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New prophylaxis regimen for SARS-CoV-2 infection in health professionals with low doses of Hydroxychloroquine and Bromhexine. A randomized, double-blind placebo-controlled clinical trial (ELEVATE Trial).

Julio Granados Montiel[1*], Eric Joseph Hazan Lasri[2], Raigam J. Martinez-Portilla[3] Rafael Franco Cendejas[4], Tatiana Chávez Heres[5], Phaedra Suriel Silva Bermúdez[1], Rocío Aguilar Gaytán[1], Natalia Manzano-León[6].

*Corresponding author
Julio Granados-Montiel.
Av. México-Xochimilco 289 col. Arenal de Guadalupe, Tlalpan. Mexico City, Mexico. C.P. 14389.
juliogram@gmail.com

Author affiliations
1 Tissue Engineering and Regenerative Medicine Unit. National Institute of Rehabilitation. Mexico City, Mexico.
2 Division of Traumatology, Emergencies and Bone Infections. National Institute of Rehabilitation. Mexico City, Mexico.
5 División de Investigación Clinica. Instituto Nacional de Perinatologia Isidro Espinosa de los Reyes (INPer). México.
3 Infectology Laboratory. National Institute of Rehabilitation. Mexico City, Mexico.
4 Service, Hospital Epidemiological Surveillance Unit. National Institute of Rehabilitation. Mexico City, Mexico.
5 División de Investigación Básica. Instituto Nacional de Cancerología (INCan). México.
6 Managing Director. National Institute of Rehabilitation. Mexico City, Mexico.

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ABSTRACT

Introduction In December 2019, the presence of pneumonia cases caused by the SARS-CoV-2 virus was reported in the city of Wuhan in Hubei province, China. This highly infectious new virus is the cause of severe acute respiratory syndrome (SARS). SARS-CoV-2 infection produces mild symptoms in 80% of those infected, severe symptoms in 15%, while 5% will require admission to the intensive care unit, and artificial respirator use. As of April 15, 2020, SARS-CoV-2 has caused 1,918,138 confirmed cases of infection and 123,126 deaths. In Mexico, it has produced 8,261 confirmed cases and 686 deaths. It is estimated, according to the experience of Italy, that 20% of health workers will be infected by this virus, causing 4.4% of deaths among them.

The SARS-CoV-2 virus binds to the pneumocyte through the angiotensin-converting enzyme (ACE) receptor. Two of the most promising treatments work by inhibiting binding to this receptor. These drugs are hydroxychloroquine and bromhexine. This study`s objective is to assess the efficacy of hydroxychloroquine + bromhexine as a prophylactic treatment for SARS-CoV-2 infection in healthy health workers exposed to the first line of care in patients with suspected or confirmed infection by this virus.

Methods and analysis A simple, double-blind, randomized clinical study of parallel allocation in a 1:1 ratio, placebo control, low doses of hydroxychloroquine (200 mg every 24 hours for 60 days + bromhexine syrup 8 mg every 8 hours for 60 days) will be administered. The control group will receive 8 mg bromhexine syrup every 8 hours for 60 days + placebo, which will be identical to hydroxychloroquine. The primary endpoint will be the efficacy of the intervention determined as the proportion of infected personnel in the intervention group divided by the proportion of infected in the control group in a period of 60 days and expressed in relative risk, absolute risk and analysis of survival. The results will be evaluated 30 days after the start of treatment, and close monitoring will be maintained for possible adverse events related to the use of the drugs. At least a 16% reduction in absolute risk is expected between the intervention and control group. At least a minimum of 20% infected is expected in the control group. The sample was calculated using a type 1 error of 0.0501 and a power of 80% plus 10% of possible losses, for a total of 140 volunteers assigned in parallel to two groups of 70 participants each. Adherence to treatment of more than 50% is expected. The study will be carried out for six months from its beginning. During this time is expected to finish the collection of the sample and adequate follow-up of the participants. The study will begin on May 1, 2020, and will end in November 2020. The statistical analysis will be available in December 2020 and the final results in January 2021.

Ethics and dissemination The protocol is approved by the local medical ethical review committee at the National Institute of Rehabilitation Luis Guillermo Ibarra Ibarra with the internal number INRLGII/25/20 and by The Federal Commission for Protection against Sanitary Risk (in Spanish, Comisión Federal para la Protección contra Riesgos Sanitarios, COFEPRIS) approval number 203300410A0058/2020. The protocol was peer reviewed to obtain funding from Mexico City Government.

Trial registration number NCT04340349.

