et al.* Modelling the allocation and impact of a COVID-19 vaccine. Published Online First: 25 September 2020. doi:https://doi.org/10.25561/82822
- 3 Phillips N. The coronavirus is here to stay — here's what that means. *Nature* 2021;**590**:382–4. doi:10.1038/d41586-021-00396-2
- 4 Torjesen I. Covid-19 will become endemic but with decreased potency over time, scientists believe. *BMJ* 2021;**372**:n494. doi:10.1136/bmj.n494
- 5 Hockham C, Kotwal S, Wilcox A, *et al.* Protocol for the Controlled evaluation of Angiotensin Receptor blockers for COVID-19 respiratory disease (CLARITY): a randomised controlled trial. *Trials* 2021;**22**:573. doi:10.1186/s13063-021-05521-0
- 6 Teshome A, Adane A, Girma B, *et al.* The Impact of Vitamin D Level on COVID-19 Infection: Systematic Review and Meta-Analysis. *Front Public Health* 2021;**9**:624559. doi:10.3389/fpubh.2021.624559
- 7 Bouillon R, Marcocci C, Carmeliet G, *et al.* Skeletal and Extraskeletal Actions of Vitamin D: Current Evidence and Outstanding Questions. *Endocr Rev* 2019;**40**:1109–51. doi:10.1210/er.2018-00126
- 8 Pearce SH, Cheetham TD. Diagnosis and management of vitamin D deficiency. *BMJ* 2010;**340**:b5664. doi:10.1136/bmj.b5664
- 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
- 11 Greiller CL, Martineau AR. Modulation of the immune response to respiratory viruses by vitamin D. *Nutrients* 2015;**7**:4240–70. doi:10.3390/nu7064240
- 12 Greiller CL, Suri R, Jolliffe DA, *et al.* Vitamin D attenuates rhinovirus-induced expression of intercellular adhesion molecule-1 (ICAM-1) and platelet-activating factor receptor (PAFR) in respiratory epithelial cells. *J Steroid Biochem Mol Biol* 2019;**187**:152–9. doi:10.1016/j.jsbmb.2018.11.013
- 13 Telcian AG, Zdrenghea MT, Edwards MR, *et al.* Vitamin D increases the antiviral activity of bronchial epithelial cells in vitro. *Antiviral Res* 2017;**137**:93–101. doi:10.1016/j.antiviral.2016.11.004
- 14 Hansdottir S, Monick MM, Lovan N, *et al.* Vitamin D decreases respiratory syncytial virus induction of NF-kappaB-linked chemokines and cytokines in airway epithelium while maintaining the antiviral state. *J Immunol* 2010;**184**:965–74. doi:10.4049/jimmunol.0902840
- 15 Hansdottir S, Monick MM, Hinde SL, *et al.* Respiratory epithelial cells convert inactive vitamin D to its active form: potential effects on host defense. *J Immunol* 2008;**181**:7090–9. doi:10.4049/jimmunol.181.10.7090

- 1
2 161 16 Zhou J, Du J, Huang L, *et al.* Preventive Effects of Vitamin D on Seasonal Influenza A in Infants: A
3 162 Multicenter, Randomized, Open, Controlled Clinical Trial. *The Pediatric Infectious Disease Journal*
4 163 2018;**37**:749–54. doi:10.1097/INF.0000000000001890
5 163
6
7 164 17 Abioye AI, Bromage S, Fawzi W. Effect of micronutrient supplements on influenza and other respiratory
8 165 tract infections among adults: a systematic review and meta-analysis. *BMJ Glob Health* 2021;**6**:e003176.
9 166 doi:10.1136/bmjgh-2020-003176
10
11 167 18 International Zinc Nutrition Consultative Group (IZiNCG), Brown KH, Rivera JA, *et al.* International Zinc
12 168 Nutrition Consultative Group (IZiNCG) technical document #1. Assessment of the risk of zinc deficiency in
13 169 populations and options for its control. *Food Nutr Bull* 2004;**25**:S99-203.
14
15 170 19 Smith MR, DeFries R, Chhatre A, *et al.* Inadequate Zinc Intake in India: Past, Present, and Future. *Food Nutr*
16 171 *Bull* 2019;**40**:26–40. doi:10.1177/0379572118825176
17
18
19 172 20 GBD Compare. Institute for Health Metrics and Evaluation. 2014. [https://www.healthdata.org/data-](https://www.healthdata.org/data-visualization/gbd-compare)
20 173 [visualization/gbd-compare](https://www.healthdata.org/data-visualization/gbd-compare) (accessed 17 Dec 2021).
21
22 174 21 Hemilä H, Fitzgerald JT, Petrus EJ, *et al.* Zinc Acetate Lozenges May Improve the Recovery Rate of Common
23 175 Cold Patients: An Individual Patient Data Meta-Analysis. *Open Forum Infect Dis* 2017;**4**:ofx059.
24 176 doi:10.1093/ofid/ofx059
25
26 177 22 Roth DE, Richard SA, Black RE. Zinc supplementation for the prevention of acute lower respiratory infection
27 178 in children in developing countries: meta-analysis and meta-regression of randomized trials. *Int J Epidemiol*
28 179 2010;**39**:795–808. doi:10.1093/ije/dyp391
29
30
31 180 23 Bhatnagar S, Wadhwa N, Aneja S, *et al.* Zinc as adjunct treatment in infants aged between 7 and 120 days
32 181 with probable serious bacterial infection: a randomised, double-blind, placebo-controlled trial. *Lancet*
33 182 2012;**379**:2072–8. doi:10.1016/S0140-6736(12)60477-2
34
35 183 24 Banupriya N, Bhat BV, Benet BD, *et al.* Short Term Oral Zinc Supplementation among Babies with Neonatal
36 184 Sepsis for Reducing Mortality and Improving Outcome - A Double-Blind Randomized Controlled Trial. *Indian*
37 185 *J Pediatr* 2018;**85**:5–9. doi:10.1007/s12098-017-2444-8
38
39 186 25 Zhang L, Liu Y. Potential interventions for novel coronavirus in China: A systematic review. *J Med Virol*
40 187 2020;**92**:479–90. doi:10.1002/jmv.25707
41
42 188 26 Skalny AV, Rink L, Ajsuvakova OP, *et al.* Zinc and respiratory tract infections: Perspectives for COVID-19
43 189 (Review). *Int J Mol Med* 2020;**46**:17–26. doi:10.3892/ijmm.2020.4575
44
45
46 190 27 te Velthuis AJW, van den Worm SHE, Sims AC, *et al.* Zn(2+) inhibits coronavirus and arterivirus RNA
47 191 polymerase activity in vitro and zinc ionophores block the replication of these viruses in cell culture. *PLoS*
48 192 *Pathog* 2010;**6**:e1001176. doi:10.1371/journal.ppat.1001176
49
50 193 28 COVID19 STATEWISE STATUS. MyGov.in. 2020. <https://mygov.in/corona-data/covid19-statewise-status/>
51 194 (accessed 17 Dec 2021).
52
53 195 29 Tambe MP, Parande MA, Tapare VS, *et al.* An epidemiological study of laboratory confirmed COVID-19 cases
54 196 admitted in a tertiary care hospital of Pune, Maharashtra. *Indian J Public Health* 2020;**64**:S183–7.
55 197 doi:10.4103/ijph.IJPH_522_20
56
57
58 198 30 Kodge BG. A review on current status of COVID19 cases in Maharashtra state of India using GIS: a case
59 199 study. *Spatial Information Research* 2020;**1**–7. doi:10.1007/s41324-020-00349-3
60

- 1
2 200 31 India: WHO Coronavirus Disease (COVID-19) Dashboard With Vaccination Data. <https://covid19.who.int>
3 (accessed 17 Dec 2021).
4 201
- 5
6 202 32 ODK - Collect data anywhere. <https://getodk.org> (accessed 17 Dec 2021).
7
- 8 203 33 Trang HM, Cole DE, Rubin LA, *et al.* Evidence that vitamin D3 increases serum 25-hydroxyvitamin D more
9 204 efficiently than does vitamin D2. *Am J Clin Nutr* 1998;**68**:854–8. doi:10.1093/ajcn/68.4.854
10
- 11 205 34 Armas LAG, Hollis BW, Heaney RP. Vitamin D2 is much less effective than vitamin D3 in humans. *J Clin*
12 206 *Endocrinol Metab* 2004;**89**:5387–91. doi:10.1210/jc.2004-0360
13
- 14 207 35 Kearns MD, Binongo JNG, Watson D, *et al.* The effect of a single, large bolus of vitamin D in healthy adults
15 208 over the winter and following year: a randomized, double-blind, placebo-controlled trial. *Eur J Clin Nutr*
16 209 2015;**69**:193–7. doi:10.1038/ejcn.2014.209
17
- 18
19 210 36 Kearns MD, Alvarez JA, Tangpricha V. Large, single-dose, oral vitamin D supplementation in adult
20 211 populations: a systematic review. *Endocr Pract* 2014;**20**:341–51. doi:10.4158/EP13265.RA
21
- 22 212 37 Vieth R, Bischoff-Ferrari H, Boucher BJ, *et al.* The urgent need to recommend an intake of vitamin D that is
23 213 effective. *Am J Clin Nutr* 2007;**85**:649–50. doi:10.1093/ajcn/85.3.649
24
- 25 214 38 Dong Y, Stallmann-Jorgensen IS, Pollock NK, *et al.* A 16-week randomized clinical trial of 2000 international
26 215 units daily vitamin D3 supplementation in black youth: 25-hydroxyvitamin D, adiposity, and arterial
27 216 stiffness. *J Clin Endocrinol Metab* 2010;**95**:4584–91. doi:10.1210/jc.2010-0606
28
- 29 217 39 Schleithoff SS, Zittermann A, Tenderich G, *et al.* Vitamin D supplementation improves cytokine profiles in
30 218 patients with congestive heart failure: a double-blind, randomized, placebo-controlled trial. *Am J Clin Nutr*
31 219 2006;**83**:754–9. doi:10.1093/ajcn/83.4.754
32
- 33
34 220 40 Pappa HM, Mitchell PD, Jiang H, *et al.* Treatment of vitamin D insufficiency in children and adolescents with
35 221 inflammatory bowel disease: a randomized clinical trial comparing three regimens. *J Clin Endocrinol Metab*
36 222 2012;**97**:2134–42. doi:10.1210/jc.2011-3182
37
- 38 223 41 Abu-Mouch S, Fireman Z, Jarchofsky J, *et al.* Vitamin D supplementation improves sustained virologic
39 224 response in chronic hepatitis C (genotype 1)-naïve patients. *World J Gastroenterol* 2011;**17**:5184–90.
40 225 doi:10.3748/wjg.v17.i47.5184
41
- 42 226 42 Institute of Medicine (US) Panel on Micronutrients. *Dietary Reference Intakes for Vitamin A, Vitamin K,*
43 227 *Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and*
44 228 *Zinc.* Washington (DC): : National Academies Press (US) 2001.
45 229 <http://www.ncbi.nlm.nih.gov/books/NBK222310/> (accessed 2 Mar 2022).
46 229
47
- 48 230 43 AIIMS/ICMR National Task Force/Joint Monitoring Group. Clinical Guidelines for Management of Adult
49 231 COVID-19 Patients: Revised on 14/01/2022. New Delhi: : Ministry of Health and Family Welfare,
50 232 Government of India
51 233 [https://www.mohfw.gov.in/pdf/ClinicalGuidanceforManagementofAdultCovid19Patientsupdatedason17thJ](https://www.mohfw.gov.in/pdf/ClinicalGuidanceforManagementofAdultCovid19Patientsupdatedason17thJanuary2022.pdf)
52 234 [anuary2022.pdf](https://www.mohfw.gov.in/pdf/ClinicalGuidanceforManagementofAdultCovid19Patientsupdatedason17thJanuary2022.pdf) (accessed 28 Feb 2022).
53
- 54 235 44 MOHFW, GOI. Clinical Management Protocol for COVID-19 (In Adults) - Version 6 (24.05.21). New Delhi: :
55 236 Ministry of Health and Family Welfare, Government of India 2021.
56 237 [https://www.mohfw.gov.in/pdf/UpdatedDetailedClinicalManagementProtocolforCOVID19adultsdated2405](https://www.mohfw.gov.in/pdf/UpdatedDetailedClinicalManagementProtocolforCOVID19adultsdated24052021.pdf)
57 238 [2021.pdf](https://www.mohfw.gov.in/pdf/UpdatedDetailedClinicalManagementProtocolforCOVID19adultsdated24052021.pdf) (accessed 28 Feb 2022).
58 238
59
60

