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A randomised controlled trial for high-dose intravenous zinc as adjunctive therapy in SARS-CoV-2 (COVID-19) positive critically ill patients: trial protocol

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Abstract

Introduction

SARS-CoV-2 (COVID 19) has caused an international pandemic of respiratory illness, resulting in significant healthcare and economic turmoil. To date, no robust vaccine or treatment has been identified. Elemental Zinc has previously been demonstrated to have beneficial effects on coronaviruses and other viral respiratory infections due to its' effect on RNA polymerase. Additionally, Zinc has well demonstrated protective effects against hypoxic injury – a clear mechanism of end-organ injury in respiratory distress syndrome. We aimed to assess the effect of high dose intravenous zinc (HDIVZn) on SARS-CoV-2 infection. The end of study analyses evaluated the reduction of impact of oxygen saturations or requirement of oxygen supplementation.

Methods and Analysis

We designed a double-blind randomised controlled trial of daily HDIVZn (0.5mg/kg) versus placebo. Primary outcome measures are lowest oxygen saturations (or greatest supplemental oxygenation) for non-ventilated patients and worst PaO_2/FiO_2 for ventilated patients. Following power calculations, 100 hospitalised patients and 160 ventilated patients will be recruited to demonstrate a 20% difference. The duration of the followup is up to the point of discharge.

Ethics and dissemination

Ethical approval was sought through the independent Human Research Ethics Committee. Participant recruitment will commence in May 2020. Results will be published in peer-reviewed medical journals.

Trial Registration

Clinical trial registered with Australian New Zealand Clinical Trials Registry (ACTRN126200000454976)

Strengths and Limitations of this study

- COVID19 associated respiratory infection results in severe respiratory distress syndrome and potentially death
- High dose Zinc has previous demonstrated efficacy in patients with alternate corona virus and further demonstrates protection in end-organ hypoxia
- We aimed to determine the efficacy of high dose intra-venous zinc on respiratory and end-organ outcomes in patients infected with COVID19
- We designed a double-blind placebo-controlled randomised clinical trial with well-defined universal outcome measures
- High dose intravenous zinc has been proven safe in previous studies of critically ill patients.

Introduction

Since December 2019, a new coronavirus, designated SARS-CoV-2, has caused an international pandemic of respiratory illness termed COVID-19, posing significant threats to global health and the economy [1-3]. In more severe cases, COVID-19 enters the lungs, causing respiratory complications such as bronchitis and pneumonia [4 5]. Development of pneumonia leads to a reduced ability for oxygenation and in some cases the development of acute respiratory distress syndrome (ARDS), requiring mechanical ventilation. In the most severe cases, patients can develop multiple organ failure and hypoxic brain injury. In the absence of an effective vaccine or robust treatment for people with the disease [6], there is an urgent need to find a treatment that inhibits virus replication or reduces the progression of the disease.

Zinc is a naturally occurring essential heavy metal, and zinc deficiency is associated with a range of pathological conditions, including retarded growth and delayed wound healing and tissue repair. Zinc is also important for the maintenance and development of the immune system and plays a role in cell division and growth. Zinc deficiency results in reduced immunity and increases susceptibility to infectious diseases [7 8]. Numerous studies report the potential of zinc and zinc salts to inhibit viral infections in clinical and experimental settings. Viral infections that have been successfully inhibited by zinc include the common cold (a type of coronavirus) [9], respiratory syncytial virus infections [10], cytomegalovirus infections [11] and herpes labialis [12]. More importantly, zinc is a potent inhibitor of the replication of SARS-coronavirus (SARS-CoV) and equine arteritis virus (EAV) in cell culture [13].

Furthermore, our published studies have shown that high dose intravenous zinc (HDIVZn) protects various organs, including the heart, kidneys and liver against the damage caused by hypoxia [7 14-16]. In addition to being efficacious, the dose of elemental zinc at the 0.25mg/kg (~0.5mg/kg $ZnCl_2$) dose used for our HDIVZn and applied in our preclinical study in sheep is very safe and well-tolerated in humans as verified from multiple observational reports including the treatment of very sick children in an ICU setting with suspected zinc deficiency [17-20].

We plan to perform a randomised controlled trial to test the efficacy and safety of HDIVZn administered as a daily injection in subjects with COVID-19 infection to assess whether giving HDIVZn to patients improves clinical outcomes.

Methods and Design

Ethical approval was sought through the institutional Human Research Ethics Committee. This clinical trial was registered with Australian New Zealand Clinical Trials Registry (ACTRN126200000454976). The protocol design was produced in adherence with the Standard Protocol Otems for Randomised Trials (SPIRIT) [21].

Patient and public involvement

The development of the research question stemmed from the urgent global crisis inflicted by COVID-19, and the urgent need for a therapy to reduce the impact of the disease on the individual patients. Patients were not intrinsically involved in the design, recruitment or conduct of the study. Results of the study will be disseminated to patients after publication in peer-review journal. While the burden of the treatment or placebo was not assessed directly by patients, it was deemed minimal due to the lack of deviation from standard care.

Study design

Institutional ethical approval was sought through the Human Research Ethics Committee. This is a Phase 2, double-blind, placebo-controlled, randomised study at a single institution. The study plans to randomise 160 participants and is performed on hospitalised or critically-ill patients with confirmed COVID-19 (as detailed in Table 2). The study plans to evaluate the efficacy and safety of HDIVZn over a seven-day period of treatment.

Dosing rationale

The upper limit for daily zinc intake in an adult is 40mg. However, when 30 mg of elemental zinc was given orally to humans, it resulted in an only 1.8-fold increase in plasma zinc in the first 4 hours [22]. A similar study in humans where elemental Zn was given orally at 30 mg/d for six months showed a statistically significant but clinically marginal increase in the plasma zinc from 14.18 ± 1.75 µmol/L in the placebo group to 17.18 ± 3.48 µmol/L in zinc group [23]. Oral delivery of zinc is affected by several factors, including normal variations in gut zinc absorption, dietary factors such as the presence of phytate, and interactions with other metal ions [24]. Also, repeated high oral zinc intake causes a rapid and significant upregulation of intestinal metallothioneins which markedly decreases subsequent gut zinc, and importantly copper, absorption [25]. The latter may lead to copper deficiency in patients administered zinc for prolonged periods.

In our recently published sheep study, we determined that a single IV dose of $ZnCl_2$

at 0.5mg/kg increased the plasma zinc concentration by 4-fold from a baseline concentration of 11.3 ± 0.4 µmol/L to 46.5 ± 1.5 µmol/L within two hours [14]. Interestingly, two doses of IV $ZnCl_2$ at 0.5mg/kg increased the plasma zinc concentration 7-fold from a baseline concentration of 11.3 ± 0.4 µmol/L to 70.1 ± 5.8 µmol/L.

Translation of a drug's dose from animals to humans depends on the drug dose correction factor (K_m) which is calculated by dividing the average body weight (kg) of a species by its body surface area (m^2). For humans, K_m is 37 [26]. Direct carryover of a drug's pharmacologic dosage, from animals to humans depends on how similar the K_m value of the animal species in which efficacy was tested, is to human K_m. As published in [26], K_m for rats is 6, and for rabbit, K_m is 12. Moreover, for sheep K_m is 36 (average body weight of sheep is 40kg, and body surface area is 1.10m^2 [27]). Therefore, the near similar K_m value of sheep (36) to that of humans (37) has allowed us to conclude that human equivalent elemental zinc dosage would equate to 0.25mg/kg, the same dose that was shown to be protective in the sheep study and which we plan to use in our proposed trial.

Information regarding the safety of HDIVZn can be derived from published reports where humans were treated with high doses of zinc [17-20]. The estimated elemental HDIVZn dosage for an average 70kg human to be used in our proposed study will be 0.25mg/d x 70kg=17mg/d. Elemental zinc has been administered at a substantially higher dose (ranging from 26.4 to 37.5mg/d for eight successive days) in the treatment of human burns without any side effects [17-19]. In fact, zinc at doses ranging from 5-22mg/d has been administered in humans routinely as a component of parenteral nutrition without any reported side effects [28]. Furthermore, a recently published phase I clinical trial in critically ill children with suspected zinc deficiency involved administration of zinc intravenously at a dose 3-times higher than is proposed in the current study (0.75mg/kg/d for seven days), without any adverse effects [20].

Study objective and endpoints

The endpoints of this study are listed in Table 1. The primary objective is to assess the effect of 7 days of HDIVZn on oxygenation in comparison with placebo in patients with confirmed COVID19. Oxygenation (litres/minute) will be measured by either the highest level of supplemental oxygen (non-ventilated patients) or lowest PaO_2/FiO2 ratio in ventilated patients.

Secondary outcome measures include ICU and in-hospital mortality, length of stay in ICU or hospital, duration of oxygenation, severe adverse drug events and changes based on

WHO R&D Blueprint "Novel Coronavirus COVID-19 Therapeutic Trial synopsis". The following is based on an eight-point ordinal scale consisting of:

0, not hospitalized, no clinical or virological evidence of infection;

1, not hospitalized, Infected, and able to resume normal activities;

2, not hospitalized, Infected, but unable to resume normal activities;

3, hospitalized, no requirement of supplemental oxygen;

4, hospitalized, requiring oxygen therapy via mast or nasal prongs;

5, hospitalized, non-invasive ventilation, requiring high flow oxygen;

6, hospitalized, intubation and mechanical ventilation

7, hospitalized, requiring ECMO, invasive mechanical ventilation, additional organ support, RRT;

8, death;

Sample size

The primary outcome of this study is related to the effect of zinc therapy on the level of oxygenation expressed either as worst (highest) oxygen flow (in litres/min) in non-ventilated patients or worst (lowest) PaO_2 (in mmHg)/FiO_2 (as a fraction of 1) ratio in ventilated patients. We hypothesize that zinc therapy will decrease the worst level of oxygenation during the first week (of the treatment period) by 20% compared to placebo from a mean worst value of 10L/min (placebo) to a mean of 8L/min (zinc) or for PaO_2/FiO_2 ratio from a mean worst value of 150 (placebo) to a mean worst value of 180 (zinc). If patients transition from non-ventilated to ventilated during the study period, the PaO_2/FiO_2 ratio will be used.

To have an 80% power to see such an effect at an alpha of 0.05 in non-ventilated patients, assuming a standard deviation (SD) for O_2 flow of 4L/min 28 patients would have to be randomized in each arm. In ventilated patients, to have an 80% power to see such an effect at an alpha of 0.05, assuming an SD for the PaO_2/FiO_2 ratio of 60, 49 patients would have to randomized in each arm.

Rounding off the first group to 30 per arm and the second group to 50 per arm to account for withdrawals, we estimate that a study of 160 patients would provide a suitable sample size to test our primary hypothesis.

Study procedures

Eligibility

Consenting COVID-19 symptomatic confirmed hospitalized adult patients who fulfil World Health Organisation's case definition which includes a positive polymerase chain reaction (PCR) for COVID-19 from any specimen (e.g. respiratory, blood, urine, stool, other bodily fluid). Inclusion and exclusion criteria are summarised in Table 2.

Randomization

This pilot study will be conducted as a randomised double-blinded placebo-controlled study using a stratified randomization method. Ward and ICU patients will be enrolled as soon as possible after fulfilling the criteria of stratification. Patients will be stratified based on whether they required mechanical ventilation. Thereafter, a permuted block randomisation method with variable block sizes of 2, 4 and 6 will be used to allocate eligible patients to either the treatment group, receiving HDIVZn or to the control group in a 1:1 ratio. Randomisation will be performed by the randomisation module in Research Electronic Data Capture (REDCap, Vanderbilt University, USA), which is a secure web application for managing online data collection. Assessment of the unblinding procedures by the project research officer in the case of adverse event will be performed if necessary. The clinical staff involved in patient care will administer the trial drugs as soon as possible after enrolment.

Study drug administration and blinding

Pharmaceutical grade zinc chloride stock solution obtained from an Australian company (Phebra Pty Ltd, Lane Cove West, NSW, Australia) will be diluted in 250ml of normal saline and infused, resulting in a final dosage of 0.5mg/kg/d. Patients will be administered zinc daily for seven days. To standardise administration time, zinc infusions will commence early morning. Zinc will be administered via central venous or peripheral access over 3-6 hrs. The clinical trial nurse (allocation concealment) will use a web-based randomisation program to determine the allocation of patients and then prepare the coded zinc solution or placebo. Each coded solution bag, which will be indistinguishable irrespective of study group, will then be dispensed for administration to the patient as per protocol. This coded identifying study number will also be labelled on the patient case report form (CRF). The investigators, study coordinators, treating physicians, bedside nurses and patients/family will remain blinded to the allocated study solution.

Study assessment

Screening procedures

The screening log is designed to monitor patient recruitment at the participating site. A screening log will be maintained at each participating site by the research coordinator to document patients evaluated for enrolment. The log will provide a record of all patients assessed for eligibility and deemed ineligible for the study. When a patient is considered ineligible, the reason(s) will be noted on the log. The log will also be used to assess patient recruitment targets.

Physical examination, measurements and laboratory tests

All data will be collected by trained staff at each study site using a case report form (CRF) worksheet developed by the coordinating centre. Data will then be entered into the REDCap web database (electronic CRF [eCRF]). Randomised patients will be followed up to discharge, death or 28 days post-randomisation whichever occurs first.

Study day 1 commences on randomisation and concludes at the expiry of the calendar day. Data collection will be restricted primarily to those variables necessary to define clinical patient characteristics including baseline demographics, primary diagnoses, physiological parameters, diagnostic interventions, therapeutic interventions and documentation of deaths and other serious adverse events. A complete list of collected data is summarised in Table 3.

Adverse events

An adverse reaction is defined as any untoward and unintended response to an investigational medicinal product related to any dose administered. All adverse events judged by either the reporting investigator or the sponsor as having a reasonable possibility of a causal relationship to an investigational medicinal product will qualify as adverse reactions. All adverse events which are considered to be potentially causally related to the study intervention or are otherwise of concern in the investigator's judgement will be reported. An interim analysis is planned after 50% (80) of the patients have been randomised.

Criteria for withdrawal

The study treatment will be discontinued in cases where the participant wishes to withdraw from the trial or when a Grade 4 severity adverse event occurs.

Outcome measures

Safety evaluations

An independent Data Monitoring Committee (DMC), consisting of experts in intensive care, clinical research and biostatistics will be established before patient enrolment and will review all trial protocols. The role of the DMC will be to provide study oversight to ensure that the rights and safety of patients involved in the study are protected by reviewing reported Adverse Events and making recommendations to the Management Committee (MC).

Statistical evaluation

The descriptive analysis of the data will include the calculation of means, standard deviations, and absolute and relative frequencies of the baseline and follow-up data. Randomisation will be checked by suitable two-sided statistical tests (Chi-Square, or Fisher's exact test for categorical data, Student's t-Test or Mann-Whitney-U tests for continuous data). If normality of the data is not given, non-parametric methods will be used. Potentially confounding factors will be checked for using multivariable logistic regression analysis. All data will be analysed according to the intention-to-treat principle. Continuous data will be tested for normal distribution using histograms. Between-group comparisons for continuous data will be performed with the use of the Student's t-test or the Mann-Whitney U test and for categorical data with the use of Fisher's exact test or chi-square test where appropriate. A p-value 0.05 will indicate statistical significance. A full model with clinical relevant covariates (e.g. sex, age, previous heart surgery, preoperative creatinine) will be used for a stepwise backward variable selection procedure to identify independent risk factors for AKI. Secondary endpoints will be analysed in the ITT collective using Fishers' exact test, or chi-square tests for categorical data, Student's t-tests and Mann-Whitney-U tests for continuous data.

A senior statistician will perform data analysis on an intention-to-treat basis. An interim analysis on the safety and the primary outcomes will be performed when 50% (80/160) of the patients have received zinc for at least seven days. Summary statistics will be used to describe the clinical data and presented as mean ± SD, median with interquartile range (IQR) or percentages as appropriate. Chi-squared analysis with Fisher's exact test (when appropriate) and Student's t-test (Mann Whitney U test for non-normal distributions) will be used to compare data between the active treatment group and the control group

with statistical significance declared for probability values of 0.05 or less. Analysis of the outcome of excluded patients due to other trials etc. will be in accordance with the CONSORT guidelines.

Discussion

This single centre, double-blind, randomised controlled trial will assess the role of HDIVZn in protection against the SARS-CoV-2 virus. The beneficial effects of Zinc on viral infections have been previously demonstrated. Further, HDIVZn may provide additional protection to other end organs that may be indirectly affected by pulmonary injury and impaired oxygenation.

In coronaviruses, Zn^{2+} inhibits both the proteolytic processing of replicase polyproteins and the RNA-dependent RNA polymerase (RdRp) activity. Although mechanisms of action of zinc are unknown, several possibilities exist. Firstly, DNA and RNA polymerases use divalent metal ions like Mg^{2+} as a co-factor, and one possible mechanism is that Zn^{2+} displaces Mg^{2+} and subsequently inhibits RdRp activity. In support is the observation that various divalent metals ions sustained the activity of poliovirus RdRp in the following preference Mn^{2+}> Co^{2+}> Ni^{2+}> Fe^{2+}> Mg^{2+}> Ca^{2+}> Cu^{2+} [29]. In contrast, Zn^{2+} was incapable of sustaining RdRp catalyzed nucleotide incorporation [29]. Secondly, a zinc-binding pocket has been identified in the Dengue virus and SARS-coronavirus RdRp. Therefore, it is possible that binding of zinc may induce a structural change in the conformation of RdRp which disables RdRp to catalyze nucleotide incorporation. Finally, adding high concentrations of zinc ions to cells impairs viral polyprotein processing which is integral to virus replication [30].

