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| **High-dose intravenous zinc (HDIVZn) as adjunctive therapy in COVID-19 positive critically ill patients: A pilot randomized controlled trial** |
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| **Statement of Compliance**  This document is a protocol for a research project. This study will be conducted in compliance with all stipulation of this protocol, the conditions of the ethics committee approval, the NHMRC National Statement on Ethical Conduct in Human Research (2007) and the Note for Guidance on Good Clinical Practice (CPMP/ICH-135/95). |

# ABBREVIATIONS

|  |  |
| --- | --- |
| AE | Adverse event |
| AKI | Acute kidney injury |
| APACHE | Acute Physiology and Chronic Health Evaluation |
| AR | Adverse reaction |
| CRF | Case report form |
| eCRF | Electronic case report form |
| GCP | Good clinical practice |
| HRC | Health Research Council |
| HREC | Human Research Ethics Committee |
| ICU | Intensive care unit |
| IRI | Ischaemia reperfusion injury |
| NHMRC | National Health and Medical Research Council |
| RCT | Randomised controlled trial |
| RRT | Renal replacement therapy |
| SAE | Serious adverse event |
| SAR | Serious adverse reaction |
| SUSAR | Suspected unexpected severe adverse reaction |
| TGA | Therapeutic Goods Administration |
| Zn | Zinc |
| HIDVZn | High dose intravenous Zinc |
|  |  |

# BACKGROUND

## Lay Summary

An outbreak of a new virus called novel coronavirus (COVID‐19 or 2019‐CoV) infection has posed significant threats to the health of people worldwide and the global economy. There are no vaccines for this virus, and there is no specific treatment for people infected with the virus. Therefore, we must urgently find a new treatment to inhibit the growth of the virus in the body and to prevent people with the virus from becoming more unwell.

In more severe cases, COVID-19 virus can spread to the lungs. As the virus continues to replicate, it can cause more respiratory problems like bronchitis and pneumonia. Development of pneumonia can mean that the lungs cannot absorb enough oxygen. In a small number of severe cases, people can become extremely unwell and develop extreme difficulty with their breathing. We call this acute respiratory distress syndrome (ARDS), which often requires a patient to be placed on a machine called a ventilator to help them breath and to supply oxygen. If the lungs are damaged so that not enough oxygen can get around the rest of the body, damage to other major organs, including the liver, kidney and brain can occur.

Zinc is a naturally occurring essential metal required for the normal function of the body. Zinc deficiency is associated with a range of abnormal conditions, including slow growth and poor wound healing. Furthermore, zinc is essential for the normal function of the immune system and the growth of cells in your body. Zinc deficiency results in reduced immunity and increases your chance of catching infections [[1](#_ENREF_1)] [[2](#_ENREF_2)].

Numerous studies have been done showing the potential of zinc (in its various salts including chloride or sulphate) to inhibit viral infections in clinical trials and experiments. These virus infections include the common cold (often a type of coronavirus) [[3](#_ENREF_3)], respiratory syncytial virus infections [[4](#_ENREF_4)], cytomegalovirus infections [[5](#_ENREF_5)] and herpes labialis [[6](#_ENREF_6)]. More importantly, zinc can prevent the growth of SARS-coronavirus (SARS-CoV) and equine arteritis virus (EAV) in cells grown in the lab [[7](#_ENREF_7)].

Furthermore, our published studies have shown that zinc (delivered as Zn chloride) protects various organs, including the heart, kidneys and liver against the damage caused by a lack of oxygen [[1](#_ENREF_1), [8-10](#_ENREF_8)].