- 1
2 239 45 MOHFW, GOI. Revised Discharge Policy for COVID-19: Updated on 9th January 2022. New Delhi: : Ministry
3 240 of Health and Family Welfare, Government of India
4 241 <https://www.mohfw.gov.in/pdf/RevisedDischargePolicyforCOVID19updatedon9thJanuary2022.pdf>
5 242 (accessed 28 Feb 2022).
6 242
7
8 243 46 Kumar N, Hameed SKS, Babu GR, *et al.* Descriptive epidemiology of SARS-CoV-2 infection in Karnataka state,
9 244 South India: Transmission dynamics of symptomatic vs. asymptomatic infections. *eClinicalMedicine* 2021;**32**.
10 245 doi:10.1016/j.eclinm.2020.100717
11
12 246 47 Laxminarayan R, B CM, G VT, *et al.* SARS-CoV-2 infection and mortality during the first epidemic wave in
13 247 Madurai, south India: a prospective, active surveillance study. *The Lancet Infectious Diseases* 2021;**21**:1665–
14 248 76. doi:10.1016/S1473-3099(21)00393-5
15
16 249 48 Greenland S. Modeling and variable selection in epidemiologic analysis. *Am J Public Health* 1989;**79**:340–9.
17 250 doi:10.2105/ajph.79.3.340
18 250
19
20 251 49 Ellenberg SS, Finkelstein DM, Schoenfeld DA. Statistical Issues Arising in AIDS Clinical Trials. *Journal of the*
21 252 *American Statistical Association* 1992;**87**:562–9. doi:10.1080/01621459.1992.10475240
22
23 253 50 Martineau AR, Jolliffe DA, Hooper RL, *et al.* Vitamin D supplementation to prevent acute respiratory tract
24 254 infections: systematic review and meta-analysis of individual participant data. *BMJ* 2017;**356**:i6583.
25 255 doi:10.1136/bmj.i6583
26
27 256 51 Schoenfeld DA, Richter JR. Nomograms for calculating the number of patients needed for a clinical trial with
28 257 survival as an endpoint. *Biometrics* 1982;**38**:163–70.
29
30
31 258 52 Dorigatti I, Okell L, Cori A, *et al.* Report 4: Severity of 2019-novel coronavirus (nCoV). Imperial College
32 259 London 2020. doi:10.25561/77154
33
34 260 53 Blenkinsop A, Parmar MK, Choodari-Oskooei B. Assessing the impact of efficacy stopping rules on the error
35 261 rates under the multi-arm multi-stage framework. *Clin Trials* 2019;**16**:132–41.
36 262 doi:10.1177/1740774518823551
37
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5 265 **FIGURE LEGENDS**
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8 266 **Figure 1:** Map of India (grey) with Maharashtra highlighted in red, and Mumbai (black dot) and Pune (blue dot)
9 identified.
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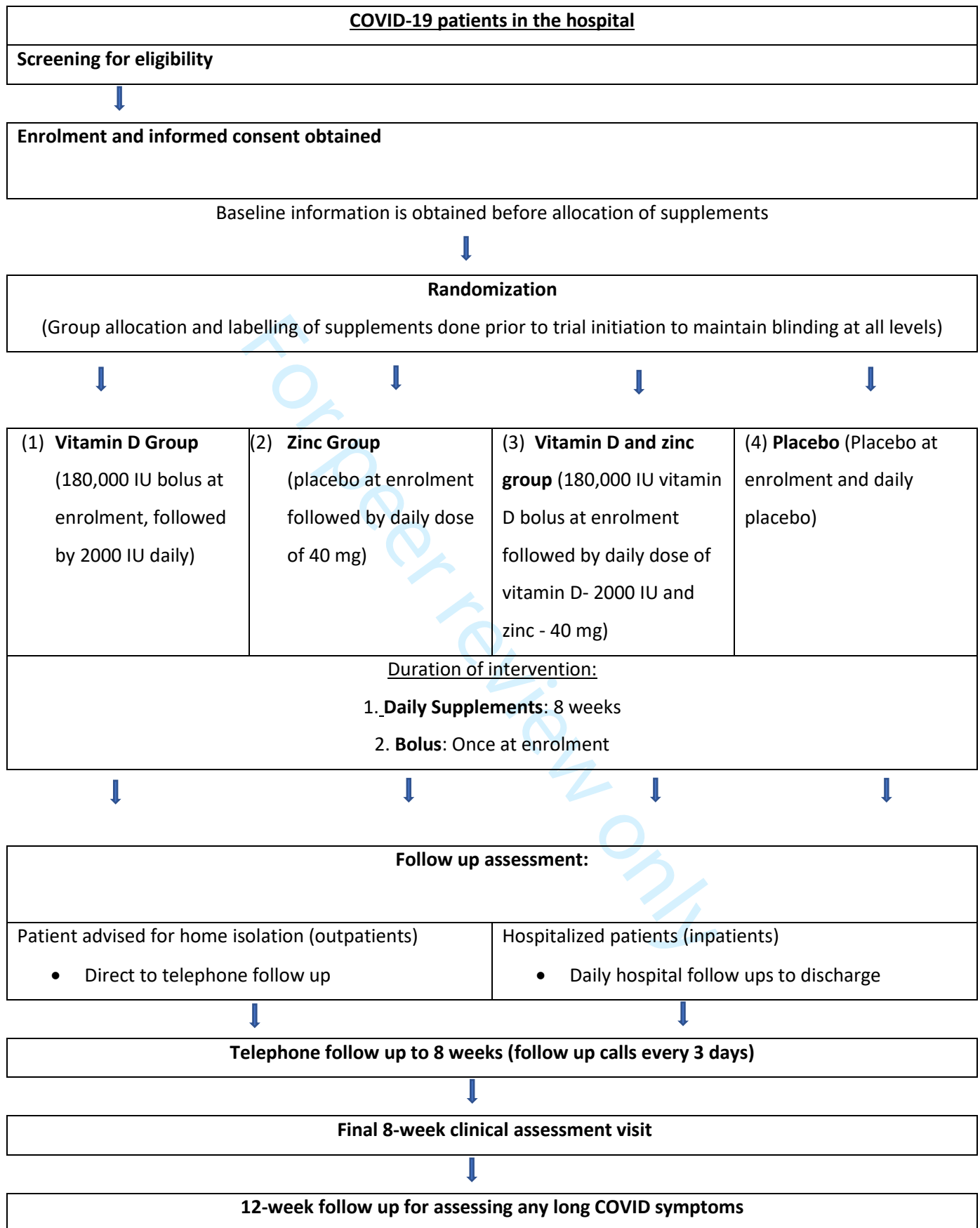
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13 268 Map created with mapchart.net.
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15 269 **Figure 2:** Overview of trial procedures. RAT: Rapid Antigen Test.
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Figure 1: Map of India (grey) with Maharashtra highlighted in red, and Mumbai (black dot) and Pune (blue dot) identified.
Map created with mapchart.net.
156x188mm (330 x 330 DPI)





SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description
Administrative information		
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym Title page (p1): “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”
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry Abstract (p2): “Trial Registration number: NCT04641195, CTRI/2021/04/032593, HMSC (GOI)-2021-0060”
	2b	All items from the World Health Organization Trial Registration Data Set Manuscript: Items from the WHO Trial Registration Data Set (including elements such as trial registration, financial support, study contacts, study title, countries of recruitment and details on design and recruitment status) are noted throughout the manuscript.
Protocol version	3	Date and version identifier NA: This is a manuscript of a study protocol.
Funding	4	Sources and types of financial, material, and other support Funding (p14): “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). The funding body has no role in study design and procedures, or the decision to submit manuscripts for publication.”
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors Author contributions (p14): “KCK, YD, WWF and NM conceptualised the project, and designed the study along with SB, KKS, ECH, YM and UP.”

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- 5b Name and contact information for the trial sponsor
The name of the study sponsor is included in the clinical trial registration records (NCT04641195, CTRI/2021/04/032593).
- 5c Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities
Funding (p14):
“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). The funding body has no role in study design and procedures, or the decision to submit manuscripts for publication.”
- 5d Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)
NA

Introduction

Background and rationale

6a

Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention

Introduction (p4-5):

“[...] Additionally, with limited proven treatment regimens for COVID-19 to date, it is essential to continue exploring low cost and commonly available effective interventions which can be implemented as standardized therapeutic treatment regimens at large.[6] This is especially important in the context of low and middle-income countries in South Asia and Africa, which are particularly vulnerable given weak health systems and the co-existence of malnutrition and other co-morbidities. This includes India, which continues to report a substantial number of COVID-19 cases.[1]

[...]

Vitamin D shows promise as a novel, cost-effective prevention and adjunctive treatment for respiratory infections. [...] In laboratory studies, vitamin D metabolites support innate immune responses to rhinoviruses and respiratory syncytial virus.[10–14] In participants with influenza, high-dose vitamin D supplementation shortened durations of fever, cough and wheezing, particularly among those with low vitamin D levels.[15] In a recent systematic review and meta-analysis of randomised controlled trials, vitamin D supplementation was associated with decreased risk of acute respiratory infections and shortened duration of symptoms.[16]

[...]

Multiple meta-analyses and pooled analyses of randomized controlled trials conducted in the US and low- and middle-income countries have shown that oral zinc supplementation reduces incidence of acute respiratory infections by 35%, shortens duration of symptoms, and improves recovery rate.[16,20–23] Zinc is a potential treatment in COVID-19, due to its immune modulatory effect, as well as direct antiviral effect.[24] The mechanisms by which zinc may serve as adjunct therapy in COVID-19 has been recently reviewed by Skalny et al. 2020,[25] who note that Zn²⁺ cations, especially in combination with zinc ionophore pyrithione inhibit SARS-coronavirus RNA polymerase activity by decreasing replication.[26]

Vitamin D and zinc are safe, inexpensive, and widely available therapies; therefore, experimental evidence that these nutrient supplements are effective against COVID-19 would readily support their inclusion in standard of care. Therefore, we are undertaking a randomized controlled trial to determine the effect of vitamin D and zinc supplementation on treatment outcomes among individuals with COVID-19 in India.”

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2		6b	Explanation for choice of comparators
3			Methods and analysis // Study procedures // Intervention (p8):
4			“A placebo was chosen as the comparator group given that there is
5			currently no widespread consensus on the use of any nutritional
6			supplement as part of standard or routine treatment for COVID-
7			19.[16]”
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10	Objectives	7	Specific objectives or hypotheses
11			Objectives (p5):
12			“The primary objectives of this trial are:
13			□ To determine the effect of vitamin D supplementation versus
14			placebo on time to recovery among patients with COVID-19
15			□ To determine the effect of zinc supplementation versus
16			placebo on time to recovery among patients with COVID-19
17			Secondary objectives include:
18			□ To determine the effect of vitamin D or zinc supplementation
19			on duration of hospital stay, all-cause mortality, necessity for assisted
20			ventilation, and individual symptoms duration
21			□ To examine the effect of vitamin D or zinc supplementation on
22			key blood biomarkers, including serum vitamin D and zinc, and
23			immunological and inflammatory markers”
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28	Trial design	8	Description of trial design including type of trial (eg, parallel group,
29			crossover, factorial, single group), allocation ratio, and framework (eg,
30			superiority, equivalence, noninferiority, exploratory)
31			Methods and analysis // Trial design, population and enrolment sites
32			(p5):
33			“This is a double-blind, placebo-controlled, randomized superiority trial
34			with 2x2 factorial design and 1:1:1:1 allocation ratio, being conducted
35			at two site hospitals in Mumbai and Pune, Maharashtra, India (Figure
36			1).”
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Methods: Participants, interventions, and outcomes

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	Eligibility criteria	10	<p>Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)</p> <p>Methods and analysis // Eligibility criteria (p6):</p> <p>“The original inclusion criteria for this study were as follows: (1) men and women aged ≥ 18 years, (2) RT-PCR-confirmed infection with SARS-COV-2, (3) oxygen saturation level (SpO₂) ≥ 90, and (4) written informed consent.</p> <p>The exclusion criteria were as following: (1) pregnant women, (2) individuals enrolled in other clinical trials, (3) daily use of multivitamins for the past 1 month.</p> <p>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.</p>

- 1
2 Interventions 11a Interventions for each group with sufficient detail to allow replication,
3 including how and when they will be administered
4 Methods and analysis // Study procedures // Intervention (p7-8):
5 Patients are randomized to one of four groups:
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7 1. Placebo-Placebo group will receive a placebo vitamin D3 bolus
8 at the hospital followed by placebo daily vitamin D3 maintenance
9 doses and placebo daily zinc supplements
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11 2. Vitamin D-Placebo group will receive an actual vitamin D3
12 bolus (180,000 IU) at the hospital followed by actual daily vitamin D3
13 maintenance doses (2000 IU daily) and daily placebo zinc
14 supplements
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16 3. Placebo-Zinc group will receive a placebo vitamin D3 bolus at
17 the hospital followed by placebo daily vitamin D3 maintenance doses
18 and actual daily zinc supplements (40 mg daily)
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20 4. Vitamin D3-Zinc group will receive an actual vitamin D3 bolus
21 (180,000 IU) at the hospital followed by actual daily vitamin D3
22 maintenance doses (2000 IU daily) and actual daily zinc supplements
23 (40 mg daily)
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26 A placebo was chosen as the comparator group given that there is
27 currently no widespread consensus on the use of any nutritional
28 supplement as part of standard or routine treatment for COVID-19.[16]
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31 Participants receive a pre-labelled daily supplement bottle with 60
32 tablets, and an envelope which contains three vitamin D3/placebo
33 bolus tablets to be consumed at baseline under supervision of site
34 hospital staff. Following the bolus dose, participants are instructed to
35 take supplements daily for 8 weeks. Participants are contacted daily
36 while in hospital or regularly via telephone after leaving the hospital to
37 ensure compliance. Research nurses identify barriers to compliance,
38 and assess compliance at 8 weeks via direct questioning and pill
39 count.
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43 Supplement and placebo tablets were manufactured by Excellamed
44 Laboratories Private Limited (Mumbai, India) with an external quality
45 check done by an independent service provider (Bee Pharmo Labs
46 Private Limited, Mumbai, India).
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49 All participants are provided with care and treatment consistent with
50 Indian national guidelines, and are encouraged to visit the study
51 clinics seven days a week for medical attention if they feel unwell.
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- 11b Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)
Methods and analysis // Adverse events and reporting (p11):
“All adverse events which are possibly, probably or very likely related to administration of any supplement are monitored and reported to site institutional review boards (IRBs) within 72 hours (serious adverse events) or 1 month (all other adverse events), using a standardized reporting format. The trial data and safety monitoring board (DSMB) is also notified. Site principal investigators and independent physicians are responsible for assessing the causal relationship and making the conclusive decision about continuation of the trial for a particular participant.”
- 11c Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)
Methods and analysis // Study procedures // Intervention (p8):
“Participants are contacted daily while in hospital or regularly via telephone after leaving the hospital to ensure compliance. Research nurses identify barriers to compliance, and assess compliance at 8 weeks via direct questioning and pill count.”
- 11d Relevant concomitant care and interventions that are permitted or prohibited during the trial
Methods and analysis // Study procedures // Intervention (p8):
“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.”

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- Outcomes 12 Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended
- [Methods and analysis // Study procedures // 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 and additional symptoms 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. Metrics of individual symptoms and combination of symptoms are used to identify the time point of resolution symptoms from baseline.
- Secondary outcomes include duration of hospital stay, need for assisted ventilation, individual symptoms duration, all-cause mortality, occurrence of other severe adverse events and change in blood biomarker levels, including 25-hydroxy vitamin D, zinc and calcium, and other immunological and inflammatory biomarkers. Biomarker levels are assessed using blood samples collected at baseline and at the 8-week clinical assessment. Occurrence of any other secondary endpoints between baseline to 8-week clinical assessment is recorded during follow up calls or visits as described above.”
- Participant timeline 13 Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)
- [See Methods and analysis // Study procedures section \(p6\)](#) for details on enrolment, intervention, and follow up. Table 1 and Figure 2, referred to in this section, also outline the sequence and schedule of enrolment and follow up.

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24	Sample size	14	<p>Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations</p> <p>Methods and analysis // Data analysis // Statistical power calculations (p12) and Table 2:</p> <p>“Assumptions related to treatment effects may be reasonably inferred from meta-analyses of well-designed randomized controlled trials studying these supplements in other acute respiratory illnesses.[15,16,20–23] We based power analysis on the primary outcome of time from onset of disease to clinical recovery, using methodology for survival times.[34] 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,[35] 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.”</p>
25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60	Recruitment	15	<p>Strategies for achieving adequate participant enrolment to reach target sample size</p> <p>Methods and analysis // Trial design, population and enrolment sites (p5):</p> <p>“While we initially targeted only hospitalized inpatients at each site for the study, we broadened our target population in June 2021 to include all hospital outpatients. This was done in order to increase generalizability of results and maintain enrolment in light of decreasing COVID-19 cases.[30]”</p> <p>Methods and analysis // Eligibility criteria (p6):</p> <p>“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.”</p> <p>Methods and analysis // Study procedures // Recruitment and obtaining informed consent (p6):</p> <p>“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. [...]”</p>

Methods: Assignment of interventions (for controlled trials)

Allocation:

Sequence generation	16a	<p>Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions</p> <p>Methods and analysis // Study procedures // Randomization and blinding (p7): “For randomization, a computer-generated list from 1 to 1000 was prepared by the study statistician, according to a randomization sequence in blocks of 20 and stratified by follow up clinic.”</p>
Allocation concealment mechanism	16b	<p>Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned</p> <p>Methods and analysis // Study procedures // Randomization and blinding (p7): “Supplement bottles and envelopes are pre-labelled with codes, and active tablets and placebo are indistinguishable, so that participants and investigators are blinded. For randomization, a computer-generated list from 1 to 1000 was prepared by the study statistician, according to a randomization sequence in blocks of 20 and stratified by follow up clinic.”</p>
Implementation	16c	<p>Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions</p> <p>Methods and analysis // Study procedures // Recruitment and obtaining informed consent (p6): “Potential participants are approached by trained site hospital staff members when they present to site hospitals. [...] Informed consent is obtained after responding to any raised queries.”</p> <p>Methods and analysis // Study procedures // Randomization and blinding (p7): “For randomization, a computer-generated list from 1 to 1000 was prepared by the study statistician, according to a randomization sequence in blocks of 20 and stratified by follow up clinic.”</p>

1
2 Blinding
3 (masking)
4

5 17a Who will be blinded after assignment to interventions (eg, trial
6 participants, care providers, outcome assessors, data analysts), and
7 how

8 Methods and analysis // Study procedures // Randomization and
9 blinding (p7):

10 “Supplement bottles and envelopes are pre-labelled with codes, and
11 active tablets and placebo are indistinguishable, so that participants
12 and investigators are blinded. For randomization, a computer-
13 generated list from 1 to 1000 was prepared by the study statistician,
14 according to a randomization sequence in blocks of 20 and stratified
15 by follow up clinic.”