In addition to the direct effect on viral replication and activity, HDIVZn may play a protective role in alternate organs. Zinc has been demonstrated to be beneficial in patients with severe pneumonia due to its anti-inflammatory properties [31 32]. Further, in SARS-CoV-2, respiratory compromise results in impaired oxygenation and hypoxia to various end organs. Such hypoxia may contribute to end-organ failure and increase the risk of mortality. Specifically, such Covid-19 associated hypoxia has been proposed to be contributory to cardiac injury [33], hepatic injury [34] and renal injury [35 36]. Our published studies have shown that high dose intravenous zinc (HDIVZn) protects various organs, including the heart, kidneys and liver against the damage caused by hypoxia. It should be noted that hypoxia and oxidative stress, result in an increase in reactive oxygen species (ROS), including superoxide (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical (·OH) – which results in

intracellular damage [37] [38]. Zinc appears to limit ROS production by several mechanisms. Firstly, metallothioneins (MTs), small cysteine-rich and heavy metal-binding proteins, participate in the intracellular defence against reactive oxygen and nitrogen species [39] and zinc has been shown to induce MT mRNA and protein expression. Secondly, zinc competes with Fe^{2+} and Cu^{2+} ions for binding to cell membranes and proteins – normally, these active metals catalyze the production of hydroxyl radical from H_2O_2 via Fenton chemistry. Thirdly, zinc upregulates the production and activation of antioxidant proteins, molecules and enzymes such as glutathione, catalase and superoxide dismutase (SOD), which catalyze O_2^- to oxygen or H_2O_2 [40]. Finally, zinc reduces the activation of oxidant-promoting enzymes such as inducible nitric acid synthase and NADPH enzyme, which catalyze oxygen to O_2^-. Accordingly, we hypothesize that Zinc may provide protection against the hypoxic injury that critically ill patients with Covid-19 may experience.

The specific strengths of the current protocol design are a) prolonged exposure of HDIVZn and b) assessment of critically ill patients, a population where a benefit would be observed if truly present. There is an inherent difficulty in assessing pulmonary response in clinical trials, and accordingly, the primary outcome measure is a possible criticism. However, maximal oxygen requirements and PaO_2/FiO_2 are well established surrogate markers [41-43].

The safety of HDIVZn has been addressed in previous literature [17-20]. Elemental zinc has been administered at a substantially higher dose (ranging from 26.4 to 37.5mg/d for eight successive days) in the treatment of burns and did not produce any side effects in humans [17-19]. In the setting of critically ill patients, zinc intravenously at 3-times higher dose than the current study (0.75mg/kg/d for seven days), and it did not produce any adverse effects [20].

In conclusion, we designed a single-centre, double-blind, randomised controlled trial to assess the potential benefit of HDIVZn for hospitalised or critically ill patients with SARS-CoV-2 infection and associated respiratory compromise. We believe that our well-designed trial will be able to expediently identify a potential agent that may improve outcomes for these critically ill patients.

Contributors: MP assisted in protocol design, ethical submission and manuscript production. JEK and VC were involved in data collection and manuscript production. PJ, JT, CM were involved in patient care and study procotol design. DJ, DB, RB, OP and JI were involved in project design, patient care, manuscript production and supervision.

Competing interests: Nil

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References

1. Hu Y, Sun J, Dai Z, et al. Prevalence and severity of corona virus disease 2019 (COVID-19): A systematic review and meta-analysis. *J Clin Virol* 2020;127:104371. doi: 10.1016/j.jcv.2020.104371 [published Online First: 2020/04/22]
2. Tu H, Tu S, Gao S, et al. The epidemiological and clinical features of COVID-19 and lessons from this global infectious public health event. *J Infect* 2020 doi: 10.1016/j.jinf.2020.04.011 [published Online First: 2020/04/22]
3. Weston S, Frieman MB. COVID-19: Knowns, Unknowns, and Questions. *mSphere* 2020;5(2) doi: 10.1128/mSphere.00203-20 [published Online First: 2020/03/20]
4. Zhu J, Zhong Z, Li H, et al. CT imaging features of 4,121 patients with COVID-19: a meta-analysis. *J Med Virol* 2020 doi: 10.1002/jmv.25910 [published Online First: 2020/04/22]
5. Ledford H. How does COVID-19 kill? Uncertainty is hampering doctors' ability to choose treatments. *Nature* 2020;580(7803):311-12. doi: 10.1038/d41586-020-01056-7 [published Online First: 2020/04/11]
6. Wang Y, Zhang D, Du G, et al. Remdesivir in adults with severe COVID-19: a randomised, double-blind, placebo-controlled, multicentre trial. *The Lancet* 2020 doi: 10.1016/S0140-6736(20)31022-9
7. Ischia J, Bolton DM, Patel O. Why is it worth testing the ability of zinc to protect against ischaemia reperfusion injury for human application. *Metallomics* 2019;11(8):1330-43. doi: 10.1039/c9mt00079h
8. Prasad AS. Zinc is an Antioxidant and Anti-Inflammatory Agent: Its Role in Human Health. *Frontiers in nutrition* 2014;1:14.
9. Hulisz D. Efficacy of zinc against common cold viruses: an overview. *J Am Pharm Assoc* 2004;44(5):594-603. doi: 10.1331/1544-3191.44.5.594.hulisz [published Online First: 2004/10/22]
10. Suara RO, Crowe JE, Jr. Effect of zinc salts on respiratory syncytial virus replication. *Antimicrob Agents Chemother* 2004;48(3):783-90. doi: 10.1128/aac.48.3.783-790.2004 [published Online First: 2004/02/26]
11. Li D, Wen LZ, H Yu. [Observation on clinical efficacy of combined therapy of zinc supplement and jinye baidu granule in treating human cytomegalovirus infection]. *Zhongguo Zhong Xi Yi Jie He Za Zhi* 2005;25(5):449-51. [published Online First: 2005/06/17]
12. Femiano F, Gombos F, Scully C. Recurrent herpes labialis: a pilot study of the efficacy of zinc therapy. *J Oral Pathol Med* 2005;34(7):423-5. doi:

10.1111/j.1600-0714.2005.00327.x [published Online First: 2005/07/14]
13. te Velthuis AJ, van den Worm SH, Sims AC, et al. Zn(2+) inhibits coronavirus and arterivirus RNA polymerase activity in vitro and zinc ionophores block the replication of these viruses in cell culture. *PLoS Pathog* 2010;6(11):e1001176. doi: 10.1371/journal.ppat.1001176 [published Online First: 2010/11/17]
14. O'Kane D, Gibson L, May CN, et al. Zinc preconditioning protects against renal ischaemia reperfusion injury in a preclinical sheep large animal model. *Biometals* 2018;31(5):821-34. doi: 10.1007/s10534-018-0125-3
15. Rao K, Sethi K, Ischia J, et al. Protective effect of zinc preconditioning against renal ischemia reperfusion injury is dose dependent. *PLoS ONE* 2017;12(7):e0180028. doi: 10.1371/journal.pone.0180028
16. Cheung E, Nikfarjam M, Jackett L, et al. The Protective Effect of Zinc Against Liver Ischaemia Reperfusion Injury in a Rat Model of Global Ischaemia. *Journal of Clinical and Experimental Hepatology* 2019;August 20, 2019, In press. doi: 10.1016/j.jceh.2019.07.006
17. Berger MM, Baines M, Raffoul W, et al. Trace element supplementation after major burns modulates antioxidant status and clinical course by way of increased tissue trace element concentrations. *Am J Clin Nutr* 2007;85(5):1293-300. doi: 10.1093/ajcn/85.5.1293
18. Berger MM, Binnert C, Chiolero RL, et al. Trace element supplementation after major burns increases burned skin trace element concentrations and modulates local protein metabolism but not whole-body substrate metabolism. *Am J Clin Nutr* 2007;85(5):1301-6. doi: 10.1093/ajcn/85.5.1301
19. Berger MM, Spertini F, Shenkin A, et al. Trace element supplementation modulates pulmonary infection rates after major burns: a double-blind, placebo-controlled trial. *Am J Clin Nutr* 1998;68(2):365-71. doi: 10.1093/ajcn/68.2.365
20. Cvijanovich NZ, King JC, Flori HR, et al. Safety and Dose Escalation Study of Intravenous Zinc Supplementation in Pediatric Critical Illness. *JPEN J Parenter Enteral Nutr* 2016;40(6):860-8. doi: 10.1177/0148607115572193
21. Chan AW, Tetzlaff JM, Altman DG, et al. SPIRIT 2013 statement: defining standard protocol items for clinical trials. *Ann Intern Med* 2013;158(3):200-7. doi: 10.7326/0003-4819-158-3-201302050-00583 [published Online First: 2013/01/09]
22. Solomons NW, Romero-Abal ME, Weiss G, et al. Bioavailability of zinc from NutriSet zinc tablets compared with aqueous zinc sulfate. *Eur J Clin Nutr* 2011;65(1):125-31. doi: 10.1038/ejcn.2010.198
23. Feillet-Coudray C, Meunier N, Rambeau M, et al. Long-term moderate zinc supplementation increases exchangeable zinc pool masses in late-middle-aged men: the Zenith Study. *Am J Clin Nutr* 2005;82(1):103-10. doi: 10.1093/ajcn.82.1.103
24. Keyzer JJ, Oosting E, Wolthers BG, et al. Zinc absorption after oral administration of zinc sulfate. *Pharm Weekbl Sci* 1983;5(5):252-3.

25. Hempe JM, Carlson JM, Cousins RJ. Intestinal metallothionein gene expression and zinc absorption in rats are zinc-responsive but refractory to dexamethasone and interleukin 1 alpha. *J Nutr* 1991;121(9):1389-96.
26. Nair AB, Jacob S. A simple practice guide for dose conversion between animals and human. *J Basic Clin Pharm* 2016;7(2):27-31. doi: 10.4103/0976-0105.177703
27. Bennett JW. Regional Body Surface-Area of Sheep. *J Agric Sci* 1973;81(Dec):429-32. doi: Doi 10.1017/S0021859600086469
28. Stehle P, Stoffel-Wagner B, Kuhn KS. Parenteral trace element provision: recent clinical research and practical conclusions. *Eur J Clin Nutr* 2016;70(8):886-93. doi: 10.1038/ejcn.2016.53
29. Arnold JJ, Ghosh SK, Cameron CE. Poliovirus RNA-dependent RNA polymerase (3D(pol)). Divalent cation modulation of primer, template, and nucleotide selection. *J Biol Chem* 1999;274(52):37060-9. doi: 10.1074/jbc.274.52.37060 [published Online First: 1999/12/22]
30. Butterworth BE, Korant BD. Characterization of the large picornaviral polypeptides produced in the presence of zinc ion. *J Virol* 1974;14(2):282-91. [published Online First: 1974/08/01]
31. Srinivasan MG, Ndeezi G, Mboijana CK, et al. Zinc adjunct therapy reduces case fatality in severe childhood pneumonia: a randomized double blind placebo-controlled trial. *BMC Med* 2012;10:14. doi: 10.1186/1741-7015-10-14 [published Online First: 2012/02/10]
32. Brooks WA, Yunus M, Santosham M, et al. Zinc for severe pneumonia in very young children: double-blind placebo-controlled trial. *Lancet* 2004;363(9422):1683-8. doi: 10.1016/S0140-6736(04)16252-1 [published Online First: 2004/05/26]
33. Zhu H, Rhee JW, Cheng P, et al. Cardiovascular Complications in Patients with COVID-19: Consequences of Viral Toxicities and Host Immune Response. *Curr Cardiol Rep* 2020;22(5):32. doi: 10.1007/s11886-020-01292-3 [published Online First: 2020/04/23]
34. Feng G, Zheng KI, Yan QQ, et al. COVID-19 and Liver Dysfunction: Current Insights and Emergent Therapeutic Strategies. *J Clin Transl Hepatol* 2020;8(1):18-24. doi: 10.14218/jcth.2020.00018 [published Online First: 2020/04/11]
35. Fanelli V, Fiorentino M, Cantaluppi V, et al. Acute kidney injury in SARS-CoV-2 infected patients. *Crit Care* 2020;24(1):155. doi: 10.1186/s13054-020-02872-z [published Online First: 2020/04/18]
36. Mubarak M, Nasri H. COVID-19 nephropathy; an emerging condition caused by novel coronavirus infection. *J Nephropathol* 2020;9(3):e21-e21. doi: 10.34172/jnp.2020.21
37. Castro L, Freeman BA. Reactive oxygen species in human health and disease. *Nutrition* 2001;17(2):161-65. doi: 10.1016/S0899-9007(00)00570-0
38. Lachance PA, Nakat Z, Jeong WS. Antioxidants: An integrative approach. *Nutrition* 2001;17(10):835-38. doi: 10.1016/S0899-9007(01)00636-0
39. Ruttkay-Nedecky B, Nejdl L, Gumulec J, et al. The role of metallothionein in oxidative stress. *Int J Mol Sci* 2013;14(3):6044-66. doi: 10.3390/ijms14036044 [published Online First: 2013/03/19]
40. Prasad AS. Zinc: an antioxidant and anti-inflammatory agent: role of zinc in degenerative disorders of aging. *J Trace Elem Med Biol* 2014;28(4):364-

71. doi: 10.1016/j.jtemb.2014.07.019 [published Online First: 2014/09/10]
41. Frat JP, Ricard JD, Quenot JP, et al. Non-invasive ventilation versus high-flow nasal cannula oxygen therapy with apnoeic oxygenation for preoxygenation before intubation of patients with acute hypoxaemic respiratory failure: a randomised, multicentre, open-label trial. *Lancet Respir Med* 2019;7(4):303-12. doi: 10.1016/s2213-2600(19)30048-7 [published Online First: 2019/03/23]
42. He H, Sun B, Liang L, et al. A multicenter RCT of noninvasive ventilation in pneumonia-induced early mild acute respiratory distress syndrome. *Crit Care* 2019;23(1):300. doi: 10.1186/s13054-019-2575-6 [published Online First: 2019/09/06]
43. Pennisi MA, Bello G, Congedo MT, et al. Early nasal high-flow versus Venturi mask oxygen therapy after lung resection: a randomized trial. *Crit Care* 2019;23(1):68. doi: 10.1186/s13054-019-2361-5 [published Online First: 2019/03/02]

Table 1: Primary and secondary outcomes

Key Primary Outcome	Key Secondary Outcomes
Mean change in the highest level of oxygenation (oxygen flow measured litre/min) in non-ventilated patients Mean change in lowest PaO_2 in ventilated patients	Mortality (ICU or in-hospital) Duration of mechanical ventilation Duration of oxygen therapy Duration of hospitalisation Length of Stay in ICU
Other Secondary Outcomes	
Adverse Drug Events Acute kidney injury Acute liver injury Duration of vasopressor drugs Sequential Organ Failure Assessment (SOFA) respiratory score	Clinical improvement based on an eight-point ordinal scale recommended in the document published by WHO R&D Blueprint "Novel Coronavirus COVID-19 Therapeutic Trial Synopsis". • Percentage of patients reporting each severity rating on an 8-point ordinal scale [Time Frame: Day 14] • Time to improvement in one category from admission using the 8-point ordinal scale [Time Frame: Up to day 28] • Mean change in the 8-point ordinal scale [Time Frame: Up to day 28]

Table 2: inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
• Consenting adult patients adult male or female, age ≥ 18 years old. Laboratory-confirmed SARS-CoV-2 infection as determined by polymerase chain reaction (PCR) or another commercial or public health assay • Hospitalized with an illness of any duration with evidence of pneumonia and severe disease, critical disease, or multi-system organ dysfunction at baseline • Ability to provide informed consent signed by study patient or legally acceptable representative • Willingness and ability to comply with study-related procedures/assessments • Have an oxygen saturation (SaO_2) of 94% or less while they were breathing ambient air or a ratio of the partial pressure of oxygen (PaO_2) to the fraction of inspired oxygen (FiO_2) (Pao2: Fio2) at or below 300 mg Hg. • No chronic kidney disease (CKD) defined by stage II or higher using the Kidney Disease Improving Global Outcomes (KDIGO) classification	• Age <18 or pregnant or lactating female • Allergy to zinc • Severe hepatic impairment defined as Child C liver disease. • eGFR ≤ 60 mL/min/1.73 m^2 (defined using CKD-EPI SCr formula) • History of any organ transplant which requires active immunosuppressive treatment which can interfere with kidney function • If a patient required any of the following within seven days prior to cardiac surgery: defibrillation, mechanical ventilation, left ventricular assist device (LVAD), or other forms of mechanical circulatory support (MCS) • If a patient required cardiopulmonary resuscitation (CPR) within 14 days • DNR (do not resuscitate) DNI (do not intubate) orders • Death is deemed imminent or inevitable during this admission, and either the attending physician, patient or substitute decision-maker is not committed to active treatment • Already receiving dialysis (either acute or chronic) or imminent need of dialysis at the time of enrolment • Patients with known HIV infection • Patients with a known or suspected history of oxalate nephropathy or hyperoxaluria, scurvy, chronic iron overload, G-6PD deficiency • Clinician expects to prescribe Zinc for another indication • Patients with known haemochromatosis.