Strengths and limitations of this study

- This simple, double-blind, randomized clinical study will provide important information of two of the most promising prophylactic treatment for SARS-CoV-2 infection: hydroxychloroquine and bromhexine.
- A sample of 140 participants: Healthy health workers exposed to the first line of care in patients with suspected or confirmed infection.
- The health system depends significantly on the medical personnel´s number and capacity assigned to its units, so each withdrawal (temporary or permanent) represents a significant loss. The study of prophylactic treatment in this population is of great value and would be the basis for protecting medical workers worldwide.
- All the data collected from this study will play an important role in the future reaction to a new outbreak.
- This kind of study will play a crucial role in new management and prevention of the viral outbreak in other areas. It will also play an essential role in reducing the response time to new emergencies in the hospital.

INTRODUCTION

On December 31, 2019, an outbreak of 27 pneumonia cases of unknown etiology was reported in Wuhan, Hubei province, China. A week later, the agent that caused the outbreak was identified as a new virus of the Coronaviridae family, being designated as a "severe acute respiratory syndrome coronavirus 2" (SARS-COV-2) by the International Committee on Taxonomy of Viruses. On January 12, 2020, China publicly shared the genetic sequence of COVID-19, and on January 14, the human-to-human transmission capacity of this virus was identified.[1,2] The affectation caused by this virus, the coronavirus disease 2019 (COVID-19), has already become a pandemic, implying the biggest battle in the health issue in recent years. Due to its biological characteristics and its high transmissibility, as of April 15, 2020, according to The World Health Organization (WHO), it has 1,918,138 confirmed cases of infection and 123,126 deaths worldwide. Latin America currently has 673,361 confirmed cases.[3] In Mexico, 5,847 confirmed cases have been reported, affecting 57.79% males and 42.21% females and 449 deaths have been registered.[4] The age group ranging between 30 and 79 years is the most frequently infected, where 81% present mild, 14% severe and 5% critical.

The SARS-CoV-2 is a betacoronavirus and this virus has an incubation period between 3 to 7 days,[5] it is transmitted through respiratory droplets from humans through contact with contaminated fomites and aerosols. On the other hand, it has been found that asymptomatic patients in close contact can transmit the disease,[6] and fecal-oral transmission has also been described as a transmission mechanism.[7] The infectivity of this virus is higher than the influenza virus`s infectivity, with an R0 value of 2.28.[8] It is estimated that an infected person can infect between 2 to 6.5 people.[9] The mechanism through which the virus infects the respiratory cell could be due to the angiotensin-converting enzyme protein 2 (ACE-2). This receptor is found in multiple tissues such as the oral cavity, brain, kidneys, intestine, and placenta. [10-12]

Health personnel is not exempt from contracting the disease. In China, it was reported that 3.5-4.4% of the infected population belonged to this group, and 14.8% presented characteristics of severity or critical illness.[5,13,14] In Italy, around 20% of healthcare professionals became infected.[15] The mean age of health workers who died was 55 years with a range of 29-72 years. The mean period from hospital admission to death was 19 days, with a range of 1-47 days.[14]

The Chloroquine has been used as an antimalarial agent. It blocks viral infection by increasing the endosomal pH required for virus fusion to the cell and interferes with glycosylation of SARS-CoV-2 cell receptors.[16,17] However, its use is not safe since both chloroquine and hydroxychloroquine (similar in structure but with less toxicity and better tolerability) can cause myocardial toxicity (restrictive or dilated cardiomyopathy). These drugs can cause conduction disturbances atrioventricular (AV) or Bundle Branch block since, being aminophilic cations, they bind to cardiomyocyte phospholipids, causing intracellular inhibition of cardiomyocyte lysosomal enzymes and finally altering the degradation of pathological metabolites such as phospholipids and glycogen. These effects are more frequent in elderly patients, females, drug use >3 months, pre-existing heart disease, kidney failure and use of high doses of chloroquine per mg/kg body weight/day.[18] Other toxic effects described are retinopathy and neuromyopathy.[19]

The Bromhexine, a derivative of the Adhatoda vasica plant (*Justicia adhatoda*), modifies the composition of mucus, increases ciliary clearance and decreases cough, improving

respiratory symptoms. It has also been reported to enhance the effects of some antibiotics.[20] Bromhexine has a specific inhibitory effect on human Transmembrane serine protease (TMPRSS2).[21] The mechanism by which SARS-CoV-2 enters human cells depends on the receptor for the angiotensin-converting enzyme 2 (ACE-2) and the serine protease TMPRSS2.[22] There are currently several drugs that demonstrate a specific inhibition of the serine protease TMPRSS2, among them, are Camostat, Mesylate and Nafamostat, both drugs approved by the FDA but not available in Mexico and that produce side effects and contraindications. Bromhexine turns out to be an ideal candidate since it has no contraindications, and its side effects are minimal, demonstrating an extensive margin of pharmacological safety. Bromhexine is widely available in the country and its low cost makes it an ideal therapeutic option.