16 17b If blinded, circumstances under which unblinding is permissible, and
17 procedure for revealing a participant’s allocated intervention during
18 the trial

19 Data and Safety Monitoring Board (p13):

20 “The trial DSMB will examine efficacy endpoints by study arms when
21 half of individuals are enrolled. In accordance with the Haybittle-Peto
22 rule, if the difference in the primary outcome between study arms is
23 <0.001 , unblinding of the DSMB and stopping will be considered.[36]”
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27 **Methods: Data collection, management, and analysis**
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2 Data collection 18a Plans for assessment and collection of outcome, baseline, and other
3 methods including any related processes to promote data quality (eg,
4 duplicate measurements, training of assessors) and a description of
5 study instruments (eg, questionnaires, laboratory tests) along with
6 their reliability and validity, if known. Reference to where data
7 collection forms can be found, if not in the protocol
8
9 Methods and analysis // Study procedures // Baseline data and
10 sample collection (p7):
11 “Following informed consent, participants undergo baseline data and
12 sample collection, including recording of key background and clinical
13 information as follows:
14
15 □ Screening and background: the initial screening form is
16 extended to collect information including participants’ demographic
17 background, socio-economic status, and health and prevention
18 behaviours (smoking and drinking)
19
20 □ Baseline dietary information: a food frequency questionnaire
21 (FFQ) is administered, collecting information on dietary practices and
22 habits in relation to 25 food groups. The FFQ is validated for use in
23 India and has been adapted to the Maharashtra context
24
25 □ Clinical baseline: clinical and physical measures are collected
26 alongside information on COVID-19 vaccination status, COVID-19
27 symptoms, vital signs, blood investigations, medical conditions,
28 treatment and medications, complications, and medical history
29 A blood sample is also collected at baseline. All information is
30 collected securely on electronic tablets, as described above.”
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34 Methods and analysis // Study procedures // Study outcomes and
35 follow up (p8 – 9):
36 “Following baseline assessment and provision of supplements,
37 participants are regularly followed up as described below:
38
39 □ Daily hospital follow up: Daily assessment of COVID-19
40 symptoms, vital signs, complications, medical conditions and study
41 supplement compliance is recorded for hospitalised participants
42
43 □ Telephone follow up: Assessment of COVID-19 symptoms,
44 supplement compliance and adverse events is conducted in a follow
45 up call every three days after leaving the hospital for all participants
46
47 □ 8-week clinical assessment: After completion of study
48 supplements at 8 weeks, information is gathered on results of a
49 clinical and physical examination, COVID-19 symptoms, compliance
50 with regimen (including direct questioning and pill count), vital signs,
51 blood investigations (from a collected blood sample), medical
52 conditions, treatment and medications, complications, and history.
53 This assessment is conducted in person at the hospital, or at a
54 location convenient to the participant where privacy can be ensured
55 (including an option to collect some information via telephone if an in-
56 person visit is not possible)
57
58 □ 12-week telephone follow up: A final assessment is conducted
59 of long-term COVID-19 symptoms [...]
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A list of collected data and blood investigations with time points at
baseline, during follow up visits or calls, and at 8 and 12 weeks is
summarized in Table 1. (Please also refer to Table 1)

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Data
management

- 18b Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols
 Methods and analysis // Study procedures // Intervention (p8):
 “Participants are contacted daily while in hospital or regularly via telephone after leaving the hospital to ensure compliance.”
- 19 Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol
 Methods and analysis // Study procedures // Recruitment and obtaining informed consent (p6):
 “Information regarding eligibility of potential participants is collected on a secure electronic tablet using Open Data Kit (ODK),[31] with questionnaires including built-in checks and data uploaded to a secure server. No identifiable data are collected until the participant has provided informed consent.”
- Methods and analysis // Study procedures // Baseline data and sample collection (p7):
 “All information is collected securely on electronic tablets, as described above.”
- Methods and analysis // Study procedures // Study outcomes and follow up (p8):
 “All data are collected using standardized questionnaire forms on electronic tablets,[31] as described above.”
- Methods and analysis // Data and sample management (p11):
 “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. Data are stored in linked-anonymised form, with identifiable information and the linking key stored separately. All analyses and data checks are conducted on anonymised data only.”

Statistical
methods

20a Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol

Methods and analysis // Data analysis // Planned analyses (p11):

“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. placebo and zinc vs. placebo using Cox regression. [...] We will assess the success of randomization by comparing baseline variables by treatment group using χ^2 and t-tests and use multivariate modelling to adjust for imbalances if needed.

[...]

The effect of vitamin D or zinc on dichotomous secondary outcomes will be analyzed in a similar approach.”

20b Methods for any additional analyses (eg, subgroup and adjusted analyses)

Methods and analysis // Data analysis // Planned analyses (p11-12):

“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.

[...]

The proportion of individuals experiencing hypercalcemia will be compared between treatment groups using χ^2 tests, and effects of the supplements on blood biomarkers will be compared via Wilcoxon and t-tests. This study will measure numerous risk factors for COVID-19 progression and severe treatment outcomes including hemoglobin; co-morbidities; medications including chloroquine, hydroxychloroquine, and ACE inhibitors; and socio-demographic, clinical, nutritional, and lifestyle-related risk factors. We will examine relationships of these factors in the placebo group first, to avoid complex questions concerning interactions between risk factors and treatments. Once we find a satisfactory parsimonious model using principles of model selection as detailed by Greenland,[32] we will test and modify it if needed in the whole study population, adjusting for treatment effects.

Analyses will consider sex and gender throughout, by disaggregating findings, and attempting to elucidate the roles of sex and gender in the clinical course and immune response by controlling for potential sociodemographic, nutritional, and immunological confounders.”

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2 20c Definition of analysis population relating to protocol non-adherence
3 (eg, as randomised analysis), and any statistical methods to handle
4 missing data (eg, multiple imputation)

5 [Methods and analysis // Data analysis // Planned analyses \(p11\):](#)
6 [“An intent-to-treat analysis will be used as the primary analytic](#)
7 [strategy.”](#)
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10 **Methods: Monitoring**

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12 Data monitoring 21a Composition of data monitoring committee (DMC); summary of its role
13 and reporting structure; statement of whether it is independent from
14 the sponsor and competing interests; and reference to where further
15 details about its charter can be found, if not in the protocol.

16 Alternatively, an explanation of why a DMC is not needed

17 [Data and Safety Monitoring Board \(p12-13\):](#)

18 [“The Data and Safety Monitoring Board \(DSMB\) was established prior](#)
19 [to commencement of the trial. It consists of independent experts in](#)
20 [respiratory infection and communicable diseases, public health and](#)
21 [nutrition, clinical research, and biostatistics. The role of the board is to](#)
22 [provide their inputs, recommendations, review the trial protocols and](#)
23 [progress by ensuring the rights and safety of involving participants in](#)
24 [the study through periodic trial review meetings.](#)
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29 [The trial DSMB will examine efficacy endpoints by study arms when](#)
30 [half of individuals are enrolled. In accordance with the Haybittle-Peto](#)
31 [rule, if the difference in the primary outcome between study arms is](#)
32 [<0.001, unblinding of the DSMB and stopping will be considered.\[36\]”](#)
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35 21b Description of any interim analyses and stopping guidelines, including
36 who will have access to these interim results and make the final
37 decision to terminate the trial

38 [Data and Safety Monitoring Board \(p13\):](#)

39 [“The trial DSMB will examine efficacy endpoints by study arms when](#)
40 [half of individuals are enrolled. In accordance with the Haybittle-Peto](#)
41 [rule, if the difference in the primary outcome between study arms is](#)
42 [<0.001, unblinding of the DSMB and stopping will be considered.\[36\]”](#)
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2 Harms 22 Plans for collecting, assessing, reporting, and managing solicited and
3 spontaneously reported adverse events and other unintended effects
4 of trial interventions or trial conduct
5 [Methods and analysis // Adverse events and reporting \(p11\):](#)
6 “Any undesirable circumstance or experiences reported by study
7 participants during the study are categorised as adverse events. All
8 adverse events which are possibly, probably or very likely related to
9 administration of any supplement are monitored and reported to site
10 institutional review boards (IRBs) within 72 hours (serious adverse
11 events) or 1 month (all other adverse events), using a standardized
12 reporting format. The trial data and safety monitoring board (DSMB) is
13 also notified. Site principal investigators and independent physicians
14 are responsible for assessing the causal relationship and making the
15 conclusive decision about continuation of the trial for a particular
16 participant. Additionally, medical insurance is provided to all study
17 participants to take care of any progression of severe adverse
18 events.”

23 Auditing 23 Frequency and procedures for auditing trial conduct, if any, and
24 whether the process will be independent from investigators and the
25 sponsor
26 NA
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28

29 Ethics and dissemination

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31 Research ethics 24 Plans for seeking research ethics committee/institutional review board
32 approval (REC/IRB) approval
33 [Abstract // Ethics and dissemination \(p2\):](#)
34 “Ethical approval was obtained from institutional ethical committees of
35 all participating institutions.”
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39 [Ethics and dissemination \(p13\):](#)
40 “This study is being conducted in the accordance with the Declaration
41 of Helsinki 2013. The study was approved by the Institutional Review
42 Board of the Harvard T.H. Chan School of Public Health (Protocol No.
43 IRB20-1425), the University Health Network Research Ethics Board
44 (20-5775), the Institutional Research Ethics Committee of the
45 Foundation for Medical Research (IREC No.
46 FMR/IREC/C19/02/2020), the Institutional Review Board of Saifee
47 Hospital (Project No. EC/008/2020) and the KEM Hospital Research
48 Centre Ethics Committee (KEMHRC ID No. 2027).”
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- Protocol amendments 25 Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)
NA:
As this is a manuscript of a study protocol, such detail has not been included in this specific document.
- Consent or assent 26a Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)
Methods and analysis // Recruitment and obtaining informed consent (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. These dedicated site hospital staff members determine their interest and eligibility, and provide a brief introduction including key details about the study and what participation involves. The staff members read out the participant information sheet in the appropriate conversational language (English, Hindi or Marathi), and discuss the trial components and the role of the participant in the study. Information provided includes a clear outline of potential benefits and harms, the length of the follow up period, remuneration that can be expected, future use of information and samples, and resources available to the participant such as access to study clinics. Informed consent is obtained after responding to any raised queries. [...] The informed consent process is completed once participants provide their signature on two copies of the consent document; one copy for the trial record and another provided to participants for their reference.”
- 26b Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable
Methods and analysis // Recruitment and obtaining informed consent (p6):
“The staff members read out the participant information sheet in the appropriate conversational language (English, Hindi or Marathi), and discuss the trial components and the role of the participant in the study. Information provided includes a clear outline of potential benefits and harms, the length of the follow up period, remuneration that can be expected, future use of information and samples, and resources available to the participant such as access to study clinics. Informed consent is obtained after responding to any raised queries.”

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- Confidentiality 27 How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial
Methods and analysis // Study procedures // Recruitment and obtaining informed consent (p6):
“Information regarding eligibility of potential participants is collected on a secure electronic tablet using Open Data Kit (ODK),[31] with questionnaires including built-in checks and data uploaded to a secure server. No identifiable data are collected until the participant has provided informed consent.”
- Methods and analysis // Study procedures // Baseline data and sample collection (p7):
“All information is collected securely on electronic tablets, as described above.”
- Methods and analysis // Study procedures // Study outcomes and follow up (p8):
“All data are collected using standardized questionnaire forms on electronic tablets,[31] as described above.”
- Methods and analysis // Data and sample management (p11):
“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. Data are stored in linked-anonymised form, with identifiable information and the linking key stored separately. All analyses and data checks are conducted on anonymised data only.”
- Declaration of interests 28 Financial and other competing interests for principal investigators for the overall trial and each study site
Competing interests (p15):
“All authors declare no conflicts of interest.”
- Access to data 29 Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators
Methods and analysis // Data and sample management (p11):
“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. Data are stored in linked-anonymised form, with identifiable information and the linking key stored separately.”

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2	Ancillary and	30	Provisions, if any, for ancillary and post-trial care, and for
3	post-trial care		compensation to those who suffer harm from trial participation
4			Methods and analysis // Adverse events and reporting (p11):
5			“Additionally, medical insurance is provided to all study participants to
6			take care of any progression of severe adverse events.”
7			
8	Dissemination	31a	Plans for investigators and sponsor to communicate trial results to
9	policy		participants, healthcare professionals, the public, and other relevant
10			groups (eg, via publication, reporting in results databases, or other
11			data sharing arrangements), including any publication restrictions
12			Ethics and dissemination (p13):
13			“The study findings will be presented in peer-reviewed medical
14			journals.”
15		31b	Authorship eligibility guidelines and any intended use of professional
16			writers
17			NA
18		31c	Plans, if any, for granting public access to the full protocol, participant-
19			level dataset, and statistical code
20			NA
21			
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28	Appendices		
29	Informed consent	32	Model consent form and other related documentation given to
30	materials		participants and authorised surrogates
31			NA:
32			As this is a manuscript of a study protocol, such detail has not been
33			included in this specific document.
34			
35			
36	Biological	33	Plans for collection, laboratory evaluation, and storage of biological
37	specimens		specimens for genetic or molecular analysis in the current trial and for
38			future use in ancillary studies, if applicable
39			Methods and analysis // Data and sample management (p11):
40			“Blood samples collected as part of this trial are processed at the
41			Foundation for Medical Research, Mumbai, and accredited
42			laboratories in India including at the site hospitals. Specimens are
43			linked-anonymised and are stored securely at the Foundation for
44			Medical Research for a maximum of three years.”
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*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons [“Attribution-NonCommercial-NoDerivs 3.0 Unported”](#) license.