Table 3: Collected data during trial

	All patients	
Baseline data	Baseline data • Demographics: Age, gender, place of residence • Comorbidities: Diabetes mellitus, arterial hypertension, congestive heart failure, chronic obstructive airways disease, chronic liver disease, malignancy, chronic renal failure • Pre-admission medication - ? esp ACEi / A2RB • Functional status / frailty score	
	Non-ventilated patients	Ventilated patients
Daily observations	• Daily saturations (Worst values) • Daily oxygen flow • Fluid input • Urine output • Fluid balance	• Daily PaO_2/FiO_2 ratio • Daily oxygen flow • Fluid input • Urine output • Fluid balance • Vasopressor data
Laboratory investigations (collected at 0800)	• Daily serum creatinine • Daily liver function • Daily blood count • Daily Zinc and trace metal concentration • Daily Cardiac troponin • Daily lactate level	• Daily serum creatinine • Daily liver function • Daily blood count • Daily Zinc and trace metal concentration • Daily Cardiac troponin • Daily lactate level
Primary outcome measures	• Mean change in highest level of oxygenation requirement (oxygen flow in litres/min)	• Mean change in lowest PaO_2/FiO_2 ratio (in mmHg)
Secondary outcome measures	• Mortality [Time Frame: Up to day 28] • Duration of oxygen therapy (days) • Duration of hospitalization (days) • Length of stay in the intensive care unit and hospital • Frequency of Serious Adverse Drug Events • Acute kidney injury • Acute liver injury • Time to resolution of fever for at least 48 hours without antipyretics by clinical severity • Incidence of severe or life-threatening bacterial, invasive fungal, or opportunistic infection • Number of patients admitted into an intensive care unit (ICU)	• Mortality [Time Frame: Up to day 28] • Duration of mechanical ventilation(days) • Duration of oxygen therapy (days) • Duration of hospitalization (days) • Length of stay in the intensive care unit and hospital • Frequency of Serious Adverse Drug Events • Acute kidney injury • Acute liver injury • Use, duration and dosage of vasopressor drugs • Time to resolution of fever for at least 48 hours without antipyretics by clinical severity • Incidence of severe or life-threatening bacterial, invasive fungal,

	[Time Frame: Up to day 28] • Sequential Organ Failure Assessment (SOFA) Respiratory Score [Time Frame: 28 days]. Assigned a point value from 0 (normal) to 4 (high degree of dysfunction/failure) • Clinical improvement based on an eight-point ordinal scale recommended in the document published by WHO R&D Blueprint "Novel Coronavirus COVID-19 Therapeutic Trial Synopsis". ○ Percentage of patients reporting each severity rating on an 8-point ordinal scale [Time Frame: Day 14] ○ Time to improvement in one category from admission using the 8-point ordinal scale [Time Frame: Up to day 28] ○ Mean change in the 8-point ordinal scale [Time Frame: Up to day 28]	or opportunistic infection • Number of patients admitted into an intensive care unit (ICU) [Time Frame: Up to day 28] • Sequential Organ Failure Assessment (SOFA) Respiratory Score [Time Frame: 28 days]. Assigned a point value from 0 (normal) to 4 (high degree of dysfunction/failure) • Clinical improvement based on an eight-point ordinal scale recommended in the document published by WHO R&D Blueprint "Novel Coronavirus COVID-19 Therapeutic Trial Synopsis". ○ Percentage of patients reporting each severity rating on an 8-point ordinal scale [Time Frame: Day 14] ○ Time to improvement in one category from admission using the 8-point ordinal scale [Time Frame: Up to day 28] ○ Mean change in the 8-point ordinal scale [Time Frame: Up to day 28]

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A randomised controlled trial for high-dose intravenous zinc as adjunctive therapy in SARS-CoV-2 (COVID-19) positive critically ill patients: trial protocol

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A randomised controlled trial for high-dose intravenous zinc as adjunctive therapy in SARS-CoV-2 (COVID-19) positive critically ill patients: trial protocol

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Abstract

Introduction

SARS-CoV-2 (COVID19) has caused an international pandemic of respiratory illness, resulting in significant healthcare and economic turmoil. To date, no robust vaccine or treatment has been identified. Elemental zinc has previously been demonstrated to have beneficial effects on coronaviruses and other viral respiratory infections due to its effect on RNA polymerase. Additionally, zinc has well demonstrated protective effects against hypoxic injury – a clear mechanism of end-organ injury in respiratory distress syndrome. We aimed to assess the effect of high dose intravenous zinc (HDIVZn) on SARS-CoV-2 infection. The end of study analyses will evaluate the reduction of impact of oxygen saturations or requirement of oxygen supplementation.

Methods and Analysis

We designed a double-blind randomised controlled trial of daily HDIVZn (0.5mg/kg) versus placebo. Primary outcome measures are lowest oxygen saturation (or greatest level of supplemental oxygenation) for non-ventilated patients and worst PaO_2/FiO_2 for ventilated patients. Following power calculations, 60 hospitalised patients and 100 ventilated patients will be recruited to demonstrate a 20% difference. The duration of follow-up is up to the point of discharge.

Ethics and dissemination

Ethical approval was obtained through the independent Human Research Ethics Committee. Participant recruitment will commence in May 2020. Results will be published in peer-reviewed medical journals.

Trial Registration

Clinical trial registered with Australian New Zealand Clinical Trials Registry (ACTRN126200000454976). No external funding was sought for the completion of this project.

Strengths and Limitations of this study

- COVID19 associated respiratory infection results in severe respiratory distress syndrome and potentially death
- High dose zinc has previously demonstrated efficacy in patients with alternate corona virus infection and further demonstrates protection in end-organ hypoxia
- This study aims to determine the efficacy of high dose intra-venous zinc on respiratory and end-organ outcomes in patients infected with COVID19
- We designed a double-blind placebo-controlled randomised clinical trial with well-defined universal outcome measures
- Limitations pertain to conducting the current study in Australia, a country with a relatively low prevalence of COVID19

Introduction

Since December 2019, a new coronavirus, designated SARS-CoV-2, has caused an international pandemic of respiratory illness termed COVID19, posing significant threats to global health and the economy [1-3]. In more severe cases, COVID19 enters the lungs, causing respiratory complications such as bronchitis and pneumonia [4 5]. Development of pneumonia leads to a reduced ability for oxygenation and in some cases the development of acute respiratory distress syndrome (ARDS), requiring mechanical ventilation. In the most severe cases, patients can develop multiple organ failure and hypoxic brain injury. In the absence of an effective vaccine or robust treatment for people with the disease [6], there is an urgent need to find a treatment that inhibits virus replication or reduces the progression of the disease.

Zinc is a naturally occurring essential heavy metal, and zinc deficiency is associated with a range of pathological conditions, including retarded growth and delayed wound healing and tissue repair. Zinc is also important for the maintenance and development of the immune system and plays a role in cell division and growth. Zinc deficiency results in reduced immunity and increases susceptibility to infectious diseases [7 8]. Numerous studies report the potential of zinc and zinc salts to inhibit viral infections in clinical and experimental settings. Viral infections that have been successfully inhibited by zinc include the common cold (a type of coronavirus) [9], respiratory syncytial virus infections [10], cytomegalovirus infections [11] and herpes labialis [12]. More importantly, zinc is a potent inhibitor of the replication of SARS-coronavirus (SARS-CoV) and equine arteritis virus (EAV) in cell culture [13].

Furthermore, our published studies have shown that high dose intravenous zinc (HDIVZn) protects various organs, including the heart, kidneys and liver against the damage caused by hypoxia [7 14-16]. In addition to being efficacious, the dose of elemental zinc used for this HDIVZn trial (0.25mg/kg (0.5mg/kg $ZnCl_2$) and applied in our preclinical study in sheep is very safe and well-tolerated in humans as verified from multiple observational reports including the treatment of very sick children in an ICU setting with suspected zinc deficiency [17-20].

We plan to perform a single site randomised controlled trial to test the efficacy and safety of HDIVZn administered as a daily injection in subjects with COVID19 infection to assess whether giving HDIVZn to patients improves clinical outcomes.

Methods and Design

Ethical approval was sought through the Austin Health institutional Human Research Ethics Committee. This clinical trial was registered with Australian New Zealand Clinical Trials Registry (ACTRN126200000454976). The protocol design was produced in adherence with the Standard Protocol Items for Randomised Trials (SPIRIT) [21]. No external funding was sought for the completion of this project.

Patient and public involvement

The development of the research question stemmed from the urgent global crisis inflicted by COVID19, and the urgent need for a therapy to reduce the impact of the disease on the affected patients. Patients were not intrinsically involved in the design, recruitment or conduct of the study. Results of the study will be disseminated to patients after publication in peer-review journal. While the burden of the treatment or placebo was not assessed directly by patients, it was deemed minimal due to the lack of deviation from standard care.

Study design

This is a Phase 2, double-blind, placebo-controlled, randomised study at a single institution. The study plans to randomise 160 hospitalised participants, including 100 critically-ill patients with confirmed COVID19 (as detailed in Table 2) and to evaluate the efficacy and safety of HDIVZn over a seven-day period of treatment.

Dosing rationale

The upper limit for daily zinc intake in an adult is 40mg [22]. However, when 30 mg of elemental zinc was given orally to humans, it resulted in an only 1.8-fold increase in plasma zinc in the first 4 hours [23]. A similar study in humans where elemental zinc (30 mg/d) or placebo were given orally for six months showed a statistically significant in the plasma zinc from 14.18 ± 1.75 µmol/L in the placebo group to 17.18 ± 3.48 µmol/L in the zinc group [24]. Oral delivery of zinc is affected by several factors, including normal variations in gut zinc absorption, dietary factors such as the presence of phytate, and interactions with other metal ions [25]. Also, repeated high oral zinc intake causes a rapid and significant upregulation of intestinal metallothioneins which markedly decrease subsequent gut zinc, and importantly copper, absorption [26]. The latter may lead to copper deficiency in patients

administered zinc for prolonged periods.

In our recently published sheep study, we determined that a single IV dose of $ZnCl_2$ at 0.5mg/kg increased the plasma zinc concentration by 4-fold from a baseline concentration of 11.3 ± 0.4 µmol/L to 46.5 ± 1.5 µmol/L within two hours [14]. Interestingly, two doses of IV $ZnCl_2$ at 0.5mg/kg increased the plasma zinc concentration 7-fold from a baseline concentration of 11.3 ± 0.4 µmol/L to 70.1 ± 5.8 µmol/L.

Translation of a drug's dose from animals to humans depends on the drug dose correction factor (K_m) which is calculated by dividing the average body weight (kg) of a species by its body surface area (m^2). For humans, K_m is 37 [27]. Direct carryover of a drug's pharmacologic dosage, from animals to humans depends on how similar the K_m value of the animal species in which efficacy was tested, is to human K_m. As published by Nair et al [27], K_m for rats is 6, for rabbits is 12 and for sheep is 36 (average body weight of sheep is 40kg, and body surface area is 1.10m^2 [28]). Therefore, the near similar K_m value of sheep (36) to that of humans (37) has allowed us to conclude that human equivalent elemental zinc dosage would equate to 0.25mg/kg, the same dose that was shown to be protective in the sheep study and which we plan to use in our proposed trial.

Information regarding the safety of HDIVZn can be derived from published reports where humans were treated with high doses of zinc [17-20]. The estimated elemental HDIVZn dosage for an average 70kg human to be used in our proposed study will be 0.25mg/d x 70kg=17mg/d. Elemental zinc has been administered at a substantially higher dose (ranging from 26.4 to 37.5mg/d for eight successive days) in the treatment of human burns without any side effects [17-19]. In fact, zinc at doses ranging from 5-22mg/d has been administered in humans routinely as a component of parenteral nutrition without any reported side effects [29]. Furthermore, a recently published phase I clinical trial in critically ill children with suspected zinc deficiency involved administration of zinc intravenously at a dose 3-times higher than is proposed in the current study (0.75mg/kg/d for seven days), without any adverse effects [20].

The duration of daily dosing in the current trial was based on previous research assessing dose escalation of intravenous supplemental zinc [20]. This dose escalation study in pediatric critical illness highlighted that with sufficiently high doses of intravenous zinc, a treatment course of seven days resulted in increases of serum zinc.

Participants and Randomisation

Eligibility

Patients will be eligible for randomisation in this study if they are consenting symptomatic hospitalized adult patients fulfilling World Health Organisation's case definition for COVID-19, including a positive polymerase chain reaction (PCR) for COVID19 from any specimen (e.g. respiratory, blood, urine, stool, other bodily fluid) within 14 days of presentation. Broadly speaking, patients included will be those with respiratory associated COVID19 disease. Patients with alternate acute respiratory pathology causing respiratory compromise, such as bacterial pneumonia, will not be deemed suitable for randomisation. Inclusion and exclusion criteria are summarised in Table 2. Patients enrolled in the current trial are eligible to be recruited in alternative trials and may receive other therapeutic interventions including dexamethasone or remdesivir.

Screening procedures

The screening log is designed to monitor patient recruitment. A screening log will be maintained at the participating site by the research coordinator to document patients evaluated for enrolment. The log will provide a record of all patients assessed for eligibility and deemed ineligible for the study. When a patient is considered ineligible, the reason(s) will be noted on the log. The log will also be used to assess patient recruitment targets.

Randomization

This study will be conducted as a randomised double-blinded placebo-controlled study using a stratified randomization method. Ward and ICU patients will be enrolled as soon as possible after fulfilling the criteria of stratification. Patients will be stratified based on whether they require mechanical ventilation. Thereafter, a permuted block randomisation method with variable block sizes of 2, 4 and 6 will be used to allocate eligible patients to either the treatment group, receiving HDIVZn or to the control group in a 1:1 ratio. Randomisation will be performed by the randomisation module in Research Electronic Data Capture (REDCap, Vanderbilt University, USA), which is a secure web application for managing online data collection. Assessment of the unblinding procedures by the project research officer in the case of adverse event will be performed if necessary. The clinical staff involved in patient care will administer the trial drugs as soon as possible after enrolment.

Study drug administration and blinding

Pharmaceutical grade zinc chloride stock solution obtained from an Australian company (Phebra Pty Ltd, Lane Cove West, NSW, Australia) will be diluted in 250ml of

normal saline and infused, resulting in a final dosage of 0.5mg/kg/d. Patients will be administered zinc daily for seven days. To standardise administration time, zinc infusions will commence in the early morning. Zinc will be administered via central venous or peripheral access over 3 hours. Zinc will be administered daily until either discharge or seven days of infusion (depending on which is sooner). The clinical trial nurse (allocation concealment) will use a web-based randomisation program to determine the allocation of patients and then prepare the coded zinc solution or placebo. Each coded solution bag, which will be indistinguishable irrespective of study group, will then be dispensed for administration to the patient as per protocol. This coded identifying study number will also be labelled on the patient case report form (CRF). The investigators, study coordinators, treating physicians, bedside nurses and patients/family will remain blinded to the allocated study solution.

Study assessment

Study objective and endpoints

The endpoints of this study are listed in Table 1. The primary objective is to assess the effect of 7 days of HDIVZn on oxygenation in comparison with placebo in patients with confirmed COVID19. Specifically, oxygen saturations and the requirement of supplemental oxygenation (litres/minute) to maintain acceptable saturations will be measured at various timepoints in conjunction with measurement of routine observations. If applicable, the method of supplemental oxygenation provided will be recorded (eg. nasal prongs, Hudson mask). The primary outcome will be measured by either the highest level of supplemental oxygen (non-ventilated patients) or lowest PaO_2/FiO_2 ratio in ventilated patients.

Secondary outcome measures include ICU and in-hospital mortality, length of stay in ICU or hospital, duration of supplemental oxygen, severe adverse drug events and changes based on WHO R&D Blueprint "Novel Coronavirus COVID19 Therapeutic Trial synopsis". The following is based on an eight-point ordinal scale consisting of:

0, not hospitalized, no clinical or virological evidence of infection;

1, not hospitalized, Infected, and able to resume normal activities;

2, not hospitalized, Infected, but unable to resume normal activities;

3, hospitalized, no requirement of supplemental oxygen;

4, hospitalized, requiring oxygen therapy via mask or nasal prongs;

5, hospitalized, non-invasive ventilation, requiring high flow oxygen;

6, hospitalized, intubation and mechanical ventilation

7, hospitalized, requiring ECMO, invasive mechanical ventilation, additional organ support, RRT;

8, death;

Physical examination, measurements and laboratory tests

All data will be collected by trained staff using a case report form (CRF) worksheet developed by the study team. Data will then be entered into the REDCap web database (electronic CRF [eCRF]). Randomised patients will be followed up to discharge, death or 28 days post-randomisation whichever occurs first.

Study day 1 commences on randomisation and concludes at the expiry of the calendar day. Data collection will be restricted primarily to those variables necessary to define clinical patient characteristics including baseline demographics, primary diagnoses, physiological parameters, diagnostic interventions, therapeutic interventions and documentation of deaths and other serious adverse events. A complete list of collected data is summarised in Table 3.

Adverse events

An adverse reaction is defined as any untoward and unintended response to an investigational medicinal product related to any dose administered. All adverse events judged by either the reporting investigator or the sponsor as having a reasonable possibility of a causal relationship to an investigational medicinal product will qualify as adverse reactions. Adverse events were classified per the Common Terminology Criteria for Adverse Events (CTCAE V5)[30]. All adverse events which are considered to be potentially causally related to the study intervention or are otherwise of concern in the investigator's judgement will be reported. An interim analysis is planned after 50% (80) of the patients have been randomised. Development of severe adverse events (Grade 4), such as severe kidney or hepatic injury, will result in cessation of the trial for the participant and all infusions related to the trial will be stopped. Additionally, the treatment will be discontinued in cases where the participant wishes to withdraw from the trial.

Safety evaluations

An independent Data Monitoring Committee (DMC), consisting of experts in intensive care, clinical research and biostatistics will be established before patient enrolment and will review all trial protocols. The role of the DMC will be to provide study

oversight to ensure that the rights and safety of patients involved in the study are protected by reviewing reported Adverse Events and making recommendations to the Management Committee (MC).

Study analysis

Sample size

The primary outcome of this study is related to the effect of zinc therapy on the level of oxygenation expressed either as worst (highest) oxygen flow (in litres/min) in non-ventilated patients or worst (lowest) PaO_2 (in mmHg)/FiO_2 (as a fraction of 1) ratio in ventilated patients. We hypothesize that zinc therapy will decrease the worst level of oxygenation during the seven days (of the treatment period) by 20% compared to placebo from a mean worst value of 10L/min (placebo) to a mean of 8L/min (zinc) or for PaO_2/FiO_2 ratio from a mean worst value of 150 (placebo) to a mean worst value of 180 (zinc). If patients transition from non-ventilated to ventilated during the study period, the PaO_2/FiO_2 ratio will be used.

To have an 80% power to see such an effect at an alpha of 0.05 in non-ventilated patients, assuming a standard deviation (SD) for O_2 flow of 4L/min 28 patients would have to be randomized in each arm. In ventilated patients, to have an 80% power to see such an effect at an alpha of 0.05, assuming an SD for the PaO_2/FiO_2 ratio of 60, 49 patients would have to be randomised in each arm.