Several studies have confirmed that zinc is safe in humans. The estimated dose of elemental zinc for an average 70kg human to be used in our proposed study will be 17mg per day. Previously, elemental zinc has been given at higher doses ranging from 26.4 to 37.5mg per day for eight days in a row for the treatment of burns and did this not produce any side effects. Furthermore, a recently published clinical trial in very unwell children in an intensive care unit who were thought to be low in zinc were given zinc sulphate into their veins at 3-times higher dose (equivalent elemental zinc dose 0.75mg/kg/d for seven days) than we intend to use in our study and did cause any side effects [[14](#_ENREF_14)]. Therefore, we are confident that our proposed 3-fold lower elemental zinc dose (of 0.25mg/kg/d), will be safe and not produce any major adverse effects. Finally, elemental zinc at doses ranging from 5-22mg per day is routinely given to people who require artificial feeding straight into their blood, without any reported side effects.

Therefore, we plan to perform a study to test whether zinc (given as Zn chloride) is effective and safe in subjects with coronavirus 2019 infection and to work out whether giving zinc to patients can make them get better quicker.

## The burden of COVID-19

Beginning in December 2019, a new coronavirus, designated SARS-CoV-2, has caused an international outbreak of respiratory illness termed COVID-19. Although most patients get a mild illness, some patients get pneumonia, and a small number get failure of multiple organs and die [[15-19](#_ENREF_15)]. Multiple clinical trials have been initiated to test various therapeutic agents and candidate vaccines[[19](#_ENREF_19)].  Thus far, there are no specific treatments for coronavirus infections.

## Zinc inhibits viral infections and pneumonia

Numerous studies have been published showing the potential of zinc and zinc salts to inhibit viral infections in clinical and experimental settings. Viral infections inhibited have included the common cold [[3](#_ENREF_3)], respiratory syncytial virus infections [[4](#_ENREF_4)], cytomegalovirus infections [[5](#_ENREF_5)] and herpes labialis [[6](#_ENREF_6)]. Inhibition of replication by Zn2+  observed for several picornaviruses such as rhinoviruses, foot-and-mouth disease virus, coxsackievirus, and mengovirus [[20-22](#_ENREF_20)]. More importantly, zinc is a potent inhibitor of the replication of SARS-coronavirus (SARS-CoV) and equine arteritis virus (EAV) in cell culture [[7](#_ENREF_7)].

In coronaviruses, Zn2+ 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 Mg2+ as a co-factor, and one possible mechanism is that Zn2+ displaces Mg2+ 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 Mn2+> Co2+> Ni2+> Fe2+> Mg2+> Ca2+> Cu2+ [[23](#_ENREF_23)]. In contrast, Zn2+ was incapable of sustaining RdRp catalyzed nucleotide incorporation [[23](#_ENREF_23)]. Secondly, a zinc-binding pocket has been identified in the Dengue virus and SARS-coronavirus RdRp. Therefore a possibility that binding of zinc may induce a structural change in the conformation of RdRp which no longer enables RdRp to catalyzed nucleotide incorporation. Finally, adding high concentrations of zinc ions to cells impairs viral polyprotein processing which is integral to virus replication [[24](#_ENREF_24)].

Several studies report that Zinc prevents pneumonia [[25](#_ENREF_25)]. Although the exact mechanism is unclear, it is postulated that zinc might act in the acute-phase response to infection, helping to boost the body’s immune response through a defense cascade, beginning with mobilisation and sequestration of zinc to metallothionein-rich tissue, rapid upregulation of immune defence-specific protein synthesis, activation of immune defence activity such as macrophages, lymphocytes, and natural killer cells, and antibody-dependent cytotoxicity [[25](#_ENREF_25)].