BMJ Open

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

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2022-061301.R2
Article Type:	Protocol
Date Submitted by the Author:	23-Apr-2022
Complete List of Authors:	Sharma, Kamal Kant; Foundation for Medical Research Partap, Uttara; Harvard University T H Chan School of Public Health, Department of Global Health and Population Mistry, Nerges; Foundation for Medical Research Marathe, Yogesh; Foundation for Medical Research Wang, Molin; Harvard University T H Chan School of Public Health, Departments of Epidemiology and Biostatistics Shaikh, Sanaa; Foundation for Medical Research D'Costa, Pradeep; King Edward Memorial Hospital Pune Gupta, Gaurav; Saifee Hospital Bromage, Sabri; Harvard University T H Chan School of Public Health, Department of Nutrition Hemler, Elena ; Harvard University T H Chan School of Public Health, Department of Global Health and Population Kain, Kevin; University Health Network, Department of Medicine; University of Toronto, Department of Medicine Dholakia, Yatin; Foundation for Medical Research Fawzi, Wafaie.; Harvard University T H Chan School of Public Health, Departments of Global Health and Population; Epidemiology; and Nutrition
Primary Subject Heading:	Infectious diseases
Secondary Subject Heading:	Nutrition and metabolism
Keywords:	COVID-19, Nutrition < TROPICAL MEDICINE, Clinical trials < THERAPEUTICS

SCHOLARONE™
Manuscripts

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3 1 **A randomized trial to determine the effect of vitamin D and zinc**
4
5 2 **supplementation for improving treatment outcomes among COVID-19**
6
7 3 **patients in India: trial protocol**
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10 4
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ABSTRACT

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.

Methods and analysis: We have designed a 2x2 factorial, randomized, double-blind, multi-centre placebo-controlled trial to evaluate the effect of vitamin D and zinc on COVID-19 outcomes in Maharashtra, India. COVID-19 positive individuals are recruited from hospitals in Mumbai and Pune. Participants are provided (1) vitamin D3 bolus (180,000 IU) maintained by daily dose of 2000 IU, and/or (2) zinc gluconate (40 mg daily), versus placebo for 8 weeks. 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. A final follow up telephone call occurs 12 weeks post-enrolment to assess long-term outcomes. The primary outcome of the study is to time to recovery, defined as time to resolution of all of fever, cough and shortness of breath. Secondary outcomes include: duration of hospital stay, all-cause mortality, necessity of assisted ventilation, change in blood biomarker levels, and individual symptoms duration. Participant recruitment commenced on April 2021.

Ethics and dissemination: Ethical approval was obtained from institutional ethical committees of all participating institutions. The study findings will be presented in peer-reviewed medical journals.

Trial Registration number: NCT04641195, CTRI/2021/04/032593, HMSC (GOI)-2021-0060

STRENGTHS AND LIMITATIONS OF THIS STUDY

- 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.
- As a double-blind factorial randomized controlled trial, this study enables an efficient assessment of the effect of vitamin D and zinc on COVID-19 symptoms that is less prone to confounding and bias than other observational studies on this topic.
- With frequent follow up of participants, this study collects information across a range of domains including sociodemographic and clinical measures, and biomarker data, which will allow for a detailed investigation of the effect of supplementation on disease progression.
- 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.

INTRODUCTION

COVID-19 continues to be a problem globally, with over 16 million incident cases and 200,000 deaths reported in November 2021.[1] Concerted global efforts have resulted in the development of vaccines, which may reduce the burden and impact of COVID-19, although suboptimal vaccine coverage and the rapid mutation of the virus continue to prolong the pandemic.[2–5] Additionally, with limited proven treatment regimens for COVID-19 to date, it is essential to continue exploring low cost and commonly available effective interventions which can be implemented as standardized therapeutic treatment regimens at large.[6] This is especially important in the context of low and middle-income countries in South Asia and Africa, which are particularly vulnerable given weak health systems and the co-existence of malnutrition and other co-morbidities. This includes India, which continues to report a substantial number of COVID-19 cases.[1]

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. Countrywide studies suggest VDD may affect at least 70% of the Indian population. Vitamin D shows promise as a novel, cost-effective prevention and adjunctive treatment for respiratory infections. In laboratory studies, vitamin D metabolites support innate immune responses to rhinoviruses and respiratory syncytial virus.[11–15] In participants with influenza, high-dose vitamin D supplementation shortened durations of fever, cough and wheezing, particularly among those with low vitamin D levels.[16] In a recent systematic review and meta-analysis of randomised controlled trials, vitamin D supplementation was associated with decreased risk of acute respiratory infections and shortened duration of symptoms.[17]

Zinc is an essential mineral that plays critical roles in gene expression, cell division, and immunity.[18] In India, dietary predominance of micronutrient-sparse staples, limited consumption of animal foods, and high consumption of zinc absorption inhibitors render the population at extremely high risk of inadequacy, which is exacerbated due to global climate change.[19] About 25% of the Indian population is zinc inadequate, and 4.3 million child deaths (<5 years) were attributable to zinc deficiency in 2017.[20] Multiple meta-analyses and pooled analyses of randomized controlled trials conducted in the US and low- and middle-income countries have shown that oral zinc supplementation reduces incidence of acute respiratory infections by 35%, shortens duration of symptoms, and improves recovery rate.[17,21–24] Zinc is a potential treatment in COVID-19, due to its immune modulatory effect, as well as direct antiviral effect.[25] The mechanisms by which zinc may serve as adjunct therapy in COVID-19 has been recently reviewed by Skalny et al. 2020,[26] who note that Zn²⁺ cations, especially in combination with zinc ionophore pyrithione inhibit SARS-coronavirus RNA polymerase activity by decreasing replication.[27]

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2 98 Vitamin D and zinc are safe, inexpensive, and widely available therapies; therefore, experimental evidence that
3
4 99 these nutrient supplements are effective against COVID-19 would readily support their inclusion in standard of
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6 100 care. Therefore, we are undertaking a randomized controlled trial to determine the effect of vitamin D and zinc
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8 101 supplementation on treatment outcomes among individuals with COVID-19 in India.
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10 OBJECTIVES

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13 103 The primary objectives of this trial are:

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16 104 ❖ To determine the effect of vitamin D supplementation versus placebo on time to recovery among patients
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18 105 with COVID-19
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20 106 ❖ To determine the effect of zinc supplementation versus placebo on time to recovery among patients with
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22 107 COVID-19

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24 108 Secondary objectives include:

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27 109 ❖ To determine the effect of vitamin D or zinc supplementation on duration of hospital stay, all-cause mortality,
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29 110 necessity for assisted ventilation, and individual symptoms duration
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32 111 ❖ To examine the effect of vitamin D or zinc supplementation on key blood biomarkers, including serum vitamin
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34 112 D and zinc, and immunological and inflammatory markers

35 METHODS AND ANALYSIS

36 37 114 Trial design, population, enrolment sites, and time frame

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40 115 This is a double-blind, placebo-controlled, randomized superiority trial with 2x2 factorial design and 1:1:1:1
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42 116 allocation ratio, being conducted at two site hospitals in Mumbai and Pune, Maharashtra, India (**Figure 1**).
43
44 117 Maharashtra has the highest cumulative number of COVID-19 cases and fatalities out of all states in India.[28]
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46 118 Within the state, both Pune and Mumbai have emerged as COVID-19 hotspots.[29,30]

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48 119 The two study sites (King Edward Memorial Hospital and Research Centre, Pune, and Saifee Hospital, Mumbai)
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50 120 are established medical institutions located within the cities of Pune and Mumbai. These hospitals have been
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52 121 designated as COVID-19 dedicated hospitals by local municipal corporations, where people can avail COVID-19-
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54 122 related treatment and services. The trial is targeting a sample size of 700. The study commenced in April 2021 and
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56 123 study activities are expected to continue until July 2022. While we initially targeted only hospitalized inpatients
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58 124 at each site for the study, we broadened our target population in June 2021 to include all hospital outpatients.
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60 125 This was done in order to increase generalizability of results and maintain enrolment in light of decreasing COVID-
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62 126 19 cases.[31]

Eligibility criteria

The original inclusion criteria for this study were as follows: (1) men and women aged ≥ 18 years, (2) RT-PCR-confirmed infection with SARS-COV-2, (3) oxygen saturation level (SpO₂) ≥ 90 , and (4) written informed consent.

The exclusion criteria were as following: (1) pregnant women, (2) individuals enrolled in other clinical trials, (3) daily use of multivitamins for the past 1 month.

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.

Study procedures

An overview of trial procedures is summarised in **Figure 2**.

Recruitment and obtaining informed consent

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. These dedicated site hospital staff members determine their interest and eligibility, and provide a brief introduction including key details about the study and what participation involves. The staff members read out the participant information sheet in the appropriate conversational language (English, Hindi or Marathi), and discuss the trial components and the role of the participant in the study. Information provided includes a clear outline of potential benefits and harms, the length of the follow up period, remuneration that can be expected, future use of information and samples, and resources available to the participant such as access to study clinics. Informed consent is obtained after responding to any raised queries. As part of the process, potential participants are informed that their participation is completely voluntary and they can withdraw any time at any stage of the study without providing any reasons. The informed consent process is completed once participants provide their signature on two copies of the consent document; one copy for the trial record and another provided to participants for their reference.

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3 155 Information regarding eligibility of potential participants is collected on a secure electronic tablet using Open Data
4 156 Kit (ODK),[32] with questionnaires including built-in checks and data uploaded to a secure server. No identifiable
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6 157 data are collected until the participant has provided informed consent.
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8 9 158 Baseline data and sample collection

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12 159 Following informed consent, participants undergo baseline data and sample collection, including recording of key
13 160 background and clinical information as follows:

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16 161 ➤ Screening and background: the initial screening form is extended to collect information including
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18 162 participants' demographic background, socio-economic status, and health and prevention behaviours
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20 163 (smoking and drinking), and COVID-19 vaccination status
- 21 164 ➤ Baseline dietary information: a food frequency questionnaire (FFQ) is administered, collecting
22
23 165 information on dietary practices and habits in relation to 25 food groups. The FFQ is validated for use in
24
25 166 India and has been adapted to the Maharashtra context
- 26 167 ➤ Clinical baseline: clinical and physical measures are collected alongside information on COVID-19
27
28 168 symptoms, vital signs, blood investigations, medical conditions, treatment and medications including
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30 169 those prescribed for COVID-19, nutritional supplement use, complications, and medical history

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32 170 A blood sample is also collected at baseline. All information is collected securely on electronic tablets, as described
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34 171 above.

35 36 37 172 Randomization and blinding

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40 173 Participants are assigned randomly to one of four groups: (1) vitamin D, (2) zinc, (3) vitamin D and zinc, or (4)
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42 174 placebo. For randomization, a computer-generated list was prepared by the study statistician, according to a
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44 175 randomization sequence in blocks of 20 and stratified by follow up clinic. The randomization list assigns each
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46 176 participant randomization identifier (ID) to a regimen code, with the actual regimen known only to the
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48 177 manufacturer and accessible to the statistician in a currently unopened, sealed envelope. Supplement bottles and
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50 178 envelopes are pre-labelled with codes, and active tablets and placebo are indistinguishable, so that participants
51
52 180 and all research staff including investigators remain blinded. At each site, each participant entering the trial is
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54 181 given the next available randomization ID, and is provided their corresponding regimen based on the assigned
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56 182 regimen code.

57 58 59 182 Intervention

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61 183 Patients are randomized to one of four groups:

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3 184 1. Placebo-Placebo group will receive a *placebo* vitamin D3 bolus at the hospital followed by *placebo*
4 185 daily vitamin D3 maintenance doses and *placebo* daily zinc supplements
- 5
6 186 2. Vitamin D-Placebo group will receive an *actual* vitamin D3 bolus (180,000 IU) at the hospital followed
7 187 by *actual* daily vitamin D3 maintenance doses (2000 IU daily) and daily *placebo* zinc supplements
- 8
9 188 3. Placebo-Zinc group will receive a *placebo* vitamin D3 bolus at the hospital followed by *placebo* daily
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11 189 vitamin D3 maintenance doses and *actual* daily zinc supplements (40 mg daily)
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13 190 4. Vitamin D3-Zinc group will receive an *actual* vitamin D3 bolus (180,000 IU) at the hospital followed by
14 191 *actual* daily vitamin D3 maintenance doses (2000 IU daily) and *actual* daily zinc supplements (40 mg
15
16 192 daily)

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19 193 We selected vitamin D3 as it has been shown to be more effective in raising and maintaining high levels of
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21 194 circulating 25(OH)D than vitamin D2 [33,34]. A bolus dose followed by daily doses was chosen to boost vitamin D
22 195 levels quickly and safely within the first few days and maintain levels thereafter. Previous studies have indicated
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24 196 the efficacy of large oral doses (>200 000 IU bolus, and 1,700-2,000 IU per day) in increasing and sustaining blood
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26 197 25(OH)D concentrations, with very low risk of side effects [35–41]. The 40 mg dosage of zinc is understood to be
27 198 sufficiently high to assess efficacy, while remaining within the Institute of Medicine’s tolerable upper intake level
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29 199 for adults [42]. A placebo was chosen as the comparator group given that there is currently no widespread
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31 200 consensus on the use of any nutritional supplement as part of standard or routine treatment for COVID-19.[17]

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33 201 Participants receive a pre-labelled daily supplement bottle with 60 tablets, and an envelope which contains three
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35 202 vitamin D3/placebo bolus tablets to be consumed at baseline under supervision of site hospital staff. Following
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37 203 the bolus dose, participants are instructed to take supplements daily for 8 weeks. Participants are observed taking
38 204 supplements daily while in hospital or contacted regularly via telephone after leaving the hospital to ensure
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40 205 compliance. Research staff identify barriers to compliance and aim to address these via appropriate counselling,
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42 206 and assess compliance at 8 weeks via direct questioning and pill count.

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44 207 Supplement and placebo tablets were manufactured by Excellamed Laboratories Private Limited (Mumbai, India)
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46 208 with an external quality check done by an independent service provider (Bee Pharmo Labs Private Limited,
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48 209 Mumbai, India).

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51 210 All participants are provided with care and treatment consistent with Indian national guidelines, and are
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53 211 encouraged to visit the study clinics seven days a week for medical attention if they feel unwell. Indian national
54 212 guidelines have evolved during the pandemic, and currently consist of appropriate treatment (which may include
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56 213 oxygen support, respiratory support, anti-inflammatory or immunomodulatory therapy, and anticoagulation
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58 214 therapy) according to disease severity; discharge of admitted patients from the hospital upon resolution of
59 215 symptoms and sufficient oxygen saturation (SpO₂ > 93%) for three days; and self-monitoring during home isolation
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216 [43–45].