Rounding off the first group to 30 per arm and the second group to 50 per arm to account for withdrawals, we estimate that a study of 160 patients would provide a suitable sample size to test our primary hypothesis.

Statistical evaluation

The descriptive analysis of the data will include the calculation of summary statistics including means, standard deviations, and absolute and relative frequencies of the baseline and follow-up data. If baseline variables are not approximately normally distributed, suitable transformations or medians with interquartile ranges will be presented. Any baseline variable differing by greater than 10% or 0.5 standard deviations between arms, will be subsequently investigated in a sensitivity analysis.

Any continuous variable outcomes that appear to be non-normal will be considered for transformation to improve normality of the residuals for subsequent regression analysis. Transformation will depend on: distribution of the variable, distribution of residuals, ease of

interpretation, and impact on main results. The co-primary outcome analyses will compare 1) the change in highest level of supplemental oxygenation in non-ventilated patients, and 2) the change in lowest PaO2/FiO2 ratio in ventilated patients through the use of multivariable linear regression. Secondary outcome analyses will involve a mix of linear, logistic, and ordinal logistic regression modelling. Assumptions of the relevant models will be checked along with alternative transformations or non-parametric methods as appropriate.

Tests of interaction between dichotomised variables and treatment pathway will be carried out to test for differing treatment effect between participants. Sensitivity analyses will be conducted to explore the robustness of the estimate of the effect. Analyses will include: complete case analysis; per-protocol analysis, adjustment for baseline, and adjustment for imbalance at baseline (if baseline measures differ substantially).

A p-value 0.05 will indicate statistical significance. A full model with clinical relevant covariates (e.g. sex, age, previous heart surgery, preoperative creatinine) will be used for a stepwise backward variable selection procedure to identify independent risk factors for acute kidney injury (AKI). A senior statistician will perform data analysis on an intention-to-treat basis. An interim analysis on the safety and the primary outcomes will be performed when 50% (80/160) of the patients have received zinc or placebo for at least seven days. Summary statistics will be used to describe the clinical data and presented as mean ± SD, median with interquartile range (IQR) or percentages as appropriate. Chi-squared analysis with Fisher's exact test (when appropriate) and Student's t-test (Mann Whitney U test for non-normal distributions) will be used to compare data between the active treatment group and the control group with statistical significance declared for probability values of 0.05 or less. Analysis of the outcome of excluded patients due to other trials etc. will be in accordance with the CONSORT guidelines.

Discussion

This single centre, double-blind, randomised controlled trial will assess the role of HDIVZn in protection against the sequelae associated with SARS-CoV-2 virus. The beneficial effects of zinc on viral infections have been previously demonstrated [31 32]. Further, HDIVZn may provide additional protection to other end organs that may be indirectly affected by pulmonary injury and impaired oxygenation.

In coronaviruses, zinc inhibits both the proteolytic processing of replicase polyproteins and the RNA-dependent RNA polymerase (RdRp) activity [13]. Although mechanisms of action of zinc are unknown, several possibilities exist. Firstly, DNA and RNA

polymerases use divalent metal ions like Mg^{2+} as a co-factor, and one possible mechanism is that zinc displaces Mg^{2+} and subsequently inhibits RdRp activity [33]. In support is the observation that various divalent metals ions sustained the activity of poliovirus RdRp in the following preference Mn^{2+}> Co^{2+}> Ni^{2+}> Fe^{2+}> Mg^{2+}> Ca^{2+}> Cu^{2+} [34]. In contrast, zinc was incapable of sustaining RdRp catalyzed nucleotide incorporation [34]. Secondly, a zinc-binding pocket has been identified in the Dengue virus and SARS-coronavirus RdRp [13]. Therefore, it is possible that binding of zinc may induce a structural change in the conformation of RdRp which disables RdRp to catalyze nucleotide incorporation. Finally, adding high concentrations of zinc ions to cells impairs viral polyprotein processing which is integral to virus replication [35].

In addition to the direct effect on viral replication and activity, HDIVZn may play a protective role in alternate organs. Zinc has been demonstrated to be beneficial in reducing mortality in patients with severe pneumonia[36]. Further, in SARS-CoV-2, respiratory compromise results in impaired oxygenation and hypoxia to various end organs. Such hypoxia may contribute to end-organ failure and increase the risk of mortality. Specifically, such COVID19 associated hypoxia has been proposed to be contributory to cardiac injury [37], hepatic injury [38] and renal injury [39] [40]. Our published studies have shown that HDIVZn protects various organs, including the heart, kidneys and liver against the damage caused by hypoxia. It should be noted that hypoxia and oxidative stress, result in an increase in reactive oxygen species (ROS), including superoxide (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical (·OH) – which result in intracellular damage [41] [42]. Zinc appears to limit ROS production by several mechanisms. Firstly, metallothioneins (MTs), small cysteine-rich and heavy metal-binding proteins, participate in the intracellular defence against reactive oxygen and nitrogen species [43] and zinc has been shown to induce MT mRNA and protein expression. Secondly, zinc competes with Fe^{2+} and Cu^{2+} ions for binding to cell membranes and proteins – normally, these active metals catalyze the production of hydroxyl radical from H_2O_2 via Fenton chemistry. Thirdly, zinc upregulates the production and activation of antioxidant proteins, molecules and enzymes such as glutathione, catalase and superoxide dismutase (SOD), which catalyze O_2^- to oxygen or H_2O_2 [44]. Finally, zinc reduces the activation of oxidant-promoting enzymes such as inducible nitric acid synthase and NADPH enzyme, which catalyze oxygen to O_2^-. Accordingly, we hypothesize that Zinc may provide protection against the hypoxic injury that critically ill patients with COVID19 may experience.

The specific strengths of the current protocol design are a) prolonged exposure of HDIVZn and b) assessment of critically ill patients, a population where a benefit would be

observed if truly present. There is an inherent difficulty in assessing pulmonary response in clinical trials, and accordingly, the primary outcome measure is a possible criticism. However, maximal oxygen requirements and PaO_2/FiO_2 are well established surrogate markers [45 46], and have been used in recent comparable trials [47-49].

The safety of HDIVZn has been addressed in previous literature [17-20]. Elemental zinc has been administered at a substantially higher dose (ranging from 26.4 to 37.5mg/d for eight successive days) in the treatment of burns and did not produce any side effects in humans [17-19]. In the setting of critically ill patients, zinc intravenously at 3-times higher dose than the current study (0.75mg/kg/d for seven days) did not produce any adverse effects [20].

In conclusion, we designed a single-centre, double-blind, randomised controlled trial to assess the potential benefit of HDIVZn for hospitalised or critically ill patients with SARS-CoV-2 infection and associated respiratory compromise. We believe that our well-designed trial will be able to expediently identify a potential agent that may improve outcomes for these critically ill patients.

Contributors: MP assisted in protocol design, ethical submission and manuscript production. JEK and VC were involved in data collection and manuscript production. LQ was involved in statistical design and analysis. PJ, JT, CM were involved in patient care and study procotol design. DJ, DB, RB, OP and JI were involved in project design, patient care, manuscript production and supervision.

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References

1. Hu Y, Sun J, Dai Z, et al. Prevalence and severity of corona virus disease 2019 (COVID-19): A systematic review and meta-analysis. *J Clin Virol* 2020;127:104371. doi: 10.1016/j.jcv.2020.104371 [published Online First: 2020/04/22]
2. Tu H, Tu S, Gao S, et al. The epidemiological and clinical features of COVID-19 and lessons from this global infectious public health event. *J Infect* 2020 doi: 10.1016/j.jinf.2020.04.011 [published Online First: 2020/04/22]
3. Weston S, Frieman MB. COVID-19: Knowns, Unknowns, and Questions. *mSphere* 2020;5(2) doi: 10.1128/mSphere.00203-20 [published Online First: 2020/03/20]
4. Zhu J, Zhong Z, Li H, et al. CT imaging features of 4,121 patients with COVID-19: a meta-analysis. *J Med Virol* 2020 doi: 10.1002/jmv.25910 [published Online First: 2020/04/22]
5. Ledford H. How does COVID-19 kill? Uncertainty is hampering doctors' ability to choose treatments. *Nature* 2020;580(7803):311-12. doi: 10.1038/d41586-020-01056-7 [published Online First: 2020/04/11]
6. Wang Y, Zhang D, Du G, et al. Remdesivir in adults with severe COVID-19: a randomised, double-blind, placebo-controlled, multicentre trial. *The Lancet* 2020 doi: 10.1016/S0140-6736(20)31022-9
7. Ischia J, Bolton DM, Patel O. Why is it worth testing the ability of zinc to protect against ischaemia reperfusion injury for human application. *Metallomics* 2019;11(8):1330-43. doi: 10.1039/c9mt00079h
8. Prasad AS. Zinc is an Antioxidant and Anti-Inflammatory Agent: Its Role in Human Health. *Frontiers in nutrition* 2014;1:14.
9. Hulisz D. Efficacy of zinc against common cold viruses: an overview. *J Am Pharm Assoc* 2004;44(5):594-603. doi: 10.1331/1544-3191.44.5.594.hulisz [published Online First: 2004/10/22]
10. Suara RO, Crowe JE, Jr. Effect of zinc salts on respiratory syncytial virus replication. *Antimicrob Agents Chemother* 2004;48(3):783-90. doi: 10.1128/aac.48.3.783-790.2004 [published Online First: 2004/02/26]
11. Li D, Wen LZ, H Yu. [Observation on clinical efficacy of combined therapy of zinc supplement and jinye baidu granule in treating human cytomegalovirus infection]. *Zhongguo Zhong Xi Yi Jie He Za Zhi* 2005;25(5):449-51. [published Online First: 2005/06/17]

12. Femiano F, Gombos F, Scully C. Recurrent herpes labialis: a pilot study of the efficacy of zinc therapy. *J Oral Pathol Med* 2005;34(7):423-5. doi: 10.1111/j.1600-0714.2005.00327.x [published Online First: 2005/07/14]
13. te Velthuis AJ, van den Worm SH, Sims AC, et al. Zn(2+) inhibits coronavirus and arterivirus RNA polymerase activity in vitro and zinc ionophores block the replication of these viruses in cell culture. *PLoS Pathog* 2010;6(11):e1001176. doi: 10.1371/journal.ppat.1001176 [published Online First: 2010/11/17]
14. O'Kane D, Gibson L, May CN, et al. Zinc preconditioning protects against renal ischaemia reperfusion injury in a preclinical sheep large animal model. *Biometals* 2018;31(5):821-34. doi: 10.1007/s10534-018-0125-3
15. Rao K, Sethi K, Ischia J, et al. Protective effect of zinc preconditioning against renal ischemia reperfusion injury is dose dependent. *PLoS ONE* 2017;12(7):e0180028. doi: 10.1371/journal.pone.0180028
16. Cheung E, Nikfarjam M, Jackett L, et al. The Protective Effect of Zinc Against Liver Ischaemia Reperfusion Injury in a Rat Model of Global Ischaemia. *Journal of Clinical and Experimental Hepatology* 2019;August 20, 2019, In press. doi: 10.1016/j.jceh.2019.07.006
17. Berger MM, Baines M, Raffoul W, et al. Trace element supplementation after major burns modulates antioxidant status and clinical course by way of increased tissue trace element concentrations. *Am J Clin Nutr* 2007;85(5):1293-300. doi: 10.1093/ajcn/85.5.1293
18. Berger MM, Binnert C, Chiolero RL, et al. Trace element supplementation after major burns increases burned skin trace element concentrations and modulates local protein metabolism but not whole-body substrate metabolism. *Am J Clin Nutr* 2007;85(5):1301-6. doi: 10.1093/ajcn/85.5.1301
19. Berger MM, Spertini F, Shenkin A, et al. Trace element supplementation modulates pulmonary infection rates after major burns: a double-blind, placebo-controlled trial. *Am J Clin Nutr* 1998;68(2):365-71. doi: 10.1093/ajcn/68.2.365
20. Cvijanovich NZ, King JC, Flori HR, et al. Safety and Dose Escalation Study of Intravenous Zinc Supplementation in Pediatric Critical Illness. *JPEN J Parenter Enteral Nutr* 2016;40(6):860-8. doi: 10.1177/0148607115572193
21. Chan AW, Tetzlaff JM, Altman DG, et al. SPIRIT 2013 statement: defining standard protocol items for clinical trials. *Ann Intern Med* 2013;158(3):200-7. doi: 10.7326/0003-4819-158-3-201302050-00583 [published Online First: 2013/01/09]
22. Institute of Medicine Panel on M. Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. Washington (DC): National Academies Press (US)

23. Solomons NW, Romero-Abal ME, Weiss G, et al. Bioavailability of zinc from NutriSet zinc tablets compared with aqueous zinc sulfate. *Eur J Clin Nutr* 2011;65(1):125-31. doi: 10.1038/ejcn.2010.198

24. Feillet-Coudray C, Meunier N, Rambeau M, et al. Long-term moderate zinc supplementation increases exchangeable zinc pool masses in late-middle-aged men: the Zenith Study. *Am J Clin Nutr* 2005;82(1):103-10. doi: 10.1093/ajcn.82.1.103
25. Keyzer JJ, Oosting E, Wolthers BG, et al. Zinc absorption after oral administration of zinc sulfate. *Pharm Weekbl Sci* 1983;5(5):252-3.
26. Hempe JM, Carlson JM, Cousins RJ. Intestinal metallothionein gene expression and zinc absorption in rats are zinc-responsive but refractory to dexamethasone and interleukin 1 alpha. *J Nutr* 1991;121(9):1389-96.
27. Nair AB, Jacob S. A simple practice guide for dose conversion between animals and human. *J Basic Clin Pharm* 2016;7(2):27-31. doi: 10.4103/0976-0105.177703
28. Bennett JW. Regional Body Surface-Area of Sheep. *J Agric Sci* 1973;81(Dec):429-32. doi: Doi 10.1017/S0021859600086469
29. Stehle P, Stoffel-Wagner B, Kuhn KS. Parenteral trace element provision: recent clinical research and practical conclusions. *Eur J Clin Nutr* 2016;70(8):886-93. doi: 10.1038/ejcn.2016.53
30. Common Terminology Criteria for Adverse Events (CTCAE v5.0). *National Cancer Institute* 2017:https://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_8.5x11.pdf.
31. Science M, Johnstone J, Roth DE, et al. Zinc for the treatment of the common cold: a systematic review and meta-analysis of randomized controlled trials. *CMAJ* 2012;184(10):E551-61. doi: 10.1503/cmaj.111990 [published Online First: 2012/05/09]
32. Bhatnagar S, Wadhwa N, Aneja S, et al. Zinc as adjunct treatment in infants aged between 7 and 120 days with probable serious bacterial infection: a randomised, double-blind, placebo-controlled trial. *Lancet* 2012;379(9831):2072-8. doi: 10.1016/S0140-6736(12)60477-2 [published Online First: 2012/06/05]
33. Ferrari E, Wright-Minogue J, Fang JW, et al. Characterization of soluble hepatitis C virus RNA-dependent RNA polymerase expressed in Escherichia coli. *J Virol* 1999;73(2):1649-54. doi: 10.1128/jvi.73.2.1649-1654.1999 [published Online First: 1999/01/09]
34. Arnold JJ, Ghosh SK, Cameron CE. Poliovirus RNA-dependent RNA polymerase (3D(pol)). Divalent cation modulation of primer, template, and nucleotide selection. *J Biol Chem* 1999;274(52):37060-9. doi: 10.1074/jbc.274.52.37060 [published Online First: 1999/12/22]
35. Butterworth BE, Korant BD. Characterization of the large picornaviral polypeptides produced in the presence of zinc ion. *J Virol* 1974;14(2):282-91. [published Online First: 1974/08/01]
36. Wang L, Song Y. Efficacy of zinc given as an adjunct to the treatment of severe pneumonia: A meta-analysis of randomized, double-blind and placebo-controlled trials. *Clin Respir J* 2018;12(3):857-64. doi: 10.1111/crj.12646 [published Online First: 2017/05/11]
37. Zhu H, Rhee JW, Cheng P, et al. Cardiovascular Complications in Patients with COVID-19: Consequences of Viral Toxicities and Host Immune Response. *Curr Cardiol Rep* 2020;22(5):32. doi: 10.1007/s11886-020-01292-3 [published Online First: 2020/04/23]

38. Feng G, Zheng KI, Yan QQ, et al. COVID-19 and Liver Dysfunction: Current Insights and Emergent Therapeutic Strategies. *J Clin Transl Hepatol* 2020;8(1):18-24. doi: 10.14218/jcth.2020.00018 [published Online First: 2020/04/11]
39. Fanelli V, Fiorentino M, Cantaluppi V, et al. Acute kidney injury in SARS-CoV-2 infected patients. *Crit Care* 2020;24(1):155. doi: 10.1186/s13054-020-02872-z [published Online First: 2020/04/18]
40. Mubarak M, Nasri H. COVID-19 nephropathy; an emerging condition caused by novel coronavirus infection. *J Nephropathol* 2020;9(3):e21-e21. doi: 10.34172/jnp.2020.21
41. Castro L, Freeman BA. Reactive oxygen species in human health and disease. *Nutrition* 2001;17(2):161-65. doi: 10.1016/S0899-9007(00)00570-0
42. Lachance PA, Nakat Z, Jeong WS. Antioxidants: An integrative approach. *Nutrition* 2001;17(10):835-38. doi: 10.1016/S0899-9007(01)00636-0
43. Ruttkay-Nedecky B, Nejdl L, Gumulec J, et al. The role of metallothionein in oxidative stress. *Int J Mol Sci* 2013;14(3):6044-66. doi: 10.3390/ijms14036044 [published Online First: 2013/03/19]
44. Prasad AS. Zinc: an antioxidant and anti-inflammatory agent: role of zinc in degenerative disorders of aging. *J Trace Elem Med Biol* 2014;28(4):364-71. doi: 10.1016/j.jtemb.2014.07.019 [published Online First: 2014/09/10]
45. Matthay MA, McAuley DF, Ware LB. Clinical trials in acute respiratory distress syndrome: challenges and opportunities. *The Lancet Respiratory Medicine* 2017;5(6):524-34. doi: 10.1016/S2213-2600(17)30188-1
46. Donahoe M. Acute respiratory distress syndrome: A clinical review. *Pulm Circ* 2011;1(2):192-211. doi: 10.4103/2045-8932.83454
47. Frat JP, Ricard JD, Quenot JP, et al. Non-invasive ventilation versus high-flow nasal cannula oxygen therapy with apnoeic oxygenation for preoxygenation before intubation of patients with acute hypoxaemic respiratory failure: a randomised, multicentre, open-label trial. *Lancet Respir Med* 2019;7(4):303-12. doi: 10.1016/s2213-2600(19)30048-7 [published Online First: 2019/03/23]
48. He H, Sun B, Liang L, et al. A multicenter RCT of noninvasive ventilation in pneumonia-induced early mild acute respiratory distress syndrome. *Crit Care* 2019;23(1):300. doi: 10.1186/s13054-019-2575-6 [published Online First: 2019/09/06]
49. Pennisi MA, Bello G, Congedo MT, et al. Early nasal high-flow versus Venturi mask oxygen therapy after lung resection: a randomized trial. *Crit Care* 2019;23(1):68. doi: 10.1186/s13054-019-2361-5 [published Online First: 2019/03/02]