In a letter to the editor in the New England Journal of Medicine, of 77,262 patients infected by COVID-19, 3387 (4.4%) were from health workers. Of these, 23 have died from this disease. The prevalence of infections in health personnel is alarming since the health services in the first world countries have been overwhelmed by this disease. Each one of the health personnel is fundamental and indispensable to reduce mortality and give opportunity care to those patients who require it. In Italy, around 20% of health professionals had a SARS-CoV-2 infection.[15] Faced with a highly contagious disease, the care of health workers, who are first line of contact and on whom the health system of each country depends, is essential. This research regarding the use of hydroxychloroquine and bromhexine versus bromhexine in health personnel will allow us to determine and compare the effectiveness of both interventions, which is of vital importance to clarify whether these treatments are effective in preventing the appearance of infection in this population. Describing for the first time that both hydroxychloroquine and bromhexine could function as disease preventives, would allow us to provide prophylaxis to all-inclusive health professionals in each country. Therefore the use of hydroxychloroquine+bromhexine in prophylactic doses in healthy health personnel exposed to the first line of care in patients confirmed or suspected with SARS-CoV-2 will significantly reduce infections. This drug combination will be more effective compared with the exclusive use of bromhexine at prophylactic doses in the same exposed population and will not have significant adverse events such as mortality, nausea, vomiting, abdominal pain, diarrhea, rash, itchy skin, hair loss, lengthening of the QT interval in the electrocardiogram (>500 msec), opacity corneal, cardiac arrhythmias, heart failure or renal failure (defined as glomerular filtration <20mL/min), when compared with the exclusive use of bromhexine at prophylactic doses.

METHODS AND ANALYSIS

Study design

Randomized, parallel-group, double-blind, placebo-controlled, 1:1 assignment to determine the efficacy of low-dose hydroxychloroquine + bromhexine vs. low dose bromhexine, for the prevention of SARS-CoV-2 infection in healthcare workers. The study will be carried out at the “Instituto Nacional de Rehabilitación, Luis Guillermo Ibarra Ibarra” (INR LGII). This institution is a third-level hospital that at the time of writing this protocol has not been designated as a COVID-19 hospital. However, at this time that we are in phase 3, it is assumed that every person who enters the hospital is a symptomatic or asymptomatic carrier. Likewise, health personnel working at the “Instituto Nacional de Ciencias Médicas y Nutrición, Salvador Zubirán” (INCMNSZ) and falls within the inclusion criteria of the

protocol will attend the "Instituto Nacional de Rehabilitación Luis Guillermo Ibarra Ibarra" on their own for participate in the clinical study.

Participants

The inclusion of participants will be assessed according to the eligibility criteria and by invitation to the same. Table 1 shows the classification of study variables according to the intervention and control groups.

Table 1 Classification of variables.

Variable	**Conceptual definition**	**Operational definition**	**Measurement scale**
Age	Date at recruitment minus date of birth	Years old	Quantitative
Sex	Male or female genotype of the person	Man or woman	Qualitative nominal
Weight	How much the patient weighs at the time of study inclusion	Weight in kilograms	Continuous quantitative
Size	How tall is the patient from head to toe at the time of study inclusion	Height centimeters	Continuous quantitative
Body mass index	The division between weight by height squared at the time of inclusion in the study	Units of kg / cm^2	Continuous quantitative
Occupation	Remunerative work performed by the participant at the time of recruitment	Unemployed, informal, unskilled employee, micro-entrepreneur or saleswoman, administrative employee, professional,	Qualitative nominal

		entrepreneur	
Civil status	Civil status of the individual	Married, single, widowed, divorced, common-law union	Qualitative nominal
Level of study	Years completed and approved at the time of study recruitment	With or without studies, primary, secondary, preparatory, technical career, undergraduate, postgraduate	Ordinal qualitative
Alcohol intake	Consumption of alcoholic beverages	Intake of alcoholic beverages	Qualitative nominal
Smoking habit	Habitual tobacco use at the time of recruitment	Number of packs of cigarettes consume per day.	Quantitative
Drug's use	Regular use of chemicals such as amphetamines, cocaine, marijuana, LSD, heroin, glass	Consumption of drugs	Qualitative nominal
Hypertension	Elevation of blood pressure >130/80	Individual risk of hypertension	Qualitative nominal
Asthma	Chronic inflammatory disease characterized by bronchial hyperactivity with recurrent episodes of bronchospasm	Individual Risk of Asthma	Qualitative nominal
Diabetes	Group of metabolic diseases, which occurs when the pancreas does not produce enough	Individual risk of diabetes	Qualitative nominal