1
2 217 Study outcomes and follow up
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5 218 Following baseline assessment and provision of supplements, participants are regularly followed up as described
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7 219 below and in **Table 1**:

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10 220 ➤ Daily hospital follow up: Daily assessment of COVID-19 symptoms, vital signs, complications, medical
11 conditions and study supplement compliance is recorded for hospitalised participants. Any new
12 prescribed medications and supplements are also recorded alongside other interventions such as need
13 for non-invasive ventilation or dialysis. Symptoms are specifically asked to participants; other measures
14 are asked, observed, assessed, or abstracted from the participants' records. Clinical measurements are
15 recorded in study-specific visits that are conducted independently after ward rounds, to minimize
16 interference in care and ensure all relevant information for the day is noted.
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18 221 ➤ Telephone follow up: Assessment of COVID-19 symptoms, supplement compliance and adverse events is
19 conducted in a follow up call every three days after leaving the hospital for all participants. All information
20 is self-reported by participants.
21
22 222 ➤ 8-week clinical assessment: After completion of study supplements at 8 weeks, information is gathered
23 on results of a clinical and physical examination, COVID-19 symptoms, compliance with regimen (including
24 direct questioning and pill count), vital signs, blood investigations (from a collected blood sample),
25 medical conditions, treatment and medications, use of any other nutritional supplements, updates to
26 COVID-19 vaccination status, complications, and history. This assessment is conducted in person at the
27 hospital, or at a location convenient to the participant where privacy can be ensured (including an option
28 to collect some information via telephone if an in-person visit is not possible). Symptoms are specifically
29 asked to participants; other measures are asked, observed, assessed, or abstracted from the participants'
30 records.
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32 223 ➤ 12-week telephone follow up: A final assessment is conducted of long-term COVID-19 symptoms, and any
33 updates to COVID-19 vaccination status. All information is self-reported by participants.
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46 241 All data are collected using standardized questionnaire forms on electronic tablets,[32] as described above.
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49 242 The primary outcome of the study is time to resolution of all of the following symptoms: (1) fever, (2) cough and
50 (3) shortness of breath. These symptoms are most commonly reported among COVID-19 patients, including in
51 Indian populations,[46,47] and have also been assessed as part of studies examining vitamin D and zinc in
52 respiratory illnesses [17]. These and additional symptoms (including fatigue, headache, loss of smell and taste and
53 sore throat) are captured on multiple time points, including baseline, daily hospital follow ups for admitted patients,
54 telephone follow ups every three days after leaving the hospital until 8 weeks post-enrolment, the 8-week clinical
55 assessment, and finally at a 12-week assessment call. Data on symptoms are collected using the same structured
56 questions at each time point: (1) whether the participant has experienced X symptom today, and if so, (2) how
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3 250 many days in total including today the participant has experienced X symptom. Staff conducting in-person and
4 251 telephone follow ups are trained uniformly using a standardised telephone script with regards to collecting this
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6 252 information. Metrics of individual symptoms and combination of symptoms are used to identify the time point of
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8 253 resolution symptoms from baseline.

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10 254 Secondary outcomes include duration of hospital stay, need for assisted ventilation, individual symptoms
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12 255 duration, all-cause mortality, and blood biomarker levels, including 25-hydroxy vitamin D, zinc, and other
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14 256 immunological and inflammatory biomarkers (including interleukin 6, angiotensin-2, soluble triggering receptor
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16 257 expressed on myeloid cells-1, immunoglobulin G and immunoglobulin M). Biomarker levels are assessed using
17 258 blood samples collected at baseline and at the 8-week clinical assessment. Occurrence of any other secondary
18
19 259 endpoints between baseline to 8-week clinical assessment is recorded during follow up calls or visits as described
20
21 260 above. A list of collected data and blood investigations with time points at baseline, during follow up visits or calls,
22 261 and at 8 and 12 weeks is summarized in **Table 1**.

Table 1. Collection of data points in the trial.

Data category	Baseline (enrolment)	Follow up	8 weeks	12 weeks
Demographic and background information	Age, gender, education, marital status, occupation, socio-economic status, health and prevention behaviours, COVID-19 vaccination <i>(Self-reported by participant, assessed by staff or abstracted from participant record)</i>		COVID-19 vaccination <i>(Self-reported by participant)</i>	COVID-19 vaccination <i>(Self-reported by participant)</i>
Dietary information	Food frequency questionnaire: consumption frequency of 25 diverse food groups in last three months <i>(Self-reported by participant)</i>			
Clinical examination	Medical history, comorbidities, preadmission medications, non-intervention nutritional supplement use <i>(Self-reported by participant, assessed by staff or abstracted from participant record)</i> Clinical symptoms ¹ <i>(Self-reported by participant)</i>	Hospital and telephone follow up: Clinical symptoms ¹ <i>(Self-reported by participant)</i> Hospital follow up only: Changes in medications, changes in non-intervention nutritional supplement use <i>(Assessed by staff or abstracted from participant record)</i>	Medical history, comorbidities, pre-assessment medications, non-intervention nutritional supplement use <i>(Self-reported by participant, assessed by staff or abstracted from participant record)</i> Clinical symptoms ¹ <i>(Self-reported by participant)</i>	Clinical symptoms ¹ <i>(Self-reported by participant)</i>
Clinical measurements	Respiratory rate, pulse, auxiliary temperature, SpO ₂ , systolic and diastolic blood pressure, weight and height <i>(Assessed by staff or abstracted from participant record)</i>	Hospital follow up only: Respiratory rate, pulse, auxiliary temperature, SpO ₂ , systolic and diastolic blood pressure, weight, requirement for non-invasive ventilation or intubation/ventilator support, need for dialysis, lab investigations <i>(Assessed by staff or abstracted from participant record)</i>	Respiratory rate, pulse, auxiliary temperature, SpO ₂ , systolic and diastolic blood pressure, weight and height <i>(Assessed by staff or abstracted from participant record)</i>	
Blood and other investigations and biomarkers	SARS-COV-2 RT-PCR, chest X-Ray, complete blood count, blood glucose, serum creatinine, CRP, LDH, serum ferritin, D-dimer, vitamin D, zinc, calcium, IgG, IgM, Ang2, IL-6 and sTREM-1		CRP, LDH, serum ferritin, D-dimer, vitamin D, zinc, calcium, IgG, IgM, Ang2, IL-6 and sTREM-1 <i>(Assessed by laboratory or abstracted from participant record)</i>	

(Assessed by laboratory or abstracted from participant record)

Other information

Hospital and telephone follow up:

Compliance, adverse events
(Self-reported by participant, assessed by staff or abstracted from participant record)

Compliance (count of remaining pills)
(Assessed by staff)

SpO2: Oxygen saturation, CRP: C-reactive protein, LDH: lactate dehydrogenase, IgG: Immunoglobulin G, IgM: immunoglobulin M, Ang2: angiotensin II, IL-6: interleukin 6, sTREM-1: soluble triggering receptor expressed on myeloid cells-1.
¹Clinical symptoms include: fever, cough, shortness of breath, fatigue, headache, loss of smell, loss of taste, diarrhea, anorexia, sore throat, nasal congestion, nausea and vomiting, and any other reported by the participant.

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2 Adverse events and reporting

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4
5 3 Any undesirable circumstance or experiences reported by study participants during the study are categorised as
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7 4 adverse events. All adverse events which are possibly, probably or very likely related to administration of any
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9 5 supplement are monitored and reported to site institutional review boards (IRBs) within 72 hours (serious adverse
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11 6 events) or 1 month (all other adverse events), using a standardized reporting format. The trial data and safety
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13 7 monitoring board (DSMB) is also notified. Site principal investigators and independent physicians are responsible
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15 8 for assessing the causal relationship and making the conclusive decision about continuation of the trial for a
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17 9 particular participant. Additionally, medical insurance is provided to all study participants to take care of any
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19 10 progression of severe adverse events.

20 11 Data and sample management

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23 12 All data collected as part of this trial are entered into password-protected android electronic tablets, with pre-
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25 13 programmed questionnaires using ODK.[32] All data are automatically and directly uploaded from the tablets onto
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27 14 a secure electronic server, and entered into a password-protected database accessible only to authorised study
28
29 15 team members. Data are stored in linked-anonymised form, with identifiable information and the linking key
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31 16 stored separately. All analyses and data checks are conducted on anonymised data only.

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33 17 Blood samples collected as part of this trial are processed at the Foundation for Medical Research, Mumbai, and
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35 18 accredited laboratories in India including at the site hospitals. Specimens are linked-anonymised and are stored
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37 19 securely at the Foundation for Medical Research for a maximum of three years.

38 20 Data analysis

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41 21 Planned analyses will initially be undertaken in blinded fashion (comparing coded treatment groups); unblinding of
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43 22 investigators and research staff with respect to treatment allocation will only occur once analyses are completed.

46 23 Planned analyses

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49 24 An intent-to-treat analysis will be used as the primary analytic strategy. Time to primary outcome will be compared
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51 25 between participants randomized to vitamin D vs. no vitamin D and zinc vs. no zinc using Cox regression. We will
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53 26 investigate effect modification of either treatment effect by the other, and by third variables collected at baseline
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55 27 (including, anthropometric status, and vitamin D status). Effect modification will be assessed by including
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57 28 interaction terms in Cox regression models, and statistical significance assessed via likelihood ratio tests. There are
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59 29 no *a priori* effect modifiers hypothesized, and unless there is strong modification of a treatment effect, our power
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31 to detect these may be low. We will assess the success of randomization by comparing baseline variables by
treatment group using χ^2 and t-tests and use multivariate modelling to adjust for imbalances if needed. 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.

The effect of vitamin D or zinc on dichotomous secondary outcomes will be analyzed in a similar approach. The proportion of individuals experiencing hypercalcemia will be compared between treatment groups using χ^2 tests, and effects of the supplements on blood biomarkers will be compared via Wilcoxon and t-tests. This study will measure numerous risk factors for COVID-19 progression and severe treatment outcomes including hemoglobin; co-morbidities; medications including chloroquine, hydroxychloroquine, and ACE inhibitors; and socio-demographic, clinical, nutritional, and lifestyle-related risk factors. We will examine relationships of these factors in the placebo group first, to avoid complex questions concerning interactions between risk factors and treatments. Once we find a satisfactory parsimonious model using principles of model selection as detailed by Greenland,[48] we will test and modify it if needed in the whole study population, adjusting for treatment effects.

Analyses will consider sex and gender throughout, by disaggregating findings, and attempting to elucidate the roles of sex and gender in the clinical course and immune response by controlling for potential sociodemographic, nutritional, and immunological confounders.

Statistical power calculations

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] Power will decrease if each treatment has a moderate effect; we accounted for this in calculating the sample size. Assumptions related to treatment effects may be reasonably inferred from meta-analyses of well-designed randomized controlled trials studying these supplements in other acute respiratory illnesses.[17,21–24,50] 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] 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,[52] 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.

Table 2. Statistical power estimation.

Effect of Treatment A	True effect of Treatment B						
	0%	5%	10%	15%	20%	25%	30%
30%	99%	99%	99%	99%	98%	98%	97%
25%	95%	94%	93%	92%	90%	88%	86%
20%	81%	79%	76%	74%	71%	69%	66%

Patient and public involvement

Patients and the public were not involved in the design of this study.

DATA AND SAFETY MONITORING BOARD

The Data and Safety Monitoring Board (DSMB) was established prior to commencement of the trial. It consists of independent experts in respiratory infection and communicable diseases, public health and nutrition, clinical research, and biostatistics. The role of the board is to provide their inputs, recommendations, review the trial protocols and progress by ensuring the rights and safety of involving participants in the study through periodic trial review meetings.

The trial DSMB will examine efficacy endpoints by study arms when half of individuals are enrolled. In accordance with the Haybittle-Peto rule, if the difference in the primary outcome between study arms is <0.001 , unblinding of the DSMB and stopping will be considered.[53]

ETHICS AND DISSEMINATION

This study is being conducted in the accordance with the Declaration of Helsinki 2013. The study was approved by the Institutional Review Board of the Harvard T.H. Chan School of Public Health (Protocol No. IRB20-1425), the University Health Network Research Ethics Board (20-5775), the Institutional Research Ethics Committee of the Foundation for Medical Research (IREC No. FMR/IREC/C19/02/2020), the Institutional Review Board of Saifee Hospital (Project No. EC/008/2020) and the KEM Hospital Research Centre Ethics Committee (KEMHRC ID No. 2027). The trial is registered on ClinicalTrials.gov (NCT04641195). Permission for the study was also obtained from the Health Management and Screening Comments (HMSC), Government of India (HMSC (GOI)-2021-0060), and the study was registered prospectively in the Clinical Trials Registry India (CTRI/2021/04/032593). Since the study intervention is related to micronutrient supplementation, endorsement from the Drugs Controller General of India was non-obligatory. The study findings will be presented in peer-reviewed medical journals.

DISCUSSION

With continued high incidence of global cases, COVID-19 remains a global health challenge. Alongside vaccination and other preventative measures, low-cost and efficient interventions which may help minimize the occurrence of serious disease are needed. These would be particularly valuable in low- and middle-income countries, where health systems are more overburdened and resources much fewer. In this context, and given previous evidence regarding the role of vitamin D and zinc in the development of and recovery from respiratory infections,[17,21–24,50] there is a need to explore their potential value as part of therapeutic regimens for COVID-19.

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. The frequent follow up of participants and collection of a range of sociodemographic, clinical and biomarker measures alongside blood samples will enable a detailed investigation of the effect of supplementation on disease progression, including potentially important immunological and inflammatory pathways. Importantly, in comparison with other vitamin D or zinc COVID-19 intervention studies currently registered on ClinicalTrials.gov, this would be the first conducted outside of the U.S. or Europe and other similar high-income countries. The location of this study in two large cities, alongside the broad eligibility criteria, increases the generalizability of study results. Given the current unpredictability of COVID-19 waves, one challenge to the study is to maintain recruitment during periods where cases may be on the decline. 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. Regardless, the findings of this study will have direct relevance to many settings in South Asia and sub-Saharan Africa with weak health systems and prevalent malnutrition. Ultimately, the evidence generated as part of this trial will enhance our understanding of the role of vitamin D and zinc in COVID-19 disease, and contribute high quality evidence on the potential value of supplementation of these micronutrients for the same.

FUNDING

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). The funding bodies had no role in study design and procedures, or the decision to submit manuscripts for publication.