Table 1: Primary and secondary outcomes

Key Primary Outcome	Key Secondary Outcomes
Mean change in the highest level of oxygenation (oxygen flow measured litre/min) in non-ventilated patients Mean change in lowest PaO_2 in ventilated patients	Mortality (ICU or in-hospital) Duration of mechanical ventilation Duration of oxygen therapy Duration of hospitalisation Length of Stay in ICU
Other Secondary Outcomes	
Adverse Drug Events Acute kidney injury Acute liver injury Duration of vasopressor drugs Sequential Organ Failure Assessment (SOFA) respiratory score	Clinical improvement based on an eight-point ordinal scale recommended in the document published by WHO R&D Blueprint "Novel Coronavirus COVID-19 Therapeutic Trial Synopsis". • Percentage of patients reporting each severity rating on an 8-point ordinal scale [Time Frame: Day 14] • Time to improvement in one category from admission using the 8-point ordinal scale [Time Frame: Up to day 28] • Mean change in the 8-point ordinal scale [Time Frame: Up to day 28]

Table 2: inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
• Consenting adult patients adult male or female, age ≥ 18 years old. Laboratory-confirmed SARS-CoV-2 infection as determined by polymerase chain reaction (PCR) • Hospitalized with an illness of any duration with evidence of pneumonia and severe disease, critical disease, or multi-system organ dysfunction at baseline • Ability to provide informed consent signed by study patient or legally acceptable representative • Willingness and ability to comply with study-related procedures/assessments • Have an oxygen saturation (SaO_2) of 94% or less while they were breathing ambient air or a ratio of the partial pressure of oxygen (PaO_2) to the fraction of inspired oxygen (FiO_2) (Pao2: Fio2) at or below 300 mg Hg. • No chronic kidney disease (CKD) defined by stage II or higher using the Kidney Disease Improving Global Outcomes (KDIGO) classification	• Age <18 or pregnant or lactating female • Allergy to zinc • Severe hepatic impairment defined as Child C liver disease. • eGFR ≤ 60 mL/min/1.73 m^2 (defined using CKD-EPI SCr formula) • History of any organ transplant which requires active immunosuppressive treatment which can interfere with kidney function • If a patient required any of the following within seven days prior to cardiac surgery: defibrillation, mechanical ventilation, left ventricular assist device (LVAD), or other forms of mechanical circulatory support (MCS) • If a patient required cardiopulmonary resuscitation (CPR) within 14 days • DNR (do not resuscitate) DNI (do not intubate) orders • Death is deemed imminent or inevitable during this admission, and either the attending physician, patient or substitute decision-maker is not committed to active treatment • Already receiving dialysis (either acute or chronic) or imminent need of dialysis at the time of enrolment • Patients with known HIV infection • Patients with a known or suspected history of oxalate nephropathy or hyperoxaluria, scurvy, chronic iron overload, G-6PD deficiency • Clinician expects to prescribe Zinc for another indication • Patients with known haemochromatosis.

Table 3: Collected data during trial

	All patients	
Baseline data	Baseline data • Demographics: Age, gender, place of residence • Comorbidities: Diabetes mellitus, arterial hypertension, congestive heart failure, chronic obstructive airways disease, chronic liver disease, malignancy, chronic renal failure • Pre-admission medication • Functional status / frailty score	
	Non-ventilated patients	Ventilated patients
Daily observations	• Daily saturations (Worst values) • Daily oxygen flow • Fluid input • Urine output • Fluid balance	• Daily PaO_2/FiO_2 ratio • Daily oxygen flow • Fluid input • Urine output • Fluid balance • Vasopressor data
Laboratory investigations (collected at 0800)	• Daily serum creatinine • Daily liver function • Daily blood count • Daily zinc and trace metal concentration (copper, potassium, magnesium) • Daily Cardiac troponin • Daily lactate level	• Daily serum creatinine • Daily liver function • Daily blood count • Daily zinc and trace metal concentration (copper, potassium and magnesium) • Daily Cardiac troponin • Daily lactate level
Primary outcome measures	• Mean change in highest level of oxygenation requirement (oxygen flow in litres/min)	• Mean change in lowest PaO_2/FiO_2 ratio (in mmHg)
Secondary outcome measures	• Mortality [Time Frame: Up to day 28] • Duration of oxygen therapy (days) • Duration of hospitalization (days) • Length of stay in the intensive care unit and hospital • Frequency of Serious Adverse Drug Events • Acute kidney injury • Acute liver injury • Time to resolution of fever for at least 48 hours without antipyretics by clinical severity • Incidence of severe or life-threatening bacterial, invasive fungal, or	• Mortality [Time Frame: Up to day 28] • Duration of mechanical ventilation(days) • Duration of oxygen therapy (days) • Duration of hospitalization (days) • Length of stay in the intensive care unit and hospital • Frequency of Serious Adverse Drug Events • Acute kidney injury • Acute liver injury • Use, duration and dosage of vasopressor drugs • Time to resolution of fever for at least 48 hours without

	opportunistic infection • Number of patients admitted into an intensive care unit (ICU) [Time Frame: Up to day 28] • Sequential Organ Failure Assessment (SOFA) Respiratory Score [Time Frame: 28 days]. Assigned a point value from 0 (normal) to 4 (high degree of dysfunction/failure) • Clinical improvement based on an eight-point ordinal scale recommended in the document published by WHO R&D Blueprint "Novel Coronavirus COVID-19 Therapeutic Trial Synopsis". ○ Percentage of patients reporting each severity rating on an 8-point ordinal scale [Time Frame: Day 14] ○ Time to improvement in one category from admission using the 8-point ordinal scale [Time Frame: Up to day 28] ○ Mean change in the 8-point ordinal scale [Time Frame: Up to day 28]	antipyretics by clinical severity • Incidence of severe or life-threatening bacterial, invasive fungal, or opportunistic infection • Number of patients admitted into an intensive care unit (ICU) [Time Frame: Up to day 28] • Sequential Organ Failure Assessment (SOFA) Respiratory Score [Time Frame: 28 days]. Assigned a point value from 0 (normal) to 4 (high degree of dysfunction/failure) • Clinical improvement based on an eight-point ordinal scale recommended in the document published by WHO R&D Blueprint "Novel Coronavirus COVID-19 Therapeutic Trial Synopsis". ○ Percentage of patients reporting each severity rating on an 8-point ordinal scale [Time Frame: Day 14] ○ Time to improvement in one category from admission using the 8-point ordinal scale [Time Frame: Up to day 28] ○ Mean change in the 8-point ordinal scale [Time Frame: Up to day 28]

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A randomised controlled trial for high-dose intravenous zinc as adjunctive therapy in SARS-CoV-2 (COVID-19) positive critically ill patients: trial protocol

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A randomised controlled trial for high-dose intravenous zinc as adjunctive therapy in SARS-CoV-2 (COVID-19) positive critically ill patients: trial protocol

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Abstract

Introduction

SARS-CoV-2 (COVID19) has caused an international pandemic of respiratory illness, resulting in significant healthcare and economic turmoil. To date, no robust vaccine or treatment has been identified. Elemental zinc has previously been demonstrated to have beneficial effects on coronaviruses and other viral respiratory infections due to its effect on RNA polymerase. Additionally, zinc has well demonstrated protective effects against hypoxic injury – a clear mechanism of end-organ injury in respiratory distress syndrome. We aimed to assess the effect of high dose intravenous zinc (HDIVZn) on SARS-CoV-2 infection. The end of study analyses will evaluate the reduction of impact of oxygen saturations or requirement of oxygen supplementation.

Methods and Analysis

We designed a double-blind randomised controlled trial of daily HDIVZn (0.5mg/kg) versus placebo. Primary outcome measures are lowest oxygen saturation (or greatest level of supplemental oxygenation) for non-ventilated patients and worst PaO_2/FiO_2 for ventilated patients. Following power calculations, 60 hospitalised patients and 100 ventilated patients will be recruited to demonstrate a 20% difference. The duration of follow-up is up to the point of discharge.

Ethics and dissemination

Ethical approval was obtained through the independent Human Research Ethics Committee. Participant recruitment will commence in May 2020. Results will be published in peer-reviewed medical journals.

Trial Registration

Clinical trial registered with Australian New Zealand Clinical Trials Registry (ACTRN126200000454976). No external funding was sought for the completion of this project.

Strengths and Limitations of this study

- This is the first trial designed primarily to assess the effect of high dose intravenous zinc in SARS-CoV-2
- A strength of this study is its randomised, double-bling, placebo controlled nature of the study design
- This is an adequately powered study with objective, universal primary and secondary outcome measures
- Potential limitations pertain to conducting the current study in Australia, a country with a relatively low prevalence of COVID19 with a risk of under recruitment

Introduction

Since December 2019, a new coronavirus, designated SARS-CoV-2, has caused an international pandemic of respiratory illness termed COVID19, posing significant threats to global health and the economy [1-3]. In more severe cases, COVID19 enters the lungs, causing respiratory complications such as bronchitis and pneumonia [4 5]. Development of pneumonia leads to a reduced ability for oxygenation and in some cases the development of acute respiratory distress syndrome (ARDS), requiring mechanical ventilation. In the most severe cases, patients can develop multiple organ failure and hypoxic brain injury. In the absence of an effective vaccine or robust treatment for people with the disease [6], there is an urgent need to find a treatment that inhibits virus replication or reduces the progression of the disease.

Zinc is a naturally occurring essential heavy metal, and zinc deficiency is associated with a range of pathological conditions, including retarded growth and delayed wound healing and tissue repair. Zinc is also important for the maintenance and development of the immune system and plays a role in cell division and growth. Zinc deficiency results in reduced immunity and increases susceptibility to infectious diseases [7 8]. Numerous studies report the potential of zinc and zinc salts to inhibit viral infections in clinical and experimental settings. Viral infections that have been successfully inhibited by zinc include the common cold (a type of coronavirus) [9], respiratory syncytial virus infections [10], cytomegalovirus infections [11] and herpes labialis [12]. More importantly, zinc is a potent inhibitor of the replication of SARS-coronavirus (SARS-CoV) and equine arteritis virus (EAV) in cell culture [13].

Furthermore, our published studies have shown that high dose intravenous zinc (HDIVZn) protects various organs, including the heart, kidneys and liver against the damage caused by hypoxia [7 14-16]. In addition to being efficacious, the dose of elemental zinc used for this HDIVZn trial (0.25mg/kg (0.5mg/kg ZnCl2) and applied in our preclinical study in sheep is very safe and well-tolerated in humans as verified from multiple observational reports including the treatment of very sick children in an ICU setting with suspected zinc deficiency [17-20].

We plan to perform a single site randomised controlled trial to test the efficacy and safety of HDIVZn administered as a daily injection in subjects with COVID19 infection to assess whether giving HDIVZn to patients improves clinical outcomes.

Methods and Design

Ethical approval was sought through the Austin Health institutional Human Research Ethics Committee (Version 2, 4th April 2020). Any modifications to the trial protocol will be approved prospectively by the associated Ethical Committee. This clinical trial was registered with Australian New Zealand Clinical Trials Registry (ACTRN126200000454976). The protocol design was produced in adherence with the Standard Protocol Items for Randomised Trials (SPIRIT) [21]. No external funding was sought for the completion of this project.

Patient and public involvement

The development of the research question stemmed from the urgent global crisis inflicted by COVID19, and the urgent need for a therapy to reduce the impact of the disease on the affected patients. Patients were not intrinsically involved in the design, recruitment or conduct of the study. Results of the study will be disseminated to patients after publication in peer-review journal. While the burden of the treatment or placebo was not assessed directly by patients, it was deemed minimal due to the lack of deviation from standard care.

Study design

This is a Phase 2, double-blind, placebo-controlled, randomised study at a single institution. The study plans to randomise 160 hospitalised participants, including 100 critically-ill patients with confirmed COVID19 (as detailed in Table 1) and to evaluate the efficacy and safety of HDIVZn over a seven-day period of treatment.

Dosing rationale

The upper limit for daily zinc intake in an adult is 40mg [22]. However, when 30 mg of elemental zinc was given orally to humans, it resulted in an only 1.8-fold increase in plasma zinc in the first 4 hours [23]. A similar study in humans where elemental zinc (30 mg/d) or placebo were given orally for six months showed a statistically significant in the plasma zinc from 14.18 ± 1.75 µmol/L in the placebo group to 17.18 ± 3.48 µmol/L in the zinc group [24]. Oral delivery of zinc is affected by several factors, including normal variations in gut zinc absorption, dietary factors such as the presence of phytate, and interactions with other metal ions [25]. Also, repeated high oral zinc intake causes a rapid and significant upregulation of intestinal metallothioneins which markedly decrease subsequent gut zinc, and importantly copper, absorption [26]. The latter may lead to copper deficiency in patients

administered zinc for prolonged periods.

In our recently published sheep study, we determined that a single IV dose of $ZnCl_2$ at 0.5mg/kg increased the plasma zinc concentration by 4-fold from a baseline concentration of 11.3 ± 0.4 µmol/L to 46.5 ± 1.5 µmol/L within two hours [14]. Interestingly, two doses of IV $ZnCl_2$ at 0.5mg/kg increased the plasma zinc concentration 7-fold from a baseline concentration of 11.3 ± 0.4 µmol/L to 70.1 ± 5.8 µmol/L.

Translation of a drug's dose from animals to humans depends on the drug dose correction factor (K_m) which is calculated by dividing the average body weight (kg) of a species by its body surface area (m^2). For humans, K_m is 37 [27]. Direct carryover of a drug's pharmacologic dosage, from animals to humans depends on how similar the K_m value of the animal species in which efficacy was tested, is to human K_m. As published by Nair et al [27], K_m for rats is 6, for rabbits is 12 and for sheep is 36 (average body weight of sheep is 40kg, and body surface area is 1.10m^2 [28]). Therefore, the near similar K_m value of sheep (36) to that of humans (37) has allowed us to conclude that human equivalent elemental zinc dosage would equate to 0.25mg/kg, the same dose that was shown to be protective in the sheep study and which we plan to use in our proposed trial.

Information regarding the safety of HDIVZn can be derived from published reports where humans were treated with high doses of zinc [17-20]. The estimated elemental HDIVZn dosage for an average 70kg human to be used in our proposed study will be 0.25mg/d x 70kg=17mg/d. Elemental zinc has been administered at a substantially higher dose (ranging from 26.4 to 37.5mg/d for eight successive days) in the treatment of human burns without any side effects [17-19]. In fact, zinc at doses ranging from 5-22mg/d has been administered in humans routinely as a component of parenteral nutrition without any reported side effects [29]. Furthermore, a recently published phase I clinical trial in critically ill children with suspected zinc deficiency involved administration of zinc intravenously at a dose 3-times higher than is proposed in the current study (0.75mg/kg/d for seven days), without any adverse effects [20].

The duration of daily dosing in the current trial was based on previous research assessing dose escalation of intravenous supplemental zinc [20]. This dose escalation study in pediatric critical illness highlighted that with sufficiently high doses of intravenous zinc, a treatment course of seven days resulted in increases of serum zinc.

Participants and Randomisation

Eligibility

Patients will be eligible for randomisation in this study if they are consenting symptomatic hospitalized adult patients fulfilling World Health Organisation's case definition for COVID-19, including a positive polymerase chain reaction (PCR) for COVID19 from any specimen (e.g. respiratory, blood, urine, stool, other bodily fluid) within 14 days of presentation. Broadly speaking, patients included will be those with respiratory associated COVID19 disease. Patients with alternate acute respiratory pathology causing respiratory compromise, such as bacterial pneumonia, will not be deemed suitable for randomisation. Inclusion and exclusion criteria are summarised in Table 1. Patients enrolled in the current trial are eligible to be recruited in alternative trials and may receive other therapeutic interventions including dexamethasone or remdesivir.

Screening procedures

The screening log is designed to monitor patient recruitment. A screening log will be maintained at the participating site by the research coordinator to document patients evaluated for enrolment. The log will provide a record of all patients assessed for eligibility and deemed ineligible for the study. When a patient is considered ineligible, the reason(s) will be noted on the log. The log will also be used to assess patient recruitment targets. Patients will be consented by trial investigators by completion of consent and assent forms from the patient directly or surrogates if the patient does not have the ability to consent (Supplementary 1).

Randomization

This study will be conducted as a randomised double-blinded placebo-controlled study using a stratified randomization method. Ward and ICU patients will be enrolled as soon as possible after fulfilling the criteria of stratification. Consenting patients will be stratified based on whether they require mechanical ventilation. Thereafter, a permuted block randomisation method with variable block sizes of 2, 4 and 6 will be used to allocate eligible patients to either the treatment group, receiving HDIVZn or to the control group in a 1:1 ratio. Randomisation will be performed by the randomisation module in Research Electronic Data Capture (REDCap, Vanderbilt University, USA), which is a secure web application for managing online data collection. Patients, clinical staff, outcome assessors and data analysts will be blinded. Assessment of the unblinding procedures by the project research officer in the case of adverse event will be performed if necessary. The clinical staff involved in patient care will administer the trial drugs as soon as possible after enrolment.