## Limitations of oral delivery of zinc

The upper limit for daily Zn intake in an adult is 40 milligrams. Importantly, when 30 mg of elemental Zn was given orally to humans, it resulted in an only 1.8-fold increase in plasma Zn to 27.4 ± 12 μmol/L from a baseline of 15.2 ± 5.1 μmol/L in the first 4 hours [[26](#_ENREF_26)]. 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 Zn from 14.18 ± 1.75 μmol/L in the placebo group to 17.18 ± 3.48 μmol/L in Zn group [[27](#_ENREF_27)]. We believe that this may be the reason why multiple trials conducted using oral Zn supplementation has failed to demonstrate improvement against either diarrhoea, infection rates or mortality [[28](#_ENREF_28), [29](#_ENREF_29)]. Oral delivery of Zn is affected by several factors, including normal variations in gut Zn absorption and dietary factors such as phytate, and interactions with other metal ions [[30](#_ENREF_30)]. Also, repeated high oral Zn intake causes a rapid and significant upregulation of intestinal metallothioneins which markedly decreases subsequent gut Zn, and importantly copper, absorption [[31](#_ENREF_31)]. The latter may lead to copper deficiency in patients administered Zn for prolonged periods.

## Pharmacologic-dose of zinc protects against ischaemia reperfusion injury.

Ischemia reperfusions injury describes the damage to organs due to a restoration of oxygen supply following a period of hypoxia. Ischaemia’ is a lack of blood and oxygen supply to an organ or cell and can be devastating if prolonged. However, ‘reperfusion’ (the subsequent return of blood and oxygen supply) further extends the injury, damaging the cells and tissues which leads to permanent organ dysfunction. The kidney and the heart are particularly sensitive to IRI. This pathology is seen in a number of organ injuries in acutely unwell patients. For example, acute kidney injury (AKI) is one of the most common complications after cardiac surgery, affecting up to 30% of all patients [[32](#_ENREF_32), [33](#_ENREF_33)]. AKI adversely affects recovery from cardiac surgery and is associated with a 5-fold increased risk of death during hospitalisation [[34](#_ENREF_34)]. Moreover, AKI is a major risk factor for subsequent chronic kidney disease (CKD) and end‐stage renal disease (ESRD) [[35](#_ENREF_35)]. One of the leading causes of AKI in cardiac patients is renal ischaemia-reperfusion injury (IRI) [[32](#_ENREF_32), [35](#_ENREF_35)]. ‘

Data presented in Fig 1, shows that HDIVZn (delivered as Zn chloride) reduces the AKI/IRI in sheep large animal model [[8](#_ENREF_8)]. More importantly, our unpublished data shown in Fig 2 demonstrates the ability of HDIVZn to protect the sheep heart against myocardial IRI.

In our recently published sheep study, we determined that a single IV ZnCl2 at a dose of 0.5mg/kg did not protect against IRI. However, single IV ZnCl2 at a dose of 0.5mg/kg increased the plasma Zn 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 [[8](#_ENREF_8)]. Interestingly two dosage IV ZnCl2 at a dose of 0.5mg/kg regimen increased the plasma Zn concentration 7-fold from a baseline concentration of 11.3 ± 0.4 μmol/L to 70.1 ± 5.8 μmol/L and was able to reduce the IRI by 70%. These data specify that for Zn to exert its protective effect not only the plasma Zn has to increase significantly, but the increase in plasma Zn has to be sustained.

As mentioned, Zn chloride intake via oral route increased the plasma zinc by only 1.8 fold. Therefore, we concluded that reduced bioavailability following oral delivery might be the reason why multiple trials conducted using oral Zn supplementation has failed to demonstrate improvement against either diarrhoea, infection rates or mortality [[28](#_ENREF_28), [29](#_ENREF_29)]. Therefore, the scientific basis behind the protective effect of HDIVZn [[8](#_ENREF_8)] against the IRI is its ability to deliver a pharmacologic Zn chloride dose and eliminate the confounding factors which impinge on the bioavailability of Zn chloride when given orally as explained in [[1](#_ENREF_1)].

One of the significant implications of acute respiratory syndrome and pneumonia following COVID-19 infection is that it reduces oxygen availability leading to tissue hypoxia. Our data has shown that HIVZn protects multiple organs, including heart, liver [[10](#_ENREF_10)] and kidneys against IRI instigated by reduced oxygen supply. An added advantage of HDIVZn therapy might be a reduction in ischaemic organ damage, including to kidneys, heart and liver seen in COVID-19 patients.