AUTHOR CONTRIBUTIONS

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3 116 WWF, KCK, YD, and NM conceptualised the project, and designed the study along with SB, KKS, ECH, YM and UP.
4 117 YD, NM, PDC, GG, KKS, YM, and SS are involved in data acquisition, and in study monitoring along with KCK, WWF
5
6 118 and UP. MW provides statistical expertise. KKS and UP drafted the manuscript, and all authors reviewed and
7
8 119 critically revised the draft and approved the final manuscript.
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10 120 **COMPETING INTERESTS**

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13 121 All authors declare no conflicts of interest.
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16 122 **ACKNOWLEDGEMENTS**

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21 124 contribution in the trial implementation. We also thank all members of the DSMB and respective IRBs for their
22
23 125 guidance and valuable inputs in the trial.
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REFERENCES

- 1 Dong E, Du H, Gardner L. An interactive web-based dashboard to track COVID-19 in real time. *The Lancet Infectious Diseases* 2020;**20**:533–4. doi:10.1016/S1473-3099(20)30120-1
- 2 Hogan A, Winksill P, Watson O, *et al.* Modelling the allocation and impact of a COVID-19 vaccine. Published Online First: 25 September 2020. doi:https://doi.org/10.25561/82822
- 3 Phillips N. The coronavirus is here to stay — here’s what that means. *Nature* 2021;**590**:382–4. doi:10.1038/d41586-021-00396-2
- 4 Torjesen I. Covid-19 will become endemic but with decreased potency over time, scientists believe. *BMJ* 2021;**372**:n494. doi:10.1136/bmj.n494
- 5 Hockham C, Kotwal S, Wilcox A, *et al.* Protocol for the Controlled evaluation of Angiotensin Receptor blockers for COVID-19 respiratory disease (CLARITY): a randomised controlled trial. *Trials* 2021;**22**:573. doi:10.1186/s13063-021-05521-0
- 6 Teshome A, Adane A, Girma B, *et al.* The Impact of Vitamin D Level on COVID-19 Infection: Systematic Review and Meta-Analysis. *Front Public Health* 2021;**9**:624559. doi:10.3389/fpubh.2021.624559
- 7 Bouillon R, Marcocci C, Carmeliet G, *et al.* Skeletal and Extraskeletal Actions of Vitamin D: Current Evidence and Outstanding Questions. *Endocr Rev* 2019;**40**:1109–51. doi:10.1210/er.2018-00126
- 8 Pearce SH, Cheetham TD. Diagnosis and management of vitamin D deficiency. *BMJ* 2010;**340**:b5664. doi:10.1136/bmj.b5664
- 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
- 11 Greiller CL, Martineau AR. Modulation of the immune response to respiratory viruses by vitamin D. *Nutrients* 2015;**7**:4240–70. doi:10.3390/nu7064240
- 12 Greiller CL, Suri R, Jolliffe DA, *et al.* Vitamin D attenuates rhinovirus-induced expression of intercellular adhesion molecule-1 (ICAM-1) and platelet-activating factor receptor (PAFR) in respiratory epithelial cells. *J Steroid Biochem Mol Biol* 2019;**187**:152–9. doi:10.1016/j.jsbmb.2018.11.013
- 13 Telcian AG, Zdrenghea MT, Edwards MR, *et al.* Vitamin D increases the antiviral activity of bronchial epithelial cells in vitro. *Antiviral Res* 2017;**137**:93–101. doi:10.1016/j.antiviral.2016.11.004
- 14 Hansdottir S, Monick MM, Lovan N, *et al.* Vitamin D decreases respiratory syncytial virus induction of NF-kappaB-linked chemokines and cytokines in airway epithelium while maintaining the antiviral state. *J Immunol* 2010;**184**:965–74. doi:10.4049/jimmunol.0902840
- 15 Hansdottir S, Monick MM, Hinde SL, *et al.* Respiratory epithelial cells convert inactive vitamin D to its active form: potential effects on host defense. *J Immunol* 2008;**181**:7090–9. doi:10.4049/jimmunol.181.10.7090

- 1
2 165 16 Zhou J, Du J, Huang L, *et al.* Preventive Effects of Vitamin D on Seasonal Influenza A in Infants: A
3 166 Multicenter, Randomized, Open, Controlled Clinical Trial. *The Pediatric Infectious Disease Journal*
4 167 2018;**37**:749–54. doi:10.1097/INF.0000000000001890
5
6
7 168 17 Abioye AI, Bromage S, Fawzi W. Effect of micronutrient supplements on influenza and other respiratory
8 169 tract infections among adults: a systematic review and meta-analysis. *BMJ Glob Health* 2021;**6**:e003176.
9 170 doi:10.1136/bmjgh-2020-003176
10
11 171 18 International Zinc Nutrition Consultative Group (IZiNCG), Brown KH, Rivera JA, *et al.* International Zinc
12 172 Nutrition Consultative Group (IZiNCG) technical document #1. Assessment of the risk of zinc deficiency in
13 173 populations and options for its control. *Food Nutr Bull* 2004;**25**:S99-203.
14
15 174 19 Smith MR, DeFries R, Chhatre A, *et al.* Inadequate Zinc Intake in India: Past, Present, and Future. *Food Nutr*
16 175 *Bull* 2019;**40**:26–40. doi:10.1177/0379572118825176
17
18 176 20 GBD Compare. Institute for Health Metrics and Evaluation. 2014. [https://www.healthdata.org/data-](https://www.healthdata.org/data-visualization/gbd-compare)
19 177 [visualization/gbd-compare](https://www.healthdata.org/data-visualization/gbd-compare) (accessed 17 Dec 2021).
20
21 178 21 Hemilä H, Fitzgerald JT, Petrus EJ, *et al.* Zinc Acetate Lozenges May Improve the Recovery Rate of Common
22 179 Cold Patients: An Individual Patient Data Meta-Analysis. *Open Forum Infect Dis* 2017;**4**:ofx059.
23 180 doi:10.1093/ofid/ofx059
24
25 181 22 Roth DE, Richard SA, Black RE. Zinc supplementation for the prevention of acute lower respiratory infection
26 182 in children in developing countries: meta-analysis and meta-regression of randomized trials. *Int J Epidemiol*
27 183 2010;**39**:795–808. doi:10.1093/ije/dyp391
28
29 184 23 Bhatnagar S, Wadhwa N, Aneja S, *et al.* Zinc as adjunct treatment in infants aged between 7 and 120 days
30 185 with probable serious bacterial infection: a randomised, double-blind, placebo-controlled trial. *Lancet*
31 186 2012;**379**:2072–8. doi:10.1016/S0140-6736(12)60477-2
32
33 187 24 Banupriya N, Bhat BV, Benet BD, *et al.* Short Term Oral Zinc Supplementation among Babies with Neonatal
34 188 Sepsis for Reducing Mortality and Improving Outcome - A Double-Blind Randomized Controlled Trial. *Indian*
35 189 *J Pediatr* 2018;**85**:5–9. doi:10.1007/s12098-017-2444-8
36
37 190 25 Zhang L, Liu Y. Potential interventions for novel coronavirus in China: A systematic review. *J Med Virol*
38 191 2020;**92**:479–90. doi:10.1002/jmv.25707
39
40 192 26 Skalny AV, Rink L, Ajsuvakova OP, *et al.* Zinc and respiratory tract infections: Perspectives for COVID-19
41 193 (Review). *Int J Mol Med* 2020;**46**:17–26. doi:10.3892/ijmm.2020.4575
42
43 194 27 te Velthuis AJW, van den Worm SHE, Sims AC, *et al.* Zn(2+) inhibits coronavirus and arterivirus RNA
44 195 polymerase activity in vitro and zinc ionophores block the replication of these viruses in cell culture. *PLoS*
45 196 *Pathog* 2010;**6**:e1001176. doi:10.1371/journal.ppat.1001176
46
47 197 28 COVID19 STATEWISE STATUS. MyGov.in. 2020. <https://mygov.in/corona-data/covid19-statewise-status/>
48 198 (accessed 17 Dec 2021).
49
50 199 29 Tambe MP, Parande MA, Tapare VS, *et al.* An epidemiological study of laboratory confirmed COVID-19 cases
51 200 admitted in a tertiary care hospital of Pune, Maharashtra. *Indian J Public Health* 2020;**64**:S183–7.
52 201 doi:10.4103/ijph.IJPH_522_20
53
54 202 30 Kodge BG. A review on current status of COVID19 cases in Maharashtra state of India using GIS: a case
55 203 study. *Spatial Information Research* 2020;**1**–7. doi:10.1007/s41324-020-00349-3
56
57
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59
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2 204 31 India: WHO Coronavirus Disease (COVID-19) Dashboard With Vaccination Data. <https://covid19.who.int>
3 (accessed 17 Dec 2021).
4 205
- 5
6 206 32 ODK - Collect data anywhere. <https://getodk.org> (accessed 17 Dec 2021).
7
- 8 207 33 Trang HM, Cole DE, Rubin LA, *et al.* Evidence that vitamin D3 increases serum 25-hydroxyvitamin D more
9 208 efficiently than does vitamin D2. *Am J Clin Nutr* 1998;**68**:854–8. doi:10.1093/ajcn/68.4.854
10
- 11 209 34 Armas LAG, Hollis BW, Heaney RP. Vitamin D2 is much less effective than vitamin D3 in humans. *J Clin*
12 210 *Endocrinol Metab* 2004;**89**:5387–91. doi:10.1210/jc.2004-0360
13
- 14 211 35 Kearns MD, Binongo JNG, Watson D, *et al.* The effect of a single, large bolus of vitamin D in healthy adults
15 212 over the winter and following year: a randomized, double-blind, placebo-controlled trial. *Eur J Clin Nutr*
16 213 2015;**69**:193–7. doi:10.1038/ejcn.2014.209
- 17
18
19 214 36 Kearns MD, Alvarez JA, Tangpricha V. Large, single-dose, oral vitamin D supplementation in adult
20 215 populations: a systematic review. *Endocr Pract* 2014;**20**:341–51. doi:10.4158/EP13265.RA
- 21
22 216 37 Vieth R, Bischoff-Ferrari H, Boucher BJ, *et al.* The urgent need to recommend an intake of vitamin D that is
23 217 effective. *Am J Clin Nutr* 2007;**85**:649–50. doi:10.1093/ajcn/85.3.649
24
- 25 218 38 Dong Y, Stallmann-Jorgensen IS, Pollock NK, *et al.* A 16-week randomized clinical trial of 2000 international
26 219 units daily vitamin D3 supplementation in black youth: 25-hydroxyvitamin D, adiposity, and arterial
27 220 stiffness. *J Clin Endocrinol Metab* 2010;**95**:4584–91. doi:10.1210/jc.2010-0606
28
- 29
30 221 39 Schleithoff SS, Zittermann A, Tenderich G, *et al.* Vitamin D supplementation improves cytokine profiles in
31 222 patients with congestive heart failure: a double-blind, randomized, placebo-controlled trial. *Am J Clin Nutr*
32 223 2006;**83**:754–9. doi:10.1093/ajcn/83.4.754
- 33
34 224 40 Pappa HM, Mitchell PD, Jiang H, *et al.* Treatment of vitamin D insufficiency in children and adolescents with
35 225 inflammatory bowel disease: a randomized clinical trial comparing three regimens. *J Clin Endocrinol Metab*
36 226 2012;**97**:2134–42. doi:10.1210/jc.2011-3182
37
- 38 227 41 Abu-Mouch S, Fireman Z, Jarchofsky J, *et al.* Vitamin D supplementation improves sustained virologic
39 228 response in chronic hepatitis C (genotype 1)-naïve patients. *World J Gastroenterol* 2011;**17**:5184–90.
40 229 doi:10.3748/wjg.v17.i47.5184
41
- 42
43 230 42 Institute of Medicine (US) Panel on Micronutrients. *Dietary Reference Intakes for Vitamin A, Vitamin K,*
44 231 *Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and*
45 232 *Zinc.* Washington (DC): : National Academies Press (US) 2001.
46 233 <http://www.ncbi.nlm.nih.gov/books/NBK222310/> (accessed 2 Mar 2022).
47
- 48 234 43 AIIMS/ICMR National Task Force/Joint Monitoring Group. Clinical Guidelines for Management of Adult
49 235 COVID-19 Patients: Revised on 14/01/2022. New Delhi: : Ministry of Health and Family Welfare,
50 236 Government of India
51 237 <https://www.mohfw.gov.in/pdf/ClinicalGuidanceforManagementofAdultCovid19Patientsupdatedason17thjanuary2022.pdf> (accessed 28 Feb 2022).
52 238
53
- 54
55 239 44 MOHFW, GOI. Clinical Management Protocol for COVID-19 (In Adults) - Version 6 (24.05.21). New Delhi: :
56 240 Ministry of Health and Family Welfare, Government of India 2021.
57 241 <https://www.mohfw.gov.in/pdf/UpdatedDetailedClinicalManagementProtocolforCOVID19adultsdated24052021.pdf> (accessed 28 Feb 2022).
58 242
59
60

- 1
2
3 243 45 MOHFW, GOI. Revised Discharge Policy for COVID-19: Updated on 9th January 2022. New Delhi: : Ministry
4 244 of Health and Family Welfare, Government of India
5 245 <https://www.mohfw.gov.in/pdf/RevisedDischargePolicyforCOVID19updatedon9thJanuary2022.pdf>
6 246 (accessed 28 Feb 2022).
7
8 247 46 Kumar N, Hameed SKS, Babu GR, *et al.* Descriptive epidemiology of SARS-CoV-2 infection in Karnataka state,
9 248 South India: Transmission dynamics of symptomatic vs. asymptomatic infections. *eClinicalMedicine* 2021;**32**.
10 249 doi:10.1016/j.eclinm.2020.100717
11
12 250 47 Laxminarayan R, B CM, G VT, *et al.* SARS-CoV-2 infection and mortality during the first epidemic wave in
13 251 Madurai, south India: a prospective, active surveillance study. *The Lancet Infectious Diseases* 2021;**21**:1665–
14 252 76. doi:10.1016/S1473-3099(21)00393-5
15
16 253 48 Greenland S. Modeling and variable selection in epidemiologic analysis. *Am J Public Health* 1989;**79**:340–9.
17 254 doi:10.2105/ajph.79.3.340
18
19 255 49 Ellenberg SS, Finkelstein DM, Schoenfeld DA. Statistical Issues Arising in AIDS Clinical Trials. *Journal of the*
20 256 *American Statistical Association* 1992;**87**:562–9. doi:10.1080/01621459.1992.10475240
21
22 257 50 Martineau AR, Jolliffe DA, Hooper RL, *et al.* Vitamin D supplementation to prevent acute respiratory tract
23 258 infections: systematic review and meta-analysis of individual participant data. *BMJ* 2017;**356**:i6583.
24 259 doi:10.1136/bmj.i6583
25
26 260 51 Schoenfeld DA, Richter JR. Nomograms for calculating the number of patients needed for a clinical trial with
27 261 survival as an endpoint. *Biometrics* 1982;**38**:163–70.
28
29 262 52 Dorigatti I, Okell L, Cori A, *et al.* Report 4: Severity of 2019-novel coronavirus (nCoV). Imperial College
30 263 London 2020. doi:10.25561/77154
31
32 264 53 Blenkinsop A, Parmar MK, Choodari-Oskooei B. Assessing the impact of efficacy stopping rules on the error
33 265 rates under the multi-arm multi-stage framework. *Clin Trials* 2019;**16**:132–41.
34 266 doi:10.1177/1740774518823551
35
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5 269 **FIGURE LEGENDS**
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8 270 **Figure 1:** Map of India (grey) with Maharashtra highlighted in red, and Mumbai (black dot) and Pune (blue dot)
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10 271 identified.