Study drug administration and blinding

Pharmaceutical grade zinc chloride stock solution obtained from an Australian company (Phebra Pty Ltd, Lane Cove West, NSW, Australia) will be diluted in 250ml of normal saline and infused, resulting in a final dosage of 0.5mg/kg/d. Patients will be administered zinc daily for seven days. Placebo will be unadjusted 250ml of normal saline. To standardise administration time, zinc infusions or control will commence in the early morning. Zinc or placebo will be administered via central venous or peripheral access over 3 hours. Zinc or placebo will be administered daily until either discharge or seven days of infusion (depending on which is sooner). The clinical trial nurse (allocation concealment) will use a web-based randomisation program to determine the allocation of patients and then prepare the coded zinc solution or placebo. Each coded solution bag, which will be indistinguishable irrespective of study group, will then be dispensed for administration to the patient as per protocol. This coded identifying study number will also be labelled on the patient case report form (CRF). The investigators, study coordinators, treating physicians, bedside nurses and patients/family will remain blinded to the allocated study solution.

Study assessment

Study objective and endpoints

The endpoints of this study are listed in Table 2. The primary objective is to assess the effect of 7 days of HDIVZn on oxygenation in comparison with placebo in patients with confirmed COVID19. Specifically, oxygen saturations and the requirement of supplemental oxygenation (litres/minute) to maintain acceptable saturations will be measured at various timepoints in conjunction with measurement of routine observations. If applicable, the method of supplemental oxygenation provided will be recorded (eg. nasal prongs, Hudson mask). The primary outcome will be measured by either the highest level of supplemental oxygen (non-ventilated patients) or lowest PaO_2/FiO_2 ratio in ventilated patients.

Secondary outcome measures include ICU and in-hospital mortality, length of stay in ICU or hospital, duration of supplemental oxygen, severe adverse drug events and changes based on WHO R&D Blueprint "Novel Coronavirus COVID19 Therapeutic Trial synopsis". The following is based on an eight-point ordinal scale consisting of:

0, not hospitalized, no clinical or virological evidence of infection;

1, not hospitalized, Infected, and able to resume normal activities;

2, not hospitalized, Infected, but unable to resume normal activities;

3, hospitalized, no requirement of supplemental oxygen;

4, hospitalized, requiring oxygen therapy via mask or nasal prongs;

5, hospitalized, non-invasive ventilation, requiring high flow oxygen;

6, hospitalized, intubation and mechanical ventilation

7, hospitalized, requiring ECMO, invasive mechanical ventilation, additional organ support, RRT;

8, death;

Physical examination, measurements and laboratory tests

All data will be collected by blinded trained staff using a case report form (CRF) worksheet developed by the study team. Data will then be entered into the REDCap web database (electronic CRF [eCRF]). Randomised patients will be followed up to discharge, death or 28 days post-randomisation whichever occurs first.

Study day 1 commences on randomisation and concludes at the expiry of the calendar day. Data collection will be restricted primarily to those variables necessary to define clinical patient characteristics including baseline demographics, primary diagnoses, physiological parameters, diagnostic interventions, therapeutic interventions and documentation of deaths and other serious adverse events. A complete list of collected data is summarised in Table 3. Compliance of study protocol will be monitored by daily checklists confirming administration of trial infusion and collection of laboratory investigation and minimum outcome measures.

Daily patient assessment will be performed to encourage participant retention, completion of infusion and data collection. Further, daily assessment will allow for prompt recognition of adverse effects of the trial intervention.

Adverse events

An adverse reaction is defined as any untoward and unintended response to an investigational medicinal product related to any dose administered. All adverse events judged by either the reporting investigator or the sponsor as having a reasonable possibility of a causal relationship to an investigational medicinal product will qualify as adverse reactions. Adverse events were classified per the Common Terminology Criteria for Adverse Events (CTCAE V5)[30]. All adverse events which are considered to be potentially causally related to the study intervention or are otherwise of concern in the investigator's judgement will be reported. An interim analysis is planned after 50% (80) of the patients have been

randomised. Development of severe adverse events (Grade 4), such as severe kidney or hepatic injury, will result in cessation of the trial for the participant and all infusions related to the trial will be stopped. For patients suffering adverse events, aftercare will be provided by in-hospital specialists units as part of ongoing care. Additionally, the treatment will be discontinued in cases where the participant wishes to withdraw from the trial.

Safety evaluations

An independent Data Monitoring Committee (DMC), consisting of experts in intensive care, clinical research and biostatistics will be established before patient enrolment and will review all trial protocols. The role of the DMC will be to provide study oversight to ensure that the rights and safety of patients involved in the study are protected by reviewing reported Adverse Events and making recommendations to the Management Committee (MC).

Study analysis

Sample size

The primary outcome of this study is related to the effect of zinc therapy on the level of oxygenation expressed either as worst (highest) oxygen flow (in litres/min) in non-ventilated patients or worst (lowest) PaO_2 (in mmHg)/FiO_2 (as a fraction of 1) ratio in ventilated patients. We hypothesize that zinc therapy will decrease the worst level of oxygenation during the seven days (of the treatment period) by 20% compared to placebo from a mean worst value of 10L/min (placebo) to a mean of 8L/min (zinc) or for PaO_2/FiO_2 ratio from a mean worst value of 150 (placebo) to a mean worst value of 180 (zinc). If patients transition from non-ventilated to ventilated during the study period, the PaO_2/FiO_2 ratio will be used.

To have an 80% power to see such an effect at an alpha of 0.05 in non-ventilated patients, assuming a standard deviation (SD) for O_2 flow of 2.5L/min, 25 patients would have to be randomized in each arm. In ventilated patients, to have an 80% power to see such an effect at an alpha of 0.05, assuming an SD for the PaO_2/FiO_2 ratio of 50, 44 patients would have to be randomised in each arm.

Rounding off the first group to 30 per arm and the second group to 50 per arm to account for withdrawals, we estimate that a study of 160 patients would provide a suitable sample size to test our primary hypothesis.

Statistical evaluation

De-identified data will be extracted to an Microsoft Excel 2019 (Redmond, Washington, USA) and will be made available to trial investigators, senior statisticians and the principal investigator. The descriptive analysis of the data will include the calculation of summary statistics including means, standard deviations, and absolute and relative frequencies of the baseline and follow-up data. If baseline variables are not approximately normally distributed, suitable transformations or medians with interquartile ranges will be presented. Any baseline variable differing by greater than 10% or 0.5 standard deviations between arms, will be subsequently investigated in a sensitivity analysis.

Any continuous variable outcomes that appear to be non-normal will be considered for transformation to improve normality of the residuals for subsequent regression analysis. Transformation will depend on: distribution of the variable, distribution of residuals, ease of interpretation, and impact on main results. The co-primary outcome analyses will compare 1) the change in highest level of supplemental oxygenation in non-ventilated patients, and 2) the change in lowest PaO2/FiO2 ratio in ventilated patients through the use of multivariable linear regression. Secondary outcome analyses will involve a mix of linear, logistic, and ordinal logistic regression modelling. Assumptions of the relevant models will be checked along with alternative transformations or non-parametric methods as appropriate.

Tests of interaction between dichotomised variables and treatment pathway will be carried out to test for differing treatment effect between participants. Sensitivity analyses will be conducted to explore the robustness of the estimate of the effect. Analyses will include: complete case analysis; per-protocol analysis, adjustment for baseline, and adjustment for imbalance at baseline (if baseline measures differ substantially).

A p-value 0.05 will indicate statistical significance. A full model with clinical relevant covariates (e.g. sex, age, previous heart surgery, preoperative creatinine) will be used for a stepwise backward variable selection procedure to identify independent risk factors for acute kidney injury (AKI). A senior statistician will perform data analysis on an intention-to-treat basis. An interim analysis on the safety and the primary outcomes will be performed when 50% (80/160) of the patients have received zinc or placebo for at least seven days. Summary statistics will be used to describe the clinical data and presented as mean ± SD, median with interquartile range (IQR) or percentages as appropriate. Chi-squared analysis with Fisher's exact test (when appropriate) and Student's t-test (Mann Whitney U test for non-normal distributions) will be used to compare data between the active treatment group and the control group with statistical significance declared for probability values of 0.05 or

less. Analysis of the outcome of excluded patients due to other trials etc. will be in accordance with the CONSORT guidelines. Statistical coding will be made upon request.

Findings will be published in peer-review journals. Eligible authors will include trial investigators, principal investigator, statisticians and clinicians involved in manuscript preparation.

Discussion

This single centre, double-blind, randomised controlled trial will assess the role of HDIVZn in protection against the sequelae associated with SARS-CoV-2 virus. The beneficial effects of zinc on viral infections have been previously demonstrated [31 32]. Further, HDIVZn may provide additional protection to other end organs that may be indirectly affected by pulmonary injury and impaired oxygenation.

In coronaviruses, zinc inhibits both the proteolytic processing of replicase polyproteins and the RNA-dependent RNA polymerase (RdRp) activity [13]. Although mechanisms of action of zinc are unknown, several possibilities exist. Firstly, DNA and RNA polymerases use divalent metal ions like Mg^{2+} as a co-factor, and one possible mechanism is that zinc displaces Mg^{2+} and subsequently inhibits RdRp activity [33]. In support is the observation that various divalent metals ions sustained the activity of poliovirus RdRp in the following preference Mn^{2+}> Co^{2+}> Ni^{2+}> Fe^{2+}> Mg^{2+}> Ca^{2+}> Cu^{2+} [34]. In contrast, zinc was incapable of sustaining RdRp catalyzed nucleotide incorporation [34]. Secondly, a zinc-binding pocket has been identified in the Dengue virus and SARS-coronavirus RdRp [13]. Therefore, it is possible that binding of zinc may induce a structural change in the conformation of RdRp which disables RdRp to catalyze nucleotide incorporation. Finally, adding high concentrations of zinc ions to cells impairs viral polyprotein processing which is integral to virus replication [35].

In addition to the direct effect on viral replication and activity, HDIVZn may play a protective role in alternate organs. Zinc has been demonstrated to be beneficial in reducing mortality in patients with severe pneumonia[36]. Further, in SARS-CoV-2, respiratory compromise results in impaired oxygenation and hypoxia to various end organs. Such hypoxia may contribute to end-organ failure and increase the risk of mortality. Specifically, such COVID19 associated hypoxia has been proposed to be contributory to cardiac injury [37], hepatic injury [38] and renal injury [39 40]. Our published studies have shown that HDIVZn protects various organs, including the heart, kidneys and liver against the damage caused by hypoxia. It should be noted that hypoxia and oxidative stress, result in an increase in

reactive oxygen species (ROS), including superoxide (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical (·OH) – which result in intracellular damage [41 42]. Zinc appears to limit ROS production by several mechanisms. Firstly, metallothioneins (MTs), small cysteine-rich and heavy metal-binding proteins, participate in the intracellular defence against reactive oxygen and nitrogen species [43] and zinc has been shown to induce MT mRNA and protein expression. Secondly, zinc competes with Fe^{2+} and Cu^{2+} ions for binding to cell membranes and proteins – normally, these active metals catalyze the production of hydroxyl radical from H_2O_2 via Fenton chemistry. Thirdly, zinc upregulates the production and activation of antioxidant proteins, molecules and enzymes such as glutathione, catalase and superoxide dismutase (SOD), which catalyze O_2^- to oxygen or H_2O_2 [44]. Finally, zinc reduces the activation of oxidant-promoting enzymes such as inducible nitric acid synthase and NADPH enzyme, which catalyze oxygen to O_2^-. Accordingly, we hypothesize that Zinc may provide protection against the hypoxic injury that critically ill patients with COVID19 may experience.

The specific strengths of the current protocol design are a) prolonged exposure of HDIVZn and b) assessment of critically ill patients, a population where a benefit would be observed if truly present. There is an inherent difficulty in assessing pulmonary response in clinical trials, and accordingly, the primary outcome measure is a possible criticism. However, maximal oxygen requirements and PaO_2/FiO_2 are well established surrogate markers [45 46], and have been used in recent comparable trials [47-49].

The safety of HDIVZn has been addressed in previous literature [17-20]. Elemental zinc has been administered at a substantially higher dose (ranging from 26.4 to 37.5mg/d for eight successive days) in the treatment of burns and did not produce any side effects in humans [17-19]. In the setting of critically ill patients, zinc intravenously at 3-times higher dose than the current study (0.75mg/kg/d for seven days) did not produce any adverse effects [20].

In conclusion, we designed a single-centre, double-blind, randomised controlled trial to assess the potential benefit of HDIVZn for hospitalised or critically ill patients with SARS-CoV-2 infection and associated respiratory compromise. We believe that our well-designed trial will be able to expediently identify a potential agent that may improve outcomes for these critically ill patients.

Contributors: MP assisted in protocol design, ethical submission and manuscript production. JEK and VC were involved in data collection and manuscript production. LQ was involved in statistical design and analysis. PJ, JT, CM were involved in patient care and study procotol design. DJ, DB, RB, OP and JI were involved in project design, patient care, manuscript production and supervision.

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References

1. Hu Y, Sun J, Dai Z, et al. Prevalence and severity of corona virus disease 2019 (COVID-19): A systematic review and meta-analysis. *J Clin Virol* 2020;127:104371. doi: 10.1016/j.jcv.2020.104371 [published Online First: 2020/04/22]
2. Tu H, Tu S, Gao S, et al. The epidemiological and clinical features of COVID-19 and lessons from this global infectious public health event. *J Infect* 2020 doi: 10.1016/j.jinf.2020.04.011 [published Online First: 2020/04/22]
3. Weston S, Frieman MB. COVID-19: Knowns, Unknowns, and Questions. *mSphere* 2020;5(2) doi: 10.1128/mSphere.00203-20 [published Online First: 2020/03/20]
4. Zhu J, Zhong Z, Li H, et al. CT imaging features of 4,121 patients with COVID-19: a meta-analysis. *J Med Virol* 2020 doi: 10.1002/jmv.25910 [published Online First: 2020/04/22]
5. Ledford H. How does COVID-19 kill? Uncertainty is hampering doctors' ability to choose treatments. *Nature* 2020;580(7803):311-12. doi: 10.1038/d41586-020-01056-7 [published Online First: 2020/04/11]
6. Wang Y, Zhang D, Du G, et al. Remdesivir in adults with severe COVID-19: a randomised, double-blind, placebo-controlled, multicentre trial. *The Lancet* 2020 doi: 10.1016/S0140-6736(20)31022-9
7. Ischia J, Bolton DM, Patel O. Why is it worth testing the ability of zinc to protect against ischaemia reperfusion injury for human application. *Metallomics* 2019;11(8):1330-43. doi: 10.1039/c9mt00079h
8. Prasad AS. Zinc is an Antioxidant and Anti-Inflammatory Agent: Its Role in Human Health. *Frontiers in nutrition* 2014;1:14.
9. Hulisz D. Efficacy of zinc against common cold viruses: an overview. *J Am Pharm Assoc* 2004;44(5):594-603. doi: 10.1331/1544-3191.44.5.594.hulisz [published Online First: 2004/10/22]
10. Suara RO, Crowe JE, Jr. Effect of zinc salts on respiratory syncytial virus replication. *Antimicrob Agents Chemother* 2004;48(3):783-90. doi: 10.1128/aac.48.3.783-790.2004 [published Online First: 2004/02/26]
11. Li D, Wen LZ, H Yu. [Observation on clinical efficacy of combined therapy of zinc supplement and jinye baidu granule in treating human cytomegalovirus infection]. *Zhongguo Zhong Xi Yi Jie He Za Zhi* 2005;25(5):449-51. [published Online First: 2005/06/17]

12. Femiano F, Gombos F, Scully C. Recurrent herpes labialis: a pilot study of the efficacy of zinc therapy. *J Oral Pathol Med* 2005;34(7):423-5. doi: 10.1111/j.1600-0714.2005.00327.x [published Online First: 2005/07/14]
13. te Velthuis AJ, van den Worm SH, Sims AC, et al. Zn(2+) inhibits coronavirus and arterivirus RNA polymerase activity in vitro and zinc ionophores block the replication of these viruses in cell culture. *PLoS Pathog* 2010;6(11):e1001176. doi: 10.1371/journal.ppat.1001176 [published Online First: 2010/11/17]
14. O'Kane D, Gibson L, May CN, et al. Zinc preconditioning protects against renal ischaemia reperfusion injury in a preclinical sheep large animal model. *Biometals* 2018;31(5):821-34. doi: 10.1007/s10534-018-0125-3
15. Rao K, Sethi K, Ischia J, et al. Protective effect of zinc preconditioning against renal ischemia reperfusion injury is dose dependent. *PLoS ONE* 2017;12(7):e0180028. doi: 10.1371/journal.pone.0180028
16. Cheung E, Nikfarjam M, Jackett L, et al. The Protective Effect of Zinc Against Liver Ischaemia Reperfusion Injury in a Rat Model of Global Ischaemia. *Journal of Clinical and Experimental Hepatology* 2019;August 20, 2019, In press. doi: 10.1016/j.jceh.2019.07.006
17. Berger MM, Baines M, Raffoul W, et al. Trace element supplementation after major burns modulates antioxidant status and clinical course by way of increased tissue trace element concentrations. *Am J Clin Nutr* 2007;85(5):1293-300. doi: 10.1093/ajcn/85.5.1293
18. Berger MM, Binnert C, Chiolero RL, et al. Trace element supplementation after major burns increases burned skin trace element concentrations and modulates local protein metabolism but not whole-body substrate metabolism. *Am J Clin Nutr* 2007;85(5):1301-6. doi: 10.1093/ajcn/85.5.1301
19. Berger MM, Spertini F, Shenkin A, et al. Trace element supplementation modulates pulmonary infection rates after major burns: a double-blind, placebo-controlled trial. *Am J Clin Nutr* 1998;68(2):365-71. doi: 10.1093/ajcn/68.2.365
20. Cvijanovich NZ, King JC, Flori HR, et al. Safety and Dose Escalation Study of Intravenous Zinc Supplementation in Pediatric Critical Illness. *JPEN J Parenter Enteral Nutr* 2016;40(6):860-8. doi: 10.1177/0148607115572193
21. Chan AW, Tetzlaff JM, Altman DG, et al. SPIRIT 2013 statement: defining standard protocol items for clinical trials. *Ann Intern Med* 2013;158(3):200-7. doi: 10.7326/0003-4819-158-3-201302050-00583 [published Online First: 2013/01/09]
22. Institute of Medicine Panel on M. Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. Washington (DC): National Academies Press (US)