	insulin or when the body does not use the insulin it produces effectively		
Obesity	Pathological state characterized by a general excess or excessive accumulation of fat in the body	Individual risk of obesity	Qualitative nominal
SARS	A form of severe pneumonia caused by coronavirus	Individual risk for SARS	Qualitative nominal
Death	Statistical term that describes the death of an individual	Individual risk of death	Qualitative nominal
Intensive Care Unit	Special facility in a hospital area, which provides life support to critically ill patients, requiring intensive supervision and monitoring	Individual risk of admission to intensive care unit	Qualitative nominal
Severe pneumonia	Defined by the American Thoracic Society Criteria requiring at least one main criterion (need for invasive mechanical ventilation and shock with need for vasopressors), or three minor criteria (respiratory rate> 30 bpm, Pa02 / FiO2 ratio <250, Infiltrates	Presence of pneumonia	Qualitative nominal

	multilobars, confusion / disorientation, uremia [BUN> 20 mg / dL], leukopenia [<4,000], thrombocytopenia [<100,000 platelets / mm^3], hypothermia [core temperature <36ºC], or hypotension requiring aggressive fluid resuscitation		
Pneumonia	Acute infection of the lung parenchyma, accompanied by bilateral infiltrates on chest X-ray	Presence or not of pneumonia	Qualitative nominal
Confusion	Glasgow scale less than 15	Individual risk of confusion	Qualitative nominal
Hypothermia	Body temperature less than 36 degrees Celsius	Individual risk of hypothermia	Qualitative nominal
Thrombocytopenia	Total platelets less than 100,000 per mm^3.	Presence or not of thrombocytopenia	Qualitative nominal
Arterial hypotension	Systolic blood pressure less than 90 mmHg or mean arterial pressure less than 60 mmHg	Individual risk of hypotension	Qualitative nominal
Sepsis	Rapid SOFA score (qSOFA) with 2 of the following three	Individual risk of sepsis	Qualitative nominal

	clinical variables: Glasgow ≤13, systolic pressure ≤100 mm Hg, or respiratory rate ≥22 bpm		
RT-PCR for SARS-CoV2	Molecular diagnosis for SARS-CoV from viral RNA	Positive or negative	Qualitative nominal
Septic shock	Arterial hypotension that persists after resuscitation volume and that requires vasopressors to maintain MAP ≥65 mm Hg and lactate ≥2 mmol / L (18 mg / dL) in the absence of hypovolemia	Individual risk of septic shock	Qualitative nominal
Adverse events related to the use of Chloroquine	Indirect drug-related mortality, nausea, vomiting, abdominal pain, rash, itchy skin, hair loss, QT segment elongation on electrocardiogram, corneal opacity, cardiac arrhythmias, and heart failure	Individual risk of adverse events related to the use of hydroxychloroquine	Qualitative nominal
Adverse events related to the use of Bromhexine	Indirect drug-related mortality, nausea, vomiting, abdominal pain, rash, itchy skin, hair loss, QT segment elongation on electrocardiogram,	Individual risk of adverse events related to the use of Bromhexine	Qualitative nominal

	corneal opacity, cardiac arrhythmias, and heart failure		
Severe acute respiratory syndrome	A form of severe pneumonia caused by coronavirus	Individual risk of severe acute respiratory syndrome	Qualitative nominal

The study variables will be divided according to the allocation group (intervention and control). The distribution of continuous variables will be assessed using the Shapiro Wilk test and Skewness and Kurtosis. The variables of normal distribution will be compared using the Student's t-test, free distribution using the Mann-Whitney U-test. Categorical variables will be evaluated using the Chi square-test.

Inclusion criteria

- Health personnel working at "Instituto Nacional de Rehabilitación and at "Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán"
- Over 18 and under 60 years, both sex.
- Exposed to the care of patients with suspected or confirmed SARS-CoV-2
- Normal electrocardiogram.

Exclusion criteria

- Reverse transcriptase-polymerase chain reaction (RT-PCR) test for SARS-CoV-2 positive at the time of inclusion.
- Panel of IgG or IgM antibodies positive for SARS-CoV-2 at the time of inclusion.
- Development of respiratory symptoms suspicious of SARS-CoV-2 during the first 7 days after starting treatment, confirmed by RT-PCR.
- Under 18 and over 60 years.
- Health personnel with morbidities such as diabetes, hypertension, autoimmune disease (such as porphyria, psoriasis, systemic lupus erythematosus), obesity as body mass index (BMI≥30), cardiovascular disease, respiratory diseases (such as asthma, chronic bronchitis, idiopathic pulmonary fibrosis).
- Allergic to any hydroxychloroquine or bromhexine compound.
- Use of immunosuppressant for any reason.
- History of a bone marrow transplant
- Known glucose-6-phosphate dehydrogenase (G6PD) deficiency.
- Chronic kidney disease or glomerular filtration <20ml/min
- Use of other drugs as digitalis, flecainide, amiodarone, procainamide, or propafenone.
- History of long QT syndrome
- Electrocardiogram with QTc>500 msec.
- Pregnant or breastfeeding