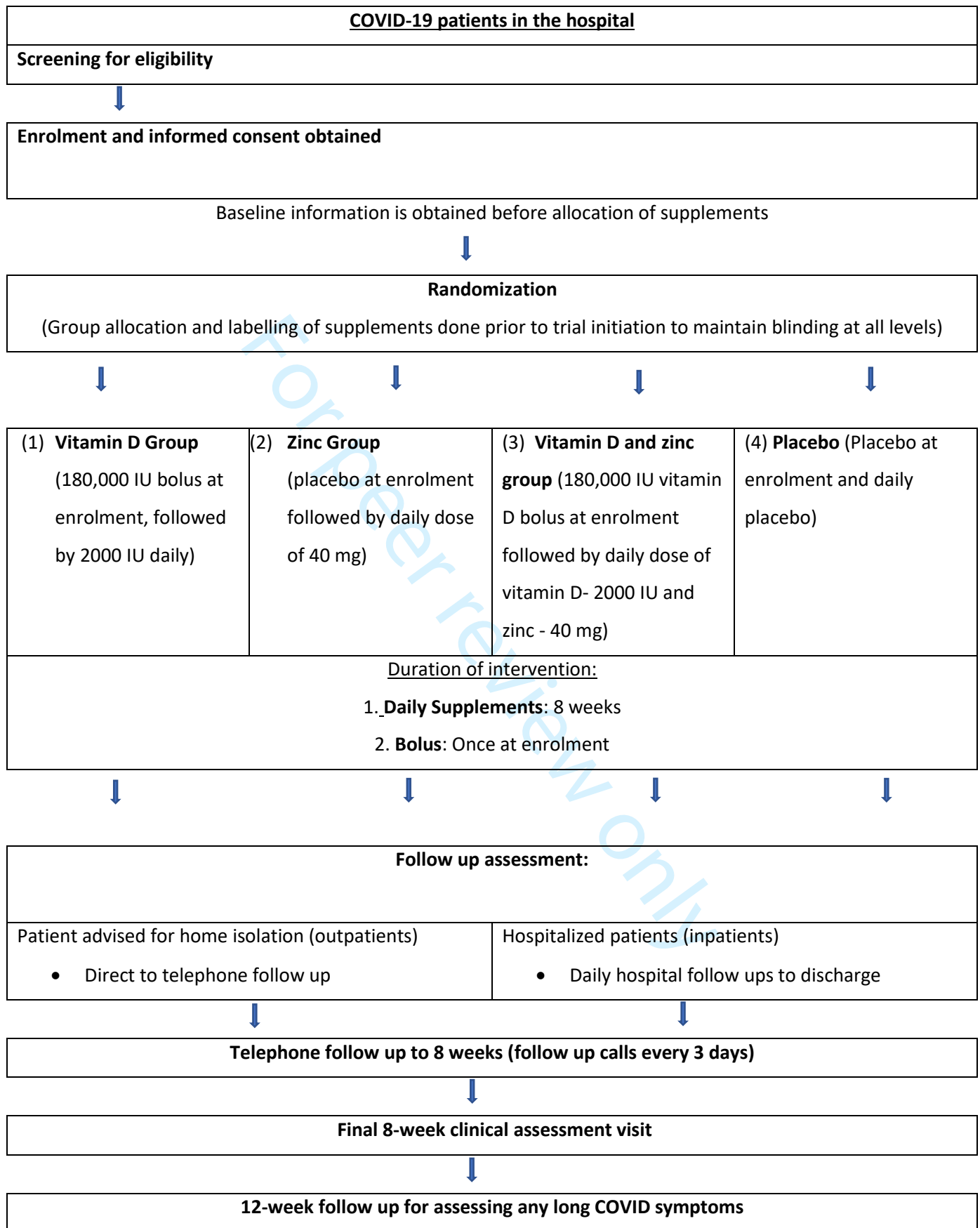
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13 272 Map created with mapchart.net.
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15 273 **Figure 2:** Overview of trial procedures. RAT: Rapid Antigen Test.
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Figure 1: Map of India (grey) with Maharashtra highlighted in red, and Mumbai (black dot) and Pune (blue dot) identified.
Map created with mapchart.net.
156x188mm (330 x 330 DPI)





SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description
Administrative information		
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym Title page (p1): “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”
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry Abstract (p2): “Trial Registration number: NCT04641195, CTRI/2021/04/032593, HMSC (GOI)-2021-0060”
	2b	All items from the World Health Organization Trial Registration Data Set Manuscript: Items from the WHO Trial Registration Data Set (including elements such as trial registration, financial support, study contacts, study title, countries of recruitment and details on design and recruitment status) are noted throughout the manuscript.
Protocol version	3	Date and version identifier NA: This is a manuscript of a study protocol.
Funding	4	Sources and types of financial, material, and other support Funding (p14): “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). The funding body has no role in study design and procedures, or the decision to submit manuscripts for publication.”
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors Author contributions (p14): “KCK, YD, WWF and NM conceptualised the project, and designed the study along with SB, KKS, ECH, YM and UP.”

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- 5b Name and contact information for the trial sponsor
The name of the study sponsor is included in the clinical trial registration records (NCT04641195, CTRI/2021/04/032593).
- 5c Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities
Funding (p14):
“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). The funding body has no role in study design and procedures, or the decision to submit manuscripts for publication.”
- 5d Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)
NA

Introduction

Background and rationale

6a

Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention

Introduction (p4-5):

“[...] Additionally, with limited proven treatment regimens for COVID-19 to date, it is essential to continue exploring low cost and commonly available effective interventions which can be implemented as standardized therapeutic treatment regimens at large.[6] This is especially important in the context of low and middle-income countries in South Asia and Africa, which are particularly vulnerable given weak health systems and the co-existence of malnutrition and other co-morbidities. This includes India, which continues to report a substantial number of COVID-19 cases.[1]

[...]

Vitamin D shows promise as a novel, cost-effective prevention and adjunctive treatment for respiratory infections. [...] In laboratory studies, vitamin D metabolites support innate immune responses to rhinoviruses and respiratory syncytial virus.[10–14] In participants with influenza, high-dose vitamin D supplementation shortened durations of fever, cough and wheezing, particularly among those with low vitamin D levels.[15] In a recent systematic review and meta-analysis of randomised controlled trials, vitamin D supplementation was associated with decreased risk of acute respiratory infections and shortened duration of symptoms.[16]

[...]

Multiple meta-analyses and pooled analyses of randomized controlled trials conducted in the US and low- and middle-income countries have shown that oral zinc supplementation reduces incidence of acute respiratory infections by 35%, shortens duration of symptoms, and improves recovery rate.[16,20–23] Zinc is a potential treatment in COVID-19, due to its immune modulatory effect, as well as direct antiviral effect.[24] The mechanisms by which zinc may serve as adjunct therapy in COVID-19 has been recently reviewed by Skalny et al. 2020,[25] who note that Zn²⁺ cations, especially in combination with zinc ionophore pyrithione inhibit SARS-coronavirus RNA polymerase activity by decreasing replication.[26]

Vitamin D and zinc are safe, inexpensive, and widely available therapies; therefore, experimental evidence that these nutrient supplements are effective against COVID-19 would readily support their inclusion in standard of care. Therefore, we are undertaking a randomized controlled trial to determine the effect of vitamin D and zinc supplementation on treatment outcomes among individuals with COVID-19 in India.”

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- 6b Explanation for choice of comparators
Methods and analysis // Study procedures // Intervention (p8):
“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.[16]”
- Objectives 7 Specific objectives or hypotheses
Objectives (p5):
“The primary objectives of this trial are:
 To determine the effect of vitamin D supplementation versus placebo on time to recovery among patients with COVID-19
 To determine the effect of zinc supplementation versus placebo on time to recovery among patients with COVID-19
Secondary objectives include:
 To determine the effect of vitamin D or zinc supplementation on duration of hospital stay, all-cause mortality, necessity for assisted ventilation, and individual symptoms duration
 To examine the effect of vitamin D or zinc supplementation on key blood biomarkers, including serum vitamin D and zinc, and immunological and inflammatory markers”
- Trial design 8 Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)
Methods and analysis // Trial design, population and enrolment sites (p5):
“This is a double-blind, placebo-controlled, randomized superiority trial with 2x2 factorial design and 1:1:1:1 allocation ratio, being conducted at two site hospitals in Mumbai and Pune, Maharashtra, India (Figure 1).”

Methods: Participants, interventions, and outcomes

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60	Study setting	9	<p>Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained</p> <p>Methods and analysis // Trial design, population and enrolment sites (p5):</p> <p>“This is a double-blind, placebo-controlled, randomized superiority trial with 2x2 factorial design and 1:1:1:1 allocation ratio, being conducted at two site hospitals in Mumbai and Pune, Maharashtra, India (Figure 1).</p> <p>[...]</p> <p>The two study sites (King Edward Memorial Hospital and Research Centre, Pune, and Saifee Hospital, Mumbai) are established medical institutions located within the cities of Pune and Mumbai. These hospitals have been designated as COVID-19 dedicated hospitals by local municipal corporations, where people can avail COVID-19-related treatment and services.”</p>
	Eligibility criteria	10	<p>Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)</p> <p>Methods and analysis // Eligibility criteria (p6):</p> <p>“The original inclusion criteria for this study were as follows: (1) men and women aged ≥ 18 years, (2) RT-PCR-confirmed infection with SARS-COV-2, (3) oxygen saturation level (SpO₂) ≥ 90, and (4) written informed consent.</p> <p>The exclusion criteria were as following: (1) pregnant women, (2) individuals enrolled in other clinical trials, (3) daily use of multivitamins for the past 1 month.</p> <p>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.</p>

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2 Interventions 11a Interventions for each group with sufficient detail to allow replication,
3 including how and when they will be administered
4 Methods and analysis // Study procedures // Intervention (p7-8):
5 Patients are randomized to one of four groups:
6
7 1. Placebo-Placebo group will receive a placebo vitamin D3 bolus
8 at the hospital followed by placebo daily vitamin D3 maintenance
9 doses and placebo daily zinc supplements
10
11 2. Vitamin D-Placebo group will receive an actual vitamin D3
12 bolus (180,000 IU) at the hospital followed by actual daily vitamin D3
13 maintenance doses (2000 IU daily) and daily placebo zinc
14 supplements
15
16 3. Placebo-Zinc group will receive a placebo vitamin D3 bolus at
17 the hospital followed by placebo daily vitamin D3 maintenance doses
18 and actual daily zinc supplements (40 mg daily)
19
20 4. Vitamin D3-Zinc group will receive an actual vitamin D3 bolus
21 (180,000 IU) at the hospital followed by actual daily vitamin D3
22 maintenance doses (2000 IU daily) and actual daily zinc supplements
23 (40 mg daily)
24
25
26 A placebo was chosen as the comparator group given that there is
27 currently no widespread consensus on the use of any nutritional
28 supplement as part of standard or routine treatment for COVID-19.[16]
29
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31 Participants receive a pre-labelled daily supplement bottle with 60
32 tablets, and an envelope which contains three vitamin D3/placebo
33 bolus tablets to be consumed at baseline under supervision of site
34 hospital staff. Following the bolus dose, participants are instructed to
35 take supplements daily for 8 weeks. Participants are contacted daily
36 while in hospital or regularly via telephone after leaving the hospital to
37 ensure compliance. Research nurses identify barriers to compliance,
38 and assess compliance at 8 weeks via direct questioning and pill
39 count.
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43 Supplement and placebo tablets were manufactured by Excellamed
44 Laboratories Private Limited (Mumbai, India) with an external quality
45 check done by an independent service provider (Bee Pharmo Labs
46 Private Limited, Mumbai, India).
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49 All participants are provided with care and treatment consistent with
50 Indian national guidelines, and are encouraged to visit the study
51 clinics seven days a week for medical attention if they feel unwell.
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- 11b Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)
Methods and analysis // Adverse events and reporting (p11):
“All adverse events which are possibly, probably or very likely related to administration of any supplement are monitored and reported to site institutional review boards (IRBs) within 72 hours (serious adverse events) or 1 month (all other adverse events), using a standardized reporting format. The trial data and safety monitoring board (DSMB) is also notified. Site principal investigators and independent physicians are responsible for assessing the causal relationship and making the conclusive decision about continuation of the trial for a particular participant.”
- 11c Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)
Methods and analysis // Study procedures // Intervention (p8):
“Participants are contacted daily while in hospital or regularly via telephone after leaving the hospital to ensure compliance. Research nurses identify barriers to compliance, and assess compliance at 8 weeks via direct questioning and pill count.”
- 11d Relevant concomitant care and interventions that are permitted or prohibited during the trial
Methods and analysis // Study procedures // Intervention (p8):
“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.”

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- Outcomes 12 Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended
- [Methods and analysis // Study procedures // 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 and additional symptoms 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. Metrics of individual symptoms and combination of symptoms are used to identify the time point of resolution symptoms from baseline.
- Secondary outcomes include duration of hospital stay, need for assisted ventilation, individual symptoms duration, all-cause mortality, occurrence of other severe adverse events and change in blood biomarker levels, including 25-hydroxy vitamin D, zinc and calcium, and other immunological and inflammatory biomarkers. Biomarker levels are assessed using blood samples collected at baseline and at the 8-week clinical assessment. Occurrence of any other secondary endpoints between baseline to 8-week clinical assessment is recorded during follow up calls or visits as described above.”
- Participant timeline 13 Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)
- [See Methods and analysis // Study procedures section \(p6\)](#) for details on enrolment, intervention, and follow up. Table 1 and Figure 2, referred to in this section, also outline the sequence and schedule of enrolment and follow up.