23. Solomons NW, Romero-Abal ME, Weiss G, et al. Bioavailability of zinc from NutriSet zinc tablets compared with aqueous zinc sulfate. *Eur J Clin Nutr* 2011;65(1):125-31. doi: 10.1038/ejcn.2010.198

24. Feillet-Coudray C, Meunier N, Rambeau M, et al. Long-term moderate zinc supplementation increases exchangeable zinc pool masses in late-middle-aged men: the Zenith Study. *Am J Clin Nutr* 2005;82(1):103-10. doi: 10.1093/ajcn.82.1.103
25. Keyzer JJ, Oosting E, Wolthers BG, et al. Zinc absorption after oral administration of zinc sulfate. *Pharm Weekbl Sci* 1983;5(5):252-3.
26. Hempe JM, Carlson JM, Cousins RJ. Intestinal metallothionein gene expression and zinc absorption in rats are zinc-responsive but refractory to dexamethasone and interleukin 1 alpha. *J Nutr* 1991;121(9):1389-96.
27. Nair AB, Jacob S. A simple practice guide for dose conversion between animals and human. *J Basic Clin Pharm* 2016;7(2):27-31. doi: 10.4103/0976-0105.177703
28. Bennett JW. Regional Body Surface-Area of Sheep. *J Agric Sci* 1973;81(Dec):429-32. doi: Doi 10.1017/S0021859600086469
29. Stehle P, Stoffel-Wagner B, Kuhn KS. Parenteral trace element provision: recent clinical research and practical conclusions. *Eur J Clin Nutr* 2016;70(8):886-93. doi: 10.1038/ejcn.2016.53
30. Common Terminology Criteria for Adverse Events (CTCAE v5.0). *National Cancer Institute* 2017:https://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_8.5x11.pdf.
31. Science M, Johnstone J, Roth DE, et al. Zinc for the treatment of the common cold: a systematic review and meta-analysis of randomized controlled trials. *CMAJ* 2012;184(10):E551-61. doi: 10.1503/cmaj.111990 [published Online First: 2012/05/09]
32. Bhatnagar S, Wadhwa N, Aneja S, et al. Zinc as adjunct treatment in infants aged between 7 and 120 days with probable serious bacterial infection: a randomised, double-blind, placebo-controlled trial. *Lancet* 2012;379(9831):2072-8. doi: 10.1016/S0140-6736(12)60477-2 [published Online First: 2012/06/05]
33. Ferrari E, Wright-Minogue J, Fang JW, et al. Characterization of soluble hepatitis C virus RNA-dependent RNA polymerase expressed in Escherichia coli. *J Virol* 1999;73(2):1649-54. doi: 10.1128/jvi.73.2.1649-1654.1999 [published Online First: 1999/01/09]
34. Arnold JJ, Ghosh SK, Cameron CE. Poliovirus RNA-dependent RNA polymerase (3D(pol)). Divalent cation modulation of primer, template, and nucleotide selection. *J Biol Chem* 1999;274(52):37060-9. doi: 10.1074/jbc.274.52.37060 [published Online First: 1999/12/22]
35. Butterworth BE, Korant BD. Characterization of the large picornaviral polypeptides produced in the presence of zinc ion. *J Virol* 1974;14(2):282-91. [published Online First: 1974/08/01]
36. Wang L, Song Y. Efficacy of zinc given as an adjunct to the treatment of severe pneumonia: A meta-analysis of randomized, double-blind and placebo-controlled trials. *Clin Respir J* 2018;12(3):857-64. doi: 10.1111/crj.12646 [published Online First: 2017/05/11]
37. Zhu H, Rhee JW, Cheng P, et al. Cardiovascular Complications in Patients with COVID-19: Consequences of Viral Toxicities and Host Immune Response. *Curr Cardiol Rep* 2020;22(5):32. doi: 10.1007/s11886-020-01292-3 [published Online First: 2020/04/23]

38. Feng G, Zheng KI, Yan QQ, et al. COVID-19 and Liver Dysfunction: Current Insights and Emergent Therapeutic Strategies. *J Clin Transl Hepatol* 2020;8(1):18-24. doi: 10.14218/jcth.2020.00018 [published Online First: 2020/04/11]
39. Fanelli V, Fiorentino M, Cantaluppi V, et al. Acute kidney injury in SARS-CoV-2 infected patients. *Crit Care* 2020;24(1):155. doi: 10.1186/s13054-020-02872-z [published Online First: 2020/04/18]
40. Mubarak M, Nasri H. COVID-19 nephropathy; an emerging condition caused by novel coronavirus infection. *J Nephropathol* 2020;9(3):e21-e21. doi: 10.34172/jnp.2020.21
41. Castro L, Freeman BA. Reactive oxygen species in human health and disease. *Nutrition* 2001;17(2):161-65. doi: 10.1016/S0899-9007(00)00570-0
42. Lachance PA, Nakat Z, Jeong WS. Antioxidants: An integrative approach. *Nutrition* 2001;17(10):835-38. doi: 10.1016/S0899-9007(01)00636-0
43. Ruttkay-Nedecky B, Nejdl L, Gumulec J, et al. The role of metallothionein in oxidative stress. *Int J Mol Sci* 2013;14(3):6044-66. doi: 10.3390/ijms14036044 [published Online First: 2013/03/19]
44. Prasad AS. Zinc: an antioxidant and anti-inflammatory agent: role of zinc in degenerative disorders of aging. *J Trace Elem Med Biol* 2014;28(4):364-71. doi: 10.1016/j.jtemb.2014.07.019 [published Online First: 2014/09/10]
45. Matthay MA, McAuley DF, Ware LB. Clinical trials in acute respiratory distress syndrome: challenges and opportunities. *The Lancet Respiratory Medicine* 2017;5(6):524-34. doi: 10.1016/S2213-2600(17)30188-1
46. Donahoe M. Acute respiratory distress syndrome: A clinical review. *Pulm Circ* 2011;1(2):192-211. doi: 10.4103/2045-8932.83454
47. Frat JP, Ricard JD, Quenot JP, et al. Non-invasive ventilation versus high-flow nasal cannula oxygen therapy with apnoeic oxygenation for preoxygenation before intubation of patients with acute hypoxaemic respiratory failure: a randomised, multicentre, open-label trial. *Lancet Respir Med* 2019;7(4):303-12. doi: 10.1016/s2213-2600(19)30048-7 [published Online First: 2019/03/23]
48. He H, Sun B, Liang L, et al. A multicenter RCT of noninvasive ventilation in pneumonia-induced early mild acute respiratory distress syndrome. *Crit Care* 2019;23(1):300. doi: 10.1186/s13054-019-2575-6 [published Online First: 2019/09/06]
49. Pennisi MA, Bello G, Congedo MT, et al. Early nasal high-flow versus Venturi mask oxygen therapy after lung resection: a randomized trial. *Crit Care* 2019;23(1):68. doi: 10.1186/s13054-019-2361-5 [published Online First: 2019/03/02]

Table 1: inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
• Consenting adult patients adult male or female, age ≥ 18 years old. Laboratory-confirmed SARS-CoV-2 infection as determined by polymerase chain reaction (PCR) • Hospitalized with an illness of any duration with evidence of pneumonia and severe disease, critical disease, or multi-system organ dysfunction at baseline • Ability to provide informed consent signed by study patient or legally acceptable representative • Willingness and ability to comply with study-related procedures/assessments • Have an oxygen saturation (SaO_2) of 94% or less while they were breathing ambient air or a ratio of the partial pressure of oxygen (PaO_2) to the fraction of inspired oxygen (FiO_2) (Pao2: Fio2) at or below 300 mg Hg. • No chronic kidney disease (CKD) defined by stage II or higher using the Kidney Disease Improving Global Outcomes (KDIGO) classification	• Age <18 or pregnant or lactating female • Allergy to zinc • Severe hepatic impairment defined as Child C liver disease. • eGFR ≤ 60 mL/min/1.73 m^2 (defined using CKD-EPI SCr formula) • History of any organ transplant which requires active immunosuppressive treatment which can interfere with kidney function • If a patient required any of the following within seven days prior to cardiac surgery: defibrillation, mechanical ventilation, left ventricular assist device (LVAD), or other forms of mechanical circulatory support (MCS) • If a patient required cardiopulmonary resuscitation (CPR) within 14 days • DNR (do not resuscitate) DNI (do not intubate) orders • Death is deemed imminent or inevitable during this admission, and either the attending physician, patient or substitute decision-maker is not committed to active treatment • Already receiving dialysis (either acute or chronic) or imminent need of dialysis at the time of enrolment • Patients with known HIV infection • Patients with a known or suspected history of oxalate nephropathy or hyperoxaluria, scurvy, chronic iron overload, G-6PD deficiency • Clinician expects to prescribe Zinc for another indication • Patients with known haemochromatosis.

Table 2: Primary and secondary outcomes

Key Primary Outcome	Key Secondary Outcomes
Mean change in the highest level of oxygenation (oxygen flow measured litre/min) in non-ventilated patients Mean change in lowest PaO_2 in ventilated patients	Mortality (ICU or in-hospital) Duration of mechanical ventilation Duration of oxygen therapy Duration of hospitalisation Length of Stay in ICU
Other Secondary Outcomes	
Adverse Drug Events Acute kidney injury Acute liver injury Duration of vasopressor drugs Sequential Organ Failure Assessment (SOFA) respiratory score	Clinical improvement based on an eight-point ordinal scale recommended in the document published by WHO R&D Blueprint "Novel Coronavirus COVID-19 Therapeutic Trial Synopsis". • Percentage of patients reporting each severity rating on an 8-point ordinal scale [Time Frame: Day 14] • Time to improvement in one category from admission using the 8-point ordinal scale [Time Frame: Up to day 28] • Mean change in the 8-point ordinal scale [Time Frame: Up to day 28]

Table 3: Collected data during trial

	All patients	
Baseline data	Baseline data • Demographics: Age, gender, place of residence • Comorbidities: Diabetes mellitus, arterial hypertension, congestive heart failure, chronic obstructive airways disease, chronic liver disease, malignancy, chronic renal failure • Pre-admission medication • Functional status / frailty score	
	Non-ventilated patients	Ventilated patients
Daily observations	• Daily saturations (Worst values) • Daily oxygen flow • Fluid input • Urine output • Fluid balance	• Daily PaO_2/FiO_2 ratio • Daily oxygen flow • Fluid input • Urine output • Fluid balance • Vasopressor data
Laboratory investigations (collected at 0800)	• Daily serum creatinine • Daily liver function • Daily blood count • Daily zinc and trace metal concentration (copper, potassium, magnesium) • Daily Cardiac troponin • Daily lactate level	• Daily serum creatinine • Daily liver function • Daily blood count • Daily zinc and trace metal concentration (copper, potassium and magnesium) • Daily Cardiac troponin • Daily lactate level
Primary outcome measures	• Mean change in highest level of oxygenation requirement (oxygen flow in litres/min)	• Mean change in lowest PaO_2/FiO_2 ratio (in mmHg)
Secondary outcome measures	• Mortality [Time Frame: Up to day 28] • Duration of oxygen therapy (days) • Duration of hospitalization (days) • Length of stay in the intensive care unit and hospital • Frequency of Serious Adverse Drug Events • Acute kidney injury • Acute liver injury • Time to resolution of fever for at least 48 hours without antipyretics by clinical severity • Incidence of severe or life-threatening bacterial, invasive fungal, or	• Mortality [Time Frame: Up to day 28] • Duration of mechanical ventilation(days) • Duration of oxygen therapy (days) • Duration of hospitalization (days) • Length of stay in the intensive care unit and hospital • Frequency of Serious Adverse Drug Events • Acute kidney injury • Acute liver injury • Use, duration and dosage of vasopressor drugs • Time to resolution of fever for at least 48 hours without

	opportunistic infection • Number of patients admitted into an intensive care unit (ICU) [Time Frame: Up to day 28] • Sequential Organ Failure Assessment (SOFA) Respiratory Score [Time Frame: 28 days]. Assigned a point value from 0 (normal) to 4 (high degree of dysfunction/failure) • Clinical improvement based on an eight-point ordinal scale recommended in the document published by WHO R&D Blueprint "Novel Coronavirus COVID-19 Therapeutic Trial Synopsis". ○ Percentage of patients reporting each severity rating on an 8-point ordinal scale [Time Frame: Day 14] ○ Time to improvement in one category from admission using the 8-point ordinal scale [Time Frame: Up to day 28] ○ Mean change in the 8-point ordinal scale [Time Frame: Up to day 28]	antipyretics by clinical severity • Incidence of severe or life-threatening bacterial, invasive fungal, or opportunistic infection • Number of patients admitted into an intensive care unit (ICU) [Time Frame: Up to day 28] • Sequential Organ Failure Assessment (SOFA) Respiratory Score [Time Frame: 28 days]. Assigned a point value from 0 (normal) to 4 (high degree of dysfunction/failure) • Clinical improvement based on an eight-point ordinal scale recommended in the document published by WHO R&D Blueprint "Novel Coronavirus COVID-19 Therapeutic Trial Synopsis". ○ Percentage of patients reporting each severity rating on an 8-point ordinal scale [Time Frame: Day 14] ○ Time to improvement in one category from admission using the 8-point ordinal scale [Time Frame: Up to day 28] ○ Mean change in the 8-point ordinal scale [Time Frame: Up to day 28]

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Participant Information Sheet/Consent Form (Enrol)

Interventional Study - *Adult providing own consent to enrol*

Title	**High-dose Zinc as Adjunctive therapy in COVID positive Critically Ill Patients: A Pilot Randomized Controlled Trial**
Short Title	**ZINC COVID**
Protocol Number	Version **3, 4th April 2020**
Local Principal Investigator	Associate Professor Joseph Ischia
Associate Investigator(s)	Dr Oneel Patel, Professor Rinaldo Bellomo, Dr Daryl Jones, Professor Damien Bolton, Dr Glenn Eastwood, Prof Paul Johnson, Prof Christine McDonald
Location	Austin Hospital

Part 1 What does my participation involve?

1 Introduction

You are invited to take part in this research project because you have been admitted to the Austin hospital with severe coronavirus infection. During your hospital stay, you may be at high risk of developing difficulty with your breathing and getting enough oxygen. Soon, you may need a machine to help you breath (mechanical ventilation). Currently, there are no standard drugs for preventing the respiratory failure associated with COVID-19 infection. It is possible that zinc may be useful in reducing the severity of the coronavirus infection. However, we really do not know if it is beneficial or not and therefore we are performing this trial.

This Participant Information and Consent Form tells you about the research project. It explains the tests and treatments involved. Knowing what is involved will help you decide if you want to take part in the research.

Please read this information carefully. Ask questions about anything that you don't understand or want to know more about. Before deciding whether or not to take part, you might want to talk about it with a relative, friend or your local doctor.

Participation in this research is voluntary. If you don't wish to take part, you don't have to. You will receive the best possible care whether or not you take part. You are also eligible for new treatments that may arise or other studies that may be suitable for you during your stay.

If you decide you want to take part in the research project, you will be asked to sign this consent form. By signing the consent section, you are telling us that you:

- Understand what you have read
- Consent to take part in the research project

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- Consent to have the tests and treatments that are described
- Consent to the use of your personal and health information as described

You will be given a copy of this Participant Information and Consent Form to keep.

2 What is the purpose of this research?

A pandemic of a novel coronavirus (COVID-19 or 2019-CoV) infection has posed significant threats to international health and the economy. In the absence of any vaccine for this virus, there is an urgent need to find a treatment that can stop the growth of the virus in the body and the complications it can cause.

In more severe cases, coronavirus enters the lungs. As the virus multiplies, it can cause problems with your breathing like bronchitis and pneumonia. Development of pneumonia leads to a reduced ability of the lungs to absorb oxygen. In a small number of severe cases, COVID-19 infections lead to the development of respiratory failure and acute respiratory distress syndrome (ARDS). Respiratory failure and ARDS are medical terms that define a condition where one can find it increasingly difficult to breathe. In such cases, the patient requires a ventilator which helps them to breathe and maintains oxygen levels in the blood. However, if lungs are damaged beyond a certain level and not enough oxygen is provided to the rest of the body, respiratory failure could lead to failure of other major organs, including the liver, kidney and brain.

Zinc is an essential nutrient which performs various vital functions in the body. Zinc is essential for the maintenance and development of the immune system, which helps fight infections. Zinc is required for wound healing and tissue repair. Zinc protects organs against injury instigated by reduced oxygen supply. Zinc has also been shown to protect against pneumonia.

Numerous research studies have shown the potential of zinc to prevent the growth of a number of other viruses (such as those that cause the common cold) that are similar to coronavirus that causes the COVID-19 illness. Furthermore, intravenous zinc has been shown to protect various organs, including the heart, kidneys and liver against the damage caused by the reduced availability of oxygen.

The amount of zinc that we plan to give is thought to be very safe with minimal side effects. This is based on earlier studies where humans with severe burns were injected with nearly double the amount of zinc that we plan to give, and it did not produce any side effects. Furthermore, in a recent study critically ill children were given a 3-times higher amount of zinc compared to what we plan to give in the current study. Again, it did not produce any adverse effects. Based on these human studies, we are confident that the dose of zinc that we plan to use in this study is very safe and well-tolerated.