- Epilepsy
- Known liver disease

Elimination criteria

- Personnel who decide to leave the study for any reason not related to adverse events.
- Personnel with incomplete information on the primary outcome (RT-PCR test for SARS-CoV-2).
- Personnel who are relocated to work in another institution.
- Personnel who do not wish to perform consecutive sample analysis for SARS-CoV-2 at 30 and 60 days after the start of pharmacological treatment.

Sample size calculation

According to the study by Remuzzi A et al.[15] the proportion of healthcare workers infected with SARS-CoV-2 and confirmed by RT-PCR was 20%. Taking this 20% as our null hypothesis, we calculate that the proportion of infections in the intervention group will be 4%. Using a two-tailed test, with a type I error of 0.0501, a power of 80%, and calculating a loss of 10% of participants for each group, we estimate that a total of 140 participants will be required, distributed in parallel groups (1:1) of 70 each. This number of volunteers will allow us to find a difference of 16% between groups with a power of 80%. To ensure that desired simple size is reached, all health workers involved in dealing with patients suspected or infected by SARS-CoV-2 will be invited personally and by institutional email.

The group's assignment will be in a centralized and straightforward random way using the Web program www.randomization.com. The randomization will be carried out independently by a researcher who will be blind to the inclusion criteria, the delivery of drugs, the participant`s follow-up, the results, the statistical analysis, and the writing of the final article. The allocation will be established, for 140 participants in blocks of 70 and 70 assigned as intervention vs. control.

Investigational product/intervention

In the experimental group, the intervention will consist of low (prophylactic) doses of hydroxychloroquine (Plaquenil) 200 mg tablets every 24 hours for 60 days + Bromhexine syrup (Bisolvón) 8 mg every 8 hours for 60 days. Hydroxychloroquine tablets and bromhexine syrup will be given to each participant in the total dose for the 60 days of the study and an extra dose if loss of the medication. The drugs will be provided directly in the hospital by an investigator blinded to group assignment process. In addition to the bromhexine, a graduate spoon will be given to ensure that participants take the appropriate dose assigned.

The intervention in the control group will consist of a placebo dose of bromhexine syrup (Bisolvón) 8 mg every 8 hours for 60 days in the control group. Bromhexine syrup will be given to each participant in the total dose for 60 days and an extra dose if the loss of the drugs. The medicine will be delivered directly by a blind researcher to the group´s assignment to which the participant belongs. A graduate spoon will also be provided to ensure that participants take the proper assigned dose.

To ensure that the intervention is carried out, each participant will be asked to keep a written record of the days and time the medication was administrated. This document will be reviewed weekly to verify that more than 50% adherence to treatment is maintained.
Participants will be asked to record any symptoms related to the use of the medication, which will be reviewed by a researcher blinded to group assignment, weekly, or at the participant`s request.
If any of the participant present symptoms of SARS-CoV-2 infection after the first 14 days after the start of the intervention and positive RT-PCT disease is confirmed, the drug will be discontinued. If the participant presents adverse events related to the drugs that are not tolerable, the treatment will be suspended. If the participants have an adherence of less than 50% of the medication, the intervention will be discontinued.
The use of drugs that interact with hydroxychloroquine or bromhexine such as flecainide, digitalis, amiodarone, procainamide or propafenone will be prohibited. If the participant has to use these drugs during the study period will be eliminated from the study.
A free diet and outdoor activity will be allowed since these do not intervene with the implementation of the treatment or have interaction with the drugs used.
Likewise, the incidence of adverse events such as mortality, nausea, vomiting, abdominal pain, rash, itchy skin, hair loss, lengthening of the QT interval in the electrocardiogram, corneal opacity, cardiac arrhythmias and, heart failure, will be determined.