***Figure 1. HDIVZn protects sheep kidneys against renal IRI.*** *Sheep were subjected to uninephrectomy and 60 min of renal ischaemia followed by 7-day reperfusion. Serum creatinine (1A) was measured before Zn pre-treatment (baseline), before ischaemia (Day 0) and for 7 days after reperfusion. Sheep were preconditioned with a single dose of 0.5mg/kg ZnCl2 (~0.25mg/kg elemental Zn) either at 24 hr or 4 hr only, or with double dose (HDIVZn) at 24 and 4 hr prior to 60 min ischaemia. Preconditioning with 0.5mg/kg ZnCl2 significantly (P = 0.001 repeated measures ANOVA) reduced the rise in serum creatinine, a biomarkers of kidney injury. Data are expressed as mean ± SEM values (n=4, except 24 hr only group, n=1). (1B) HDIVZn which consist of 0.5mg/kg ZnCl2 administered as two doses one at 24 and the other at 4 hr prior to ischaemia significantly (\*p<0.05) reduced the ischaemic burden expressed as area under curve of creatinine rise over time compared to single dose given only at either 24hr or 4hr prior to ischaemia.*



***Figure 2. HDIVZn protects sheep heart against IRI.*** *Sheep were subjected to myocardial IRI by clamping the left anterior descending coronary artery for 1 hr followed by 3 hrs of reperfusion [14]. 2A) Cross section of left ventricle rings from one representative sheep in each group. The area of myocardium at risk of*[*infarction*](https://www-sciencedirect-com.ezp.lib.unimelb.edu.au/topics/pharmacology-toxicology-and-pharmaceutical-science/infarction)*and infarct size were delineated by Evan׳s blue and*[*triphenyltetrazolium chloride*](https://www-sciencedirect-com.ezp.lib.unimelb.edu.au/topics/pharmacology-toxicology-and-pharmaceutical-science/triphenyltetrazolium)*(TTC) staining. 2B) The area at risk of infarction and the infarct size were measured by computerized planimetry. Analysis was performed blinded to treatment group. The sheep which received HDIVZn as described in figure 1, had significantly reduced (\*p<0.05)* [*infarct*](https://www-sciencedirect-com.ezp.lib.unimelb.edu.au/topics/pharmacology-toxicology-and-pharmaceutical-science/infarction)*size as compared to vehicle (saline).*

## A direct translation of HDIVZn dosage used in sheep to humans

Translation of a drug’s dose from animals to humans depends on the drug dose correction factor (Km) which is calculated by dividing the average body weight (kg) of species to its body surface area (m2). For humans, Km is 37 [[36](#_ENREF_36)]. Direct carryover of a drug’s pharmacologic dosage, from animals to humans depends on how similar the Km value of the animal species in which efficacy was tested, is to human Km. As published in [[36](#_ENREF_36)], Km for rats is 6, and for rabbit, Km is 12. Moreover, for sheep Km is 36 (average body weight of sheep is 40kg, and body surface area is 1.10m2 [[37](#_ENREF_37)]). Therefore, the near similar Km value of sheep (36) to that of humans (37) has allowed us to conclude that human equivalent elemental Zn dosage would amount to be the same 0.25mg/kg that was shown to be protective in the sheep study and which we plan to use in our proposed trial.

## Safety of HDIVZn in humans.

Any concerns regarding the safety of HDIVZn can be negated based on published reports where humans were treated with high doses of Zn [[11-14](#_ENREF_11)]. 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 Zn 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 [[11-13](#_ENREF_11)]. Besides, elemental Zn at doses ranging from 5-22mg/d has been administered routinely as a component of parenteral nutrition without any reported side effects [[38](#_ENREF_38)].