In summary, we really do not know if zinc is beneficial in people infected with the coronavirus. Therefore, the goal of this study is to test if zinc given as a daily intravenous injection through your drip in participants with coronavirus infection can reduce the severity of the disease and improve patient outcomes. A positive result of our proposed study will have an enormous impact on the health outcomes of patients who have developed coronavirus infections for which there are currently no treatments.

This research has been initiated by the study doctor, Associate Professor Joseph Ischia.

3 What does participation in this research involve?

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If you agree to participate in this study, you will be asked to sign the Participant Consent Form.

This study will be conducted over the time you are admitted to the Austin Hospital in the ward or in the intensive care unit.

In the ward or in the intensive care unit

You are participating in a randomized controlled research project. Sometimes we do not know which treatment is best for treating a condition. To find out, we need to compare different treatments. We put people into groups and give each group a different treatment. The results are compared to see if one is better. To try and make sure the groups are the same; each participant is put into a group by chance (random).

While you are admitted to the Austin hospital, you will be randomly assigned (like tossing a coin) to receive intravenous zinc or placebo**. A placebo is a medication with no active ingredients. It looks like the real thing but is not.** This means that you will have an equal (50/50) chance of receiving either zinc or placebo. Neither the doctor nor you can decide which treatment you receive.

This trial is a 'double-blind trial'. This means that following randomization, your treating doctors and other staff caring for you in the hospital and intensive care unit will not know which intravenous medicine is being given. The zinc chloride is a colourless fluid in the 250ml saline bag. Therefore, it will be impossible to tell which treatment you are receiving.

After you have been enrolled and randomized, the treating doctor will prescribe the amount (dose and frequency) of the study treatment, as they usually do for either of the study medicines: zinc chloride 0.5 mg/kg or Placebo (normal saline) administered daily around 9 am, but it is safe to give the treatment starting anywhere between 8 am to 12 pm. You will receive the zinc chloride through an exclusive lumen of the central venous catheter or peripheral catheter inserted in your vein, which is part of the standard of care. You will receive the treatment (with zinc or placebo) for 7 days, and then the treatments will stop. The treatment will be given in a 250ml bag of saline solution which will contain zinc chloride or placebo (i.e. not have zinc in it). The saline bag will be given over about a 3-6 hour period depending on what your treating doctors think is best for you.

Apart from this once daily drip, you will receive the usual medical and nursing care by the ward team or intensive care unit team. This study does involve the collection of blood samples (approximately 5-15 ml, size of a tablespoon each time). These will be collected daily as per your usual care. Some of this blood will be sent to Prof Jose Villadangos at the University of Melbourne, Parkville, Melbourne for analysis of inflammatory markers. After analysis, these samples will be destroyed as per normal Melbourne University blood product disposal protocols.

Your general hospital care will not be affected in any way by the study.

There will be no formal follow-up, but we will collect data on how you recover from the infection for the time you are in the hospital recovering for up to 28 days. We request access to your medical record to collect research related data such as blood pressure, heart rate changes and the dates you are discharged from the intensive care unit and from the hospital.

There are no additional costs associated with participating in this research project, nor will you be paid. All medication, tests and medical care required as part of the research project will be provided to you free of charge.

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4 What do I have to do?

Your involvement in this study will not have any effect on the standard of care you receive. There will be no restriction on your daily activities, or will there be any dietary restrictions. You can take your regular medication; however, we will take a note of the name of the medicine and the amount you take.

5 Other relevant information about the research project

This study will involve only those participants who are admitted to Austin Hospital. For this study, we will seek the participation of 160 people. Eighty will be randomly assigned (like tossing a coin) to receive intravenous zinc, and another 80 will receive a placebo. This project involves researchers from Austin Hospital, University of Melbourne and Monash University.

6 Do I have to take part in this research project?

Participation in any research project is voluntary. If you do not wish to take part, you do not have to. If you decide to take part and later change your mind, you are free to withdraw from the project at any stage.

If you do decide to take part, you will be given this participant Information and Consent Form to sign, and you will be given a copy to keep.

Your decision whether to take part or not to take part, or to take part and then withdraw, will not affect your routine treatment, your relationship with those treating you or your relationship with the Austin Hospital.

7 What are the alternatives to participation?

If you decide not to participate in this study, you will continue to receive standard care, and your treatment will be unaffected by your decision not to participate.

8 What are the possible benefits of taking part?

We cannot guarantee or promise that you will receive any benefits from this research. This study aims to further medical knowledge and improve the understanding of whether zinc is beneficial in critically ill patients suffering from COVID-19 infection.

9 What are the possible risks and disadvantages of taking part?

Medical treatments often cause side effects. You may have none, some or all of the effects listed below, and they may be mild, moderate or severe. If you have any of these side effects or are worried about them, talk with your study doctor. Your study doctors will also be looking out for the side effects.

Choosing to take part in this study should not pose any additional risk to you above the risks associated with your usual treatment in the hospital. Zinc is an accepted treatment for nutritional deficiency states. Although this medicine has been given to many patients over many years and is in regular current use, there may be additional unforeseen or unknown risks.

Uncommon side effects include:

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- Mild and short-lasting - Nausea and vomiting, stomach pain and diarrhoea, flu-like symptoms: fever, chills, cough, headache, a decrease in the good cholesterol (HDL) levels in your blood which is thought to be the protective cholesterol against heart disease, changes in taste perceptions (metallic taste), copper deficiency, mental confusion **(less than 5%)**
- Severe and possibly permanent- acute kidney injury and possible chronic kidney disease or need for dialysis or renal transplant **(unknown or thought to be very low- less than 1%)**

As you will be in the hospital when the study medication is given, you will be closely monitored and treated immediately if any effects were to occur.

If at any point during the study, your study doctor feels it is in your best interests not to continue receiving the study medicine; or if during the study, there is evidence to suggest beyond a reasonable doubt that the study medicine is not beneficial then your involvement in the study may be stopped.

10 What will happen to my test samples?

Some of the blood samples that you provide will be transferred to Austin Pathology for routine analysis for your standard care. Some of the blood will be sent for analysis at a laboratory that measures the levels of zinc and other metals to see if treating with zinc causes major changes in these metals. After these tests, any remaining blood samples will be destroyed.

11 What if new information arises during this research project?

Sometimes during the course of a research project, new information becomes available about the treatment that is being studied. If this happens, your study doctor will tell you about it and discuss with you whether you want to continue in the research project. If during the course of the study, a superior treatment becomes available for COVID-19, you have the right to withdraw from the study. If you decide to withdraw, your study doctor will make arrangements for your regular health care to continue. If you choose to continue in the research project, you will be asked to sign an updated consent form.

Also, on receiving new information, your study doctor might consider it to be in your best interests to withdraw you from the research project. If this happens, he/she will explain the reasons and arrange for your regular health care to continue.

12 Can I have other treatments during this research project?

Whilst you are participating in this research project, you are able to take all of the medications or treatments you have been taking for your condition or for other reasons. It is important to tell your study doctor and the study staff about any treatments or medications you may be taking, including over-the-counter medications, vitamins or herbal remedies, acupuncture or other alternative treatments. You should also tell your study doctor about any changes to these during your participation in the research project.

13 What if I withdraw from this research project?

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If you decide to withdraw from the project, please notify a member of the research team before you withdraw. This notice will allow that person or the research supervisor to discuss any health risks or special requirements linked to withdrawing.

If you do withdraw your consent during the research project, the study doctor and relevant study staff will not collect additional personal information from you, although personal information already collected will be retained to ensure that the results of the research project can be measured properly and to comply with the law. You should be aware that data collected by the sponsor up to the time you withdraw will form part of the research project results. If you do not want them to do this, you must tell them before you join the research project.

14 Could this research project be stopped unexpectedly?

This research project may be stopped unexpectedly for a variety of reasons. These may include reasons such as:

- Unacceptable side effects
- The treatment is shown not to be effective
- The treatment is shown to work or not need further testing
- Decisions made by local regulatory/health authorities.
- A superior treatment for COVID-19 is discovered.

15 What happens when the research project ends?

After recruiting the planned 160 participants and following up for 28 days, the study will be closed, and the analysis of results will begin. The results will be reviewed by the study team and a statistician to determine if zinc chloride improves outcomes in patients with COVID-19 infection. We will then publish these results in research papers for the medical and global community. No follow-up is required of the study participants.

Zinc chloride is intended as a treatment for COVID-19. Therefore, once the participants have recovered or discharged from the hospital there is no need to take Zinc chloride, and it will not be available to participants.

You are welcome to receive information about the results of the study after they have been analysed and made public. Please provide a written request to the clinical contact person (Assoc Prof Joseph Ischia) noted at the end of this consent and a copy of the research results papers will be provided to you free of charge.

Part 2 How is the research project being conducted?

16 What will happen to information about me?

By signing the consent form, you consent to the study doctor and relevant research staff collecting and using personal information about you for the research project. Any information obtained in connection with this research project that can identify you will remain confidential. Data will remain confidentially stored in research offices at the Austin Hospital. The offices will be securely locked and only accessible by the research team. Electronic data will be kept securely on a password-protected database. Only

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the ZINC COVID study team at the Austin Hospital will have the list that can link your identity to the study code. Your information will only be used for the purpose of this research project, and it will only be disclosed with your permission, except as required by law. Data collected for this study will be stored for 15 years. After this time, electronic data will be destroyed by confidential erasing and paper records will be destroyed confidential shredding.

Information about you may be obtained from your health records held at this and other health services for the purpose of this research. By signing the consent form, you agree to the study team accessing health records if they are relevant to your participation in this research project.

It is anticipated that the results of this research will be published and presented in a variety of forums. In any publication and/or presentation, the information will be provided in such a way that you cannot be identified, except with your permission. Identifiable information will not be made public in any form so that confidentiality is maintained. The study database will contain information from all study participants, but not anything that can identify you as an individual. This information may be made available to other researchers. If this happens, your identity will be protected, and you will not be identified or contacted by other researchers who request access to the study database.

Information about participation in this research may be recorded in your health records. In accordance with relevant Australian state and federal privacy laws, you have the right to request access to your information collected and stored by the research team. You also have the right to request that any information with which you disagree be corrected. Please contact the study team member named at the end of this document if you would like to access your information.

17 Complaints and compensation

If you suffer any injuries or complications as a result of this research project, you should contact the study team as soon as possible, and you will be assisted with arranging appropriate medical treatment. If you are eligible for Medicare, you can receive any medical treatment required to treat the injury or complication, free of charge, as a public patient in any Australian public hospital.

In the event of loss or injury, the parties involved in this research project have agreed to a compensation agreement. Compensation may be available if your injury or complication is caused by the study medicines or by the negligence of any of the parties involved in the study. If you receive compensation that includes an amount for medical expenses, you will be required to pay for your medical treatment from those compensation monies.

18 Who is organizing and funding the research?

The Principal Investigator for this study is Associate Professor Joseph Ischia. This research has received funding from the Australian Urologic Cancer Research Trust. All monies will be administered through Austin Health and are directed to run the study at the Austin Hospital. This money pays the Austin Hospital for the work done by its staff in this study. No money is paid directly to individual researchers.

19 Who has reviewed the research project?

All research in Australia involving humans is reviewed by an independent group of people called a Human Research Ethics Committee (HREC). The ethical aspects of this research project have been approved by the Austin Health Human Research Ethics Committee.

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This project will be carried out according to the *National Statement on Ethical Conduct in Human Research (2007)*. This statement has been developed to protect the interests of people who agree to participate in human research studies.

20 Further information and who to contact

The person you may need to contact will depend on the nature of your query.

If you want any further information concerning this study or if you have any medical problems which may be related to your involvement in the study (for example, any side effects), you can contact the following people:

Clinical contact person

Name	Assoc Prof Joseph Ischia
Position	Principal Investigator
Telephone	(03) 9496 3676

For matters relating to research at the site at which you are participating, the details of the local site complaints person are:

Complaints contact person

Position	Complaints Officer
Telephone	(03) 9496 4035 or (03) 9496 4090
Email	ethics@austin.org.au

If you have any complaints about any aspect of the project, the way it is being conducted or any questions about being a research participant in general, then you may contact:

Reviewing HREC approving this research and HREC Executive Officer details

Reviewing HREC name	Austin Health Human Research Ethics Committee
HREC Executive Officer	Mrs Lisa Pedro
Telephone	(03) 9496 4035
Email	ethics@austin.org.au

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Consent Form - *Adult providing own consent to enrol*

FAH018178

Title	**High-dose Zinc as Adjunctive therapy in COVID positive Critically Ill Patients: A Pilot Randomized Controlled Trial**
Short Title	**ZINC COVID**
Protocol Number	Version **2, 1st April 2020**
Local Principal Investigator	Associate Professor Joseph Ischia
Associate Investigator(s)	Dr Oneel Patel, Professor Rinaldo Bellomo, Dr Daryl Jones, Professor Damien Bolton, Dr Glenn Eastwood, Prof Paul Johnson, Prof Christine McDonald
Location	Austin Hospital

Declaration by Participant

- ☐ I have read the Participant Information Sheet or someone has read it to me in a language that I understand.
- ☐ I understand the purposes, procedures and risks of the research described in the project.
- ☐ I give permission for my doctors, other health professionals, hospitals or laboratories outside this hospital to release information to Austin Health concerning my condition and treatment for the purposes of this project. I understand that such information will remain confidential.
- ☐ I have had an opportunity to ask questions and I am satisfied with the answers I have received.
- ☐ I freely agree to participate in this research project as described and understand that I am free to withdraw at any time during the study without affecting my future health care.
- ☐ I understand that I will be given a signed copy of this document to keep.

Name of Participant (please print) _____

Signature _____ Date _____

Declaration by Study Doctor/Senior Researcher†

I have given a verbal explanation of the research project, its procedures and risks and I believe that the participant has understood that explanation.

Name of Study Doctor/Senior Researcher† (please print) _____

Signature _____ Date _____

† A senior member of the research team must provide the explanation of, and information concerning, the research project.
Note: All parties signing the consent section must date their own signature. I understand that, if I decide to discontinue the study treatment, a member of the research team may request my permission to obtain access to my medical records for collection of follow-up information for the purposes of research and analysis.

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Form for Withdrawal of Participation - *Adult providing own consent to enrol*

Title	**High-dose Zinc as Adjunctive therapy in COVID positive Critically Ill Patients: A Pilot Randomized Controlled Trial**
Short Title	**ZINC COVID**
Protocol Number	Version **2, 1st April 2020**
Local Principal Investigator	Associate Professor Joseph Ischia
Associate Investigator(s)	Dr Oneel Patel, Professor Rinaldo Bellomo, Dr Daryl Jones, Professor Damien Bolton, Dr Glenn Eastwood, Prof Paul Johnson, Prof Christine McDonald
Location	Austin Hospital

Declaration by Participant

I wish to withdraw from participation in the above research project and understand that such withdrawal will not affect my routine treatment, my relationship with those treating me or my relationship with Austin Health**.**

Name of Participant (please print) _____

Signature _____ Date _____

Consent provided to use data collected up to the date of withdrawal: Yes No

Consent provided to access your medical record to obtain information of health status: Yes No

In the event, the participant decided to withdraw verbally, study doctor/senior researcher to give a description of the circumstances below:

Declaration by Study Doctor/Senior Researcher†

I have given a verbal explanation of the implications of withdrawal from the research project and I believe that the participant has understood that explanation.

Name of Study Doctor/Senior Researcher† (please print) _____

Signature _____ Date _____

† A senior member of the research team must provide the explanation of and information concerning withdrawal from the research project.

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

Section/item	ItemNo	Description	Location in protocol
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	Page 1, Line 1-2
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	Page 2, Line 29 Page 5, Line 5
	2b	All items from the World Health Organization Trial Registration Data Set	Page 5, Line 5. All other items as below
Protocol version	3	Date and version identifier	Page 5, Line 3
Funding	4	Sources and types of financial, material, and other support	Page 5, Line 7
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	Page 1, Lines 4-6
	5b	Name and contact information for the trial sponsor	Not applicable
	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	Not applicable
	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)	Page10, Line 8-13
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	Page 4, Lines 30-32

	6b	Explanation for choice of comparators	Page 8, Line 6
Objectives	7	Specific objectives or hypotheses	Page 8, lines 20-Page 9, Line 7
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)	Page 5, Line 19-22
Methods: Participants, interventions, and outcomes			
Study setting	9	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	Page 5, Line 19-22
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	Page 7, Lines 1-10
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	Page 8, Line 3-16
	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)	Page 9, Lines 27 - Page10, Line 5
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	Page 9, Lines -21
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	Page 7, Line 8-10
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	Page 8, Line 20 to Page 9, Line 7
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)	Page 8, Line 5-6

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	Page 10, Line 17-33
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	Page 10, Line 31-33 Page 7, Line 13-17
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	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	Page 7, Lines 23-34
Allocation concealment mechanism	16b	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	Page 7, Lines 23-34
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	Page 8, Line 10-16
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	Page 7, Line 28
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	Page 7, Line 32-34
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	Page 9, Line 10-13

	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	Page 9, Line 22-24
Data management	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	Page 9, Line 10-13
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	Page 11, Line 1-34
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	Page 11, Line 31-34
	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)	Page 11, Line 19-22
Methods: Monitoring			
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	Page 10, Line 8-13
	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	Page 11, Line 28-29
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	Page 9, Line 27 to Page 10, Line 5
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	Page 11, Line 28-29
Ethics and dissemination			

Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	Page 5, Line 2-4
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)	Page 5, Line 3-4
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	Page 7, Line 18-20
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	Not applicable
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	Page 11, Line 2-4
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	Page 14, Line 7
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	Page 11, Line 2-4
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	Page 10, Line 3-4
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	Page 12, Line 3-5
	31b	Authorship eligibility guidelines and any intended use of professional writers	Page 12, Line 3-5
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	Page 12, Line 2
Appendices			

Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Attached
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	Not applicable

*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.