Randomization and treatment allocation

The selection of health workers will be made regardless of the hospital shift, work schedule, or assigned area. If the desired sample size is not reached, the inclusion of personnel involved in the first line of care of other referral hospitals for patients with SARS-CoV-2 will be considered.
According to the Web, an independent researcher will place the treatments for the experimental and control group to effectively hide the assignment groups. These envelopes will be correctly sealed, and inside will contain Bromhexine for both groups. In those who do not require hydroxychloroquine, the drug will be replaced by tablets identical in color and taste like hydroxychloroquine tablets, but lack active substance. In this way, the drugs used in both groups will be indistinguishable.
Researcher A will recruit the participants and assess the inclusion criteria according to the serological, electrocardiographic, and biochemical results. Once included, volunteers will go to another office with researcher B, who will be blind to the first procedure and the rest of the study. Researcher B will assign the groups independently, centrally, and through the use of a web program. This same researcher B will be the one who makes the packages indistinguishable to the person providing the drugs to the participant. Researcher C will provide treatment in a sealed envelope or box to the participant in the order of assignment without knowing each participant`s original assignment. This researcher will also be blind to the assignment and the rest of the results.
Participants will be blinded to the assignment of the treatment they will receive. Researcher A will be blinded to what the recruitment will do, researcher B who will do the group assignment and packing of the treatment, and researcher C who will deliver the treatment in packages or sealed envelopes in consecutive order without knowing what treatment each participant is receiving. The researchers in the follow-up group, the researchers in the results assessment group, and the researcher who performs the statistical analysis will be blind.

Informed consent will be obtained only by researcher A. If researcher A is not available, the study administrator may obtain informed consent for participation. The informed consent will contain the authorization to participate in the study and the authorization for the taking of biological samples, electrocardiogram, and authorization to handle personal information.

All participants will complete a written informed consent included on the first page of the questionnaire that requires permission to participate in the study. No candidate is required to participate in the study, and their participation is based on the agreement that they can withdraw at any time.

All participants have the right to withdraw from the study if they feel uncomfortable answering a question or with a test to be performed. Also, no one, including the research team, will require the reason why the participant decides to leave the study.

In order to protect the confidentiality of the participants, each one will be assigned a participation number, and all biological samples, as well as medical history information, will be identified by the participant's initials, date of birth and participant number. Part of the confidentiality protection process will include data capture only by the researcher in charge of data capture (researcher D), who will be the same for all participants and the entire study. Secondarily, the study administrator may also enter data into the database if researcher D is unwell.

The study administrator will be blind to the allocation and results of the participants. However, the administrator will be the only one who will be able to reveal the group and treatment assignment in any of the cases: major adverse events such as cardiac arrhythmias, heart failure, major following neurological abnormalities, atrial or ventricular fibrillation, kidney failure, or any adverse event related to pharmacological treatment that endangers the life or any organ of the participant´s body. The objective of revealing the assignment by the study administrator will be to be able to warm the participant of a timely treatment according to the drugs ingested.

Participant timeline and intervention

The inclusion of participants will be evaluated according to the eligibility criteria and by invitation. Volunteers who wish to participate in the study will be summoned the next day at a specialized office to carry out all the relevant studies to ensure the inclusion criteria. These include a medical history, anthropometric measurements such as weight and body mass index, electrocardiogram, hematic biometry, complete blood chemistry, and serological test for antibodies and RT-PCT for SARS-CoV-2. Volunteers will be asked for information to contact them once the serological results are obtained.

Once the results are obtained (approximately 3 days), personnel eligible to participate in the study will be contacted. They will meet in a particular office to speak with a researcher who will be in charge of carrying out the eligibility criteria and medical history checklist. This researcher will be different from the one who makes the assignment, who delivers the medicine and the one who evaluates the results and performs the statistical analysis. The assignment of the group of each participant will be done through the Web, and the participant will not know the group they have been assigned. This information will be known for the researcher in charge, unrelated to the delivery of the treatment, results, or inclusion of the participant in the study. After the assignment, the volunteers will receive the assigned treatment at the pharmacy using a code in a sealed envelope assigned by the Web.

Participants who meet the inclusion criteria and there is no reason for exclusion will proceed to the second phase of group assignment with researcher B, the next business day at a different time or office than researcher A.
The group of researchers in charge of monitoring the participants, who will be blind to the group assignment at all times, will be in charge of assessing each participant´s adverse event and treatment adherence record weekly. These follow-up researchers will be available 24 hours a day throughout the week if participants experience undesirable adverse events that require urgent attention or that do not allow them to continue with drug treatment. If this latter situation happens, the researcher in charge of the follow-up will contact the study administrator to reveal to the treating physicians the treatment received by the participant.
At the end of the first 30 days, a new RT-PCR will be requested from each participant. The same action will be carried out 60 days after the start of treatment for both groups. After 60 days, the treatment will be suspended and the results of the RT-PCR samples for SARS-CoV-2 will be evaluated.

Outcome measures
This study compares the efficacy of the use of hydroxychloroquine plus bromhexine vs. the exclusive use of bromhexine in prophylactic doses every 24 hours for 60 days in healthy health personnel exposed to the first line of care in confirmed patients with suspected infection by SARS-CoV-2.