Furthermore, a recently published phase I clinical trial in critically ill children with suspected Zn deficiency administered equivalent elemental Zn intravenously at 3-times higher dose (0.75mg/kg/d for seven days). It did not produce any adverse effects [[14](#_ENREF_14)]. Therefore, we are confident that our proposed 3-fold lower elemental Zn dosage of 0.25mg/kg/d, will be safe and not produce any adverse effects. Further, direct conversation with the authors of this Phase I study [[14](#_ENREF_14)] indicated that as elemental Zn has been given intravenously previously [[11-13](#_ENREF_11)], and the standard dose for parenteral nutrition at Cincinnati Children’s Hospital Medical Center is 0.2mg/kg/day, an Investigational New Drug (IND) application to FDA before human trials was not required.

# OBJECTIVES

## Aim

This study aims to evaluate whether the intravenous administration of high-dose intravenous zinc (HDIVZn) improves the clinical outcomes of hospitalized patients with COVID19 infection

## Hypothesis

* HDIVZn will reduce the severity of COVID-19 infection and improve the clinical outcomes of patients as measured by an eight-point ordinal scale recommended in the document published by WHO R&D Blueprint “Novel Coronavirus COVID-19 Therapeutic Trial Synopsis”.

# STUDY OUTCOME MEASURES

## Clinical efficacy outcomes:

### 1. Primary outcome

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* Mean change in the worst (highest) level of oxygenation (oxygen flow in litres/min) in non-ventilated patients
* Mean change in the worst (lowest) PaO2 /FiO2 ratio (in mmHg) in ventilated patients.

### 2. Secondary outcome [Time Frame: Up to day 28]

* 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, or opportunistic infection
* Number of patients admitted into an intensive care unit (ICU) [Time Frame: Up to day 28]
* 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”.

The eight-point ordinal scale consisted of the following categories:

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;

* 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 ]
* 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)

### 3 Measurement of safety variables

* Zn toxicity symptoms: Nausea and vomiting, stomach pain and diarrhoea, flu-like symptoms: fever, chills, cough, headache, HDL lower than 40 mg/dL, changes in taste perceptions (metallic taste).

## *Feasibility* outcomes*:*

### 1 Evaluate patient recruitment rate

* Logistical factors of recruiting patients on time
* The time required for consent and initial data collection
* Engagement and acceptability to surgical, medical and nursing hospital staff
* Define screened to eligible to randomisation patient ratios

### 2 Measurement of protocol compliance

* The primary assessment of our ability to blind treatment of the HDIVZn in a 250-ml saline preparation
* Drug availability from supplier, storage and timely delivery to a patient
* Good clinical practice documentation of drug prescription on Cerner (an electronic medical record), delivery to ICU by project research officer, double signing by nursing staff
* Appropriate preparation of drug- onsite refrigeration storage, preparation with SOPs, maintenance of sterile conditions, protocol compliance, breaches, and variation, documentation processes including patient retention and follow-up rates
* Determine the per-patient cost to estimate subsequent pivotal trial costs
* Assess the process for efficient and effective data entry and analysis

### 3 Ensure protocol safety

* Document adverse events by Clavien Dindo criteria and specifically those related to HDIVZn administration- nausea, diarrhoea, mental confusion. Importantly, determine how effectively adverse events are identified, documented and reported.

# OVERALL STUDY DESIGN

## Study design

Single centre, randomised double-blind placebo-controlled trial.

## Study Population

All consenting COVID-19 symptomatic confirmed hospitalized adult patients who fulfill World Health Organisation’s case definition which includes a positive PCR for COVID-19 from any specimen (e.g. respiratory, blood, urine, stool, other bodily fluid).

## Inclusion 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 a SARS-CoV-2 infection of any duration
* 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 (SaO2) of 94% or less while they were breathing ambient air or a ratio of the partial pressure of oxygen (PaO2) to the fraction of inspired oxygen (FiO2) (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

## Exclusion Criteria

* Age <18 or pregnant or lactating female
* Allergy to Zn
* Severe hepatic impairment defined as Child C liver disease.
* eGFR ≤ 30 mL/min/1.73 m2 (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 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.

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