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2	Sample size	14	Estimated number of participants needed to achieve study objectives
3			and how it was determined, including clinical and statistical
4			assumptions supporting any sample size calculations
5			Methods and analysis // Data analysis // Statistical power calculations
6			(p12) and Table 2:
7			“Assumptions related to treatment effects may be reasonably inferred
8			from meta-analyses of well-designed randomized controlled trials
9			studying these supplements in other acute respiratory
10			illnesses.[15,16,20–23] We based power analysis on the primary
11			outcome of time from onset of disease to clinical recovery, using
12			methodology for survival times.[34] We calculated power for detecting
13			specified hazard ratios associated with vitamin D or zinc given a
14			specified true effect of the other treatment. Assuming average time to
15			recovery of 22.2 days,[35] and a low (5%) rate of loss to follow-up,
16			enrolment of 700 patients will yield the statistical power estimates in
17			the Table 2.This analysis indicates that we will have at least 80%
18			power to detect a moderate (25-30%) effect of either treatment, given
19			a maximum 30% true effect of the other treatment.”
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25	Recruitment	15	Strategies for achieving adequate participant enrolment to reach
26			target sample size
27			Methods and analysis // Trial design, population and enrolment sites
28			(p5):
29			“While we initially targeted only hospitalized inpatients at each site for
30			the study, we broadened our target population in June 2021 to include
31			all hospital outpatients. This was done in order to increase
32			generalizability of results and maintain enrolment in light of decreasing
33			COVID-19 cases.[30]”
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37			Methods and analysis // Eligibility criteria (p6):
38			“To capture the greatest possible number and range of symptomatic
39			COVID-19 cases and increase generalizability, we made the following
40			alterations to our eligibility criteria from June 2021 (within 2 months of
41			recruitment commencement): (1) added inclusion criterion of
42			individuals with Rapid Antigen Test-confirmed SARS-COV-2 infection
43			(with confirmatory PCR tests performed subsequently on all such
44			enrolled individuals), (2) removed inclusion criterion of SpO2 ≥90, and
45			(3) removed exclusion criterion of recent daily multivitamin use.”
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50			Methods and analysis // Study procedures // Recruitment and
51			obtaining informed consent (p6):
52			“Potential participants are approached by trained site hospital staff
53			members when they present to site hospitals. Site hospital staff
54			members undergo intensive training and refresher training in order to
55			ensure that potential participants are able to make an informed
56			decision regarding participation. [...]
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Methods: Assignment of interventions (for controlled trials)

Allocation:

Sequence generation	16a	<p>Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions</p> <p>Methods and analysis // Study procedures // Randomization and blinding (p7): “For randomization, a computer-generated list from 1 to 1000 was prepared by the study statistician, according to a randomization sequence in blocks of 20 and stratified by follow up clinic.”</p>
Allocation concealment mechanism	16b	<p>Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned</p> <p>Methods and analysis // Study procedures // Randomization and blinding (p7): “Supplement bottles and envelopes are pre-labelled with codes, and active tablets and placebo are indistinguishable, so that participants and investigators are blinded. For randomization, a computer-generated list from 1 to 1000 was prepared by the study statistician, according to a randomization sequence in blocks of 20 and stratified by follow up clinic.”</p>
Implementation	16c	<p>Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions</p> <p>Methods and analysis // Study procedures // Recruitment and obtaining informed consent (p6): “Potential participants are approached by trained site hospital staff members when they present to site hospitals. [...] Informed consent is obtained after responding to any raised queries.”</p> <p>Methods and analysis // Study procedures // Randomization and blinding (p7): “For randomization, a computer-generated list from 1 to 1000 was prepared by the study statistician, according to a randomization sequence in blocks of 20 and stratified by follow up clinic.”</p>

1
2 Blinding
3 (masking)
4

5 17a Who will be blinded after assignment to interventions (eg, trial
6 participants, care providers, outcome assessors, data analysts), and
7 how

8 Methods and analysis // Study procedures // Randomization and
9 blinding (p7):

10 “Supplement bottles and envelopes are pre-labelled with codes, and
11 active tablets and placebo are indistinguishable, so that participants
12 and investigators are blinded. For randomization, a computer-
13 generated list from 1 to 1000 was prepared by the study statistician,
14 according to a randomization sequence in blocks of 20 and stratified
15 by follow up clinic.”

16 17b If blinded, circumstances under which unblinding is permissible, and
17 procedure for revealing a participant’s allocated intervention during
18 the trial

19 Data and Safety Monitoring Board (p13):

20 “The trial DSMB will examine efficacy endpoints by study arms when
21 half of individuals are enrolled. In accordance with the Haybittle-Peto
22 rule, if the difference in the primary outcome between study arms is
23 <0.001 , unblinding of the DSMB and stopping will be considered.[36]”
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27 **Methods: Data collection, management, and analysis**
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2 Data collection 18a Plans for assessment and collection of outcome, baseline, and other
3 methods including any related processes to promote data quality (eg,
4 duplicate measurements, training of assessors) and a description of
5 study instruments (eg, questionnaires, laboratory tests) along with
6 their reliability and validity, if known. Reference to where data
7 collection forms can be found, if not in the protocol
8
9 [Methods and analysis // Study procedures // Baseline data and](#)
10 [sample collection \(p7\):](#)
11 “Following informed consent, participants undergo baseline data and
12 sample collection, including recording of key background and clinical
13 information as follows:
14
15 Screening and background: the initial screening form is
16 extended to collect information including participants’ demographic
17 background, socio-economic status, and health and prevention
18 behaviours (smoking and drinking)
19
20 Baseline dietary information: a food frequency questionnaire
21 (FFQ) is administered, collecting information on dietary practices and
22 habits in relation to 25 food groups. The FFQ is validated for use in
23 India and has been adapted to the Maharashtra context
24
25 Clinical baseline: clinical and physical measures are collected
26 alongside information on COVID-19 vaccination status, COVID-19
27 symptoms, vital signs, blood investigations, medical conditions,
28 treatment and medications, complications, and medical history
29 A blood sample is also collected at baseline. All information is
30 collected securely on electronic tablets, as described above.”
31
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33
34 [Methods and analysis // Study procedures // Study outcomes and](#)
35 [follow up \(p8 – 9\):](#)
36 “Following baseline assessment and provision of supplements,
37 participants are regularly followed up as described below:
38
39 Daily hospital follow up: Daily assessment of COVID-19
40 symptoms, vital signs, complications, medical conditions and study
41 supplement compliance is recorded for hospitalised participants
42
43 Telephone follow up: Assessment of COVID-19 symptoms,
44 supplement compliance and adverse events is conducted in a follow
45 up call every three days after leaving the hospital for all participants
46
47 8-week clinical assessment: After completion of study
48 supplements at 8 weeks, information is gathered on results of a
49 clinical and physical examination, COVID-19 symptoms, compliance
50 with regimen (including direct questioning and pill count), vital signs,
51 blood investigations (from a collected blood sample), medical
52 conditions, treatment and medications, complications, and history.
53 This assessment is conducted in person at the hospital, or at a
54 location convenient to the participant where privacy can be ensured
55 (including an option to collect some information via telephone if an in-
56 person visit is not possible)
57
58 12-week telephone follow up: A final assessment is conducted
59 of long-term COVID-19 symptoms [...]
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A list of collected data and blood investigations with time points at
baseline, during follow up visits or calls, and at 8 and 12 weeks is
summarized in Table 1. (Please also refer to Table 1)

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Data
management

- 18b Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols
 Methods and analysis // Study procedures // Intervention (p8):
 “Participants are contacted daily while in hospital or regularly via telephone after leaving the hospital to ensure compliance.”
- 19 Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol
 Methods and analysis // Study procedures // Recruitment and obtaining informed consent (p6):
 “Information regarding eligibility of potential participants is collected on a secure electronic tablet using Open Data Kit (ODK),[31] with questionnaires including built-in checks and data uploaded to a secure server. No identifiable data are collected until the participant has provided informed consent.”
- Methods and analysis // Study procedures // Baseline data and sample collection (p7):
 “All information is collected securely on electronic tablets, as described above.”
- Methods and analysis // Study procedures // Study outcomes and follow up (p8):
 “All data are collected using standardized questionnaire forms on electronic tablets,[31] as described above.”
- Methods and analysis // Data and sample management (p11):
 “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. Data are stored in linked-anonymised form, with identifiable information and the linking key stored separately. All analyses and data checks are conducted on anonymised data only.”

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Statistical methods

- 20a Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol
- Methods and analysis // Data analysis // Planned analyses (p11):
“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. placebo and zinc vs. placebo using Cox regression. [...] We will assess the success of randomization by comparing baseline variables by treatment group using χ^2 and t-tests and use multivariate modelling to adjust for imbalances if needed.
[...]
The effect of vitamin D or zinc on dichotomous secondary outcomes will be analyzed in a similar approach.”
- 20b Methods for any additional analyses (eg, subgroup and adjusted analyses)
- Methods and analysis // Data analysis // Planned analyses (p11-12):
“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.
[...]
The proportion of individuals experiencing hypercalcemia will be compared between treatment groups using χ^2 tests, and effects of the supplements on blood biomarkers will be compared via Wilcoxon and t-tests. This study will measure numerous risk factors for COVID-19 progression and severe treatment outcomes including hemoglobin; co-morbidities; medications including chloroquine, hydroxychloroquine, and ACE inhibitors; and socio-demographic, clinical, nutritional, and lifestyle-related risk factors. We will examine relationships of these factors in the placebo group first, to avoid complex questions concerning interactions between risk factors and treatments. Once we find a satisfactory parsimonious model using principles of model selection as detailed by Greenland,[32] we will test and modify it if needed in the whole study population, adjusting for treatment effects.
- Analyses will consider sex and gender throughout, by disaggregating findings, and attempting to elucidate the roles of sex and gender in the clinical course and immune response by controlling for potential sociodemographic, nutritional, and immunological confounders.”

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20c Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)

Methods and analysis // Data analysis // Planned analyses (p11):
“An intent-to-treat analysis will be used as the primary analytic strategy.”

10 **Methods: Monitoring**

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12 Data monitoring 21a Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol.

Alternatively, an explanation of why a DMC is not needed

Data and Safety Monitoring Board (p12-13):

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“The Data and Safety Monitoring Board (DSMB) was established prior to commencement of the trial. It consists of independent experts in respiratory infection and communicable diseases, public health and nutrition, clinical research, and biostatistics. The role of the board is to provide their inputs, recommendations, review the trial protocols and progress by ensuring the rights and safety of involving participants in the study through periodic trial review meetings.

The trial DSMB will examine efficacy endpoints by study arms when half of individuals are enrolled. In accordance with the Haybittle-Peto rule, if the difference in the primary outcome between study arms is <0.001 , unblinding of the DSMB and stopping will be considered.[36]”

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21b Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial

Data and Safety Monitoring Board (p13):

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“The trial DSMB will examine efficacy endpoints by study arms when half of individuals are enrolled. In accordance with the Haybittle-Peto rule, if the difference in the primary outcome between study arms is <0.001 , unblinding of the DSMB and stopping will be considered.[36]”

1
2 Harms 22 Plans for collecting, assessing, reporting, and managing solicited and
3 spontaneously reported adverse events and other unintended effects
4 of trial interventions or trial conduct
5 [Methods and analysis // Adverse events and reporting \(p11\):](#)
6 “Any undesirable circumstance or experiences reported by study
7 participants during the study are categorised as adverse events. All
8 adverse events which are possibly, probably or very likely related to
9 administration of any supplement are monitored and reported to site
10 institutional review boards (IRBs) within 72 hours (serious adverse
11 events) or 1 month (all other adverse events), using a standardized
12 reporting format. The trial data and safety monitoring board (DSMB) is
13 also notified. Site principal investigators and independent physicians
14 are responsible for assessing the causal relationship and making the
15 conclusive decision about continuation of the trial for a particular
16 participant. Additionally, medical insurance is provided to all study
17 participants to take care of any progression of severe adverse
18 events.”

23 Auditing 23 Frequency and procedures for auditing trial conduct, if any, and
24 whether the process will be independent from investigators and the
25 sponsor
26 NA
27
28

29 Ethics and dissemination

30
31 Research ethics 24 Plans for seeking research ethics committee/institutional review board
32 approval (REC/IRB) approval
33 [Abstract // Ethics and dissemination \(p2\):](#)
34 “Ethical approval was obtained from institutional ethical committees of
35 all participating institutions.”
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39 [Ethics and dissemination \(p13\):](#)
40 “This study is being conducted in the accordance with the Declaration
41 of Helsinki 2013. The study was approved by the Institutional Review
42 Board of the Harvard T.H. Chan School of Public Health (Protocol No.
43 IRB20-1425), the University Health Network Research Ethics Board
44 (20-5775), the Institutional Research Ethics Committee of the
45 Foundation for Medical Research (IREC No.
46 FMR/IREC/C19/02/2020), the Institutional Review Board of Saifee
47 Hospital (Project No. EC/008/2020) and the KEM Hospital Research
48 Centre Ethics Committee (KEMHRC ID No. 2027).”
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- Protocol amendments 25 Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)
NA:
As this is a manuscript of a study protocol, such detail has not been included in this specific document.
- Consent or assent 26a Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)
Methods and analysis // Recruitment and obtaining informed consent (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. These dedicated site hospital staff members determine their interest and eligibility, and provide a brief introduction including key details about the study and what participation involves. The staff members read out the participant information sheet in the appropriate conversational language (English, Hindi or Marathi), and discuss the trial components and the role of the participant in the study. Information provided includes a clear outline of potential benefits and harms, the length of the follow up period, remuneration that can be expected, future use of information and samples, and resources available to the participant such as access to study clinics. Informed consent is obtained after responding to any raised queries. [...] The informed consent process is completed once participants provide their signature on two copies of the consent document; one copy for the trial record and another provided to participants for their reference.”
- 26b Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable
Methods and analysis // Recruitment and obtaining informed consent (p6):
“The staff members read out the participant information sheet in the appropriate conversational language (English, Hindi or Marathi), and discuss the trial components and the role of the participant in the study. Information provided includes a clear outline of potential benefits and harms, the length of the follow up period, remuneration that can be expected, future use of information and samples, and resources available to the participant such as access to study clinics. Informed consent is obtained after responding to any raised queries.”

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- Confidentiality 27 How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial
Methods and analysis // Study procedures // Recruitment and obtaining informed consent (p6):
“Information regarding eligibility of potential participants is collected on a secure electronic tablet using Open Data Kit (ODK),[31] with questionnaires including built-in checks and data uploaded to a secure server. No identifiable data are collected until the participant has provided informed consent.”
- Methods and analysis // Study procedures // Baseline data and sample collection (p7):
“All information is collected securely on electronic tablets, as described above.”
- Methods and analysis // Study procedures // Study outcomes and follow up (p8):
“All data are collected using standardized questionnaire forms on electronic tablets,[31] as described above.”
- Methods and analysis // Data and sample management (p11):
“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. Data are stored in linked-anonymised form, with identifiable information and the linking key stored separately. All analyses and data checks are conducted on anonymised data only.”
- Declaration of interests 28 Financial and other competing interests for principal investigators for the overall trial and each study site
Competing interests (p15):
“All authors declare no conflicts of interest.”
- Access to data 29 Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators
Methods and analysis // Data and sample management (p11):
“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. Data are stored in linked-anonymised form, with identifiable information and the linking key stored separately.”

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2	Ancillary and	30	Provisions, if any, for ancillary and post-trial care, and for
3	post-trial care		compensation to those who suffer harm from trial participation
4			Methods and analysis // Adverse events and reporting (p11):
5			“Additionally, medical insurance is provided to all study participants to
6			take care of any progression of severe adverse events.”
7			
8	Dissemination	31a	Plans for investigators and sponsor to communicate trial results to
9	policy		participants, healthcare professionals, the public, and other relevant
10			groups (eg, via publication, reporting in results databases, or other
11			data sharing arrangements), including any publication restrictions
12			Ethics and dissemination (p13):
13			“The study findings will be presented in peer-reviewed medical
14			journals.”
15		31b	Authorship eligibility guidelines and any intended use of professional
16			writers
17			NA
18		31c	Plans, if any, for granting public access to the full protocol, participant-
19			level dataset, and statistical code
20			NA
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28	Appendices		
29	Informed consent	32	Model consent form and other related documentation given to
30	materials		participants and authorised surrogates
31			NA:
32			As this is a manuscript of a study protocol, such detail has not been
33			included in this specific document.
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36	Biological	33	Plans for collection, laboratory evaluation, and storage of biological
37	specimens		specimens for genetic or molecular analysis in the current trial and for
38			future use in ancillary studies, if applicable
39			Methods and analysis // Data and sample management (p11):
40			“Blood samples collected as part of this trial are processed at the
41			Foundation for Medical Research, Mumbai, and accredited
42			laboratories in India including at the site hospitals. Specimens are
43			linked-anonymised and are stored securely at the Foundation for
44			Medical Research for a maximum of three years.”
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*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons [“Attribution-NonCommercial-NoDerivs 3.0 Unported”](#) license.