Primary endpoint
The primary endpoint will be the proportion of health personnel infected by SARS-CoV-2 after seven days of posterior study`s inclusion and up 60 days after starting treatment, both the control and intervention group. The infection will be diagnosed using RT-PCR for SARS-CoV-2 after day 7 of the start of treatment. The period will be 60 days. The proportion of infected will be evaluated between the control and experimental group using relative risk (RR) and absolute risk increase (ARI) in the established time. The disease-free period in the 60 days will also be evaluated by analyzing the cumulative incidence of healthy patients, and the presence of confirmed infection by RT-PCR of SARS-CoV-2 will be the outcome. The censoring variable will be the discontinuation of treatment either due to death, adverse events, or any elimination criteria. Since there is the possibility of false positives and negatives, also of PCR, we will perform qualitative measurements of IgM and IgG with the Elecsys® Anti-SARS-CoV-2 test from Roche laboratories.
Secondary outcome

Secondary endpoints

- The secondary outcome will be the proportion of health personnel infected by SARS-CoV-2 after seven days of study inclusion and up to 30 days after the start of treatment, both in the intervention and control group. The infection will be diagnosed using RT-PCR for SARS-CoV-2 after day 7 of the start of treatment. The period will be 30 days. The proportion of infected will be evaluated between the control and experimental group employing RR and ARI in the established time with their respective 95% confidence intervals. The disease-free period in the 30 days will also be evaluated by analyzing the cumulative incidence of healthy health personnel, and the presence of confirmed infection by RT-PCR of SARS-CoV-2

will be the outcome. The censoring variable will be the discontinuation of treatment either due to death, adverse events, or any elimination criteria.

- Another secondary endpoint will be adverse events, defined as the presence of any of the following during the study period: death, nausea, vomiting, abdominal pain, diarrhea, rash, itchy skin, hair loss, lengthening of the QT interval in the electrocardiogram (>500msec), corneal opacity, cardiac arrhythmias, heart failure or kidney failure (renal clearance <20ml/min). The proportion of the compound of adverse events between the experimental and control groups will be analyzed using RR and ARI for 60 days with their respective 95% confidence intervals.

The efficacy of the treatment will be established as the proportion of volunteers infected with SARS-CoV-2 in the intervention group compared with the control group. This difference should be sufficient to avoid overlapping of the 95% confidence intervals. It will be considered effective if the intervals do not overlap and ineffective if both groups have a proportion of infected whose confidence intervals overlap.
This type of evaluation will allow an adequate understanding of the efficacy of the treatment in both groups.

Handling and storage of data and documents

Before the start of the study, the researchers in charge of the recruitment, assignment, and delivery of drugs will be trained to perform the task assigned to them at least 3 days before the start of the study.
Researcher A will assess the eligibility criteria of potential participants and perform a detailed clinical examination to assess whether they can participate in the study. The data that will be collected initially will be the following:

- Medical history (includes personal data): study identifier number, history number, name, date of birth, sex, occupation, marital status, nationality, current residence, degree of studies (primary, secondary, upper secondary, bachelor`s degree, postgraduate), hospital service to which it belongs and the number of hours worked per week.
- Personal history: alcohol intake (yes/no; how many glasses of beer or alcoholic beverages do you consume per week), smoking habit (yes/ no; and number of cigarettes per day), drug use (yes/no), diet per week (dietary restrictions and number of meals per day) and number of hours of sleep per day.
- Gynecological history (in women): Number of pregnancies, number of live children, menarche, menopause.
- History of respiratory disease, history of gastrointestinal disease, nephrological, neurological, hematological, cardiovascular, allergic.
- Genetic family history, such as hypertension, diabetes, heart disease, kidney disease.
- Physical examination: blood pressure, heart rate, respiratory rate, temperature, weight, height, body mass index, skin lesions, head and neck inspection, respiratory inspection (chest symmetry, lung expansion, palpation of the bases and preserved vertices, lung percussion, auscultation for lung murmur, breath sounds). Cardiovascular inspection (palpation of the fifth intercostal space, auscultation of heart sounds, pulses that are palpable and symmetrical), abdominal inspection (palpation, percussion and auscultation of peristaltic sounds), neurological

evaluation (Glasgow, active motility, passive motility, reflex motility, cranial nerves, sensitivity).

- Hematic biometry: Hematocrit, leukocytes, segmented (%), lymphocytes (%), monocytes (%), Mean corpuscular volume (MCV), platelets.
- Blood chemistry: Glycaemia, urea, creatinine, sodium, potassium, chlorine, GOT, GPT, alkaline phosphatase AF (FAL), total bilirubin.
- Muscle enzymes.
- Clotting times: Thrombin time (PT), Prothrombin time (PTT), International normalized ratio (INR).
- Electrocardiogram: rhythm, heart rate, heart axis, evaluation of P wave, PR interval, duration of QRS, QT interval, time of T wave. The electrocardiogram will be performed using an instrument calibrated and validated for its use internationally.
- Molecular test results for IgG antibodies and IgM serology:
- The FDA approved product called Cellex qSARS-CoV-2 IgG/IgM Rapid Test will be used for serological determination. The device cassette, sample, and buffer solution must be at room temperature. The sample (10 µL) is transferred to the center of the sample well. After the sample well is free of liquid, two drops of sample diluent are added. After fifteen to twenty minutes, read the test results. Results should not be read after twenty minutes.
- A positive IgM result occurs when a colored band appears on the M test line (M) and the control line (C) and indicates that IgM against SARS-CoV-2 is present.
- A positive IgG result occurs when a colored band appears on the G test line (G) and the control line (C) and indicates that IgG against SARS-CoV-2 is present.
- A positive result for IgM and IgG occurs when colored bands occur both M and G, as well as C.
- A negative result occurs when a colored band appears in C only and indicates that IgM and IgG antibodies against SARS-CoV-2 were not detected.
- An invalid result occurs when a color band is not produced in C, and the test must be repeated.
- Official RT-PCR results (carried out by "Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán")

All this information will be collected in a pre-established medical history questionnaire for each potential participant. This information will be dumped into an online database registered on the CASTOR server in the United States of America.

The information obtained from the weekly assessment of adverse events, and the results of the RT-PCR for SARS-CoV-2 at 30 and 60 days after starting treatment will be entered into an online database. In order to ensure the quality of the data collection, the database will be built in CASTOR, a database on the Web that allows entering all the pre-defined data for each participant, thus reducing human error. This information will be stored on a server in the United States of America and can only be accessed by the study`s administrator. The data may only be entered by a researcher in charge of collecting the data sheets and emptying them.

Monitoring and quality assurance

Every possible adverse event will be noted daily by the participants in the agenda that will be delivered to them. This agenda will be evaluated weekly by the researcher in charge who

monitoring the participants (who will be blind to the group assignment). In case of unbearable adverse events for the participants or that put their health at risk, an open line will be available 24 hours a day with direct communication to the researcher in charge of monitoring the study to report any event that requires hospitalization or immediate evaluation at the hospital. All participants with adverse events that put their life or health at risk may be urgently assessed by personnel from the National Institute of Medical Science and Nutrition "Salvador Zubirán" at the National Rehabilitation Institute and if possible by the same INR staff within the INR as part of the study. The patient follow-up investigator will immediately contact the study administrator to disclose the participant's assignment to treating physicians at that institution, but the assignment will never be disclosed to other investigators related to the study. All the study expenses and/or attention of collateral effects will be covered by the current cost of the financing SECTEI/061/2020.

The audit will be carried out weekly, assessing adverse events, capturing data in the corresponding datasheets by the study administrator. Likewise, the data entered in the CASTOR web base will be valued to validate its quality. The paper data sheets must be kept in a special office dedicated to the study in folders separated by volunteers with the informed consent of each participant, the data of the medical history, laboratory results, eligibility criteria, adverse event sheet and results, molecular tests, as well as electrocardiogram. The letter of revocation of informed consent will also be protected if required. As part of the audit, an interim analysis will be carried out 30 days after the study starts to assess the possible adverse effects and whether these outweigh the potential benefits of the intervention. In the adverse event outweigh the potential benefits, termination of the study will be assessed. The approval of the research ethics committee of the National Institute of Rehabilitation of Mexico will be sought.

Statistical analysis

The data analysis will be carried out by intention to treat, which means that each participant will be analyzed according to the group assigned regardless of whether they modified their treatment. The study variables will be divided according to the allocation group (intervention and control). The distribution of continuous variables will be assessed using the Shapiro Wilk test and Skewness and kurtosis. The variables of normal distribution will be compared using the Student's t-test, free distribution using the Mann-Whitney U test. Categorical variables will be evaluated using the Chi-square test.

The primary objective will be expressed in number and proportion for each group. The relative risk will be obtained as the division between the proportion of primary outcomes in the intervention group by the proportion of primary outcomes in the control group. It will be expressed as RR with its respective 95% confidence interval for the initial time, which is 60 days. Likewise, the result will be expressed as absolute risk, which will be derived from the proportion of the primary outcome in the intervention group minus the proportion of the primary outcome in the control group. Secondarily, the primary objective will be analyzed with the non-parametric estimate of the survival and risk function using Kaplan-Meier curves for 60 days according to the allocation group. The primary endpoint will be SARS-CoV-2 infection within the 60-day period, and the silencing variable will be dropping out of the study for any reason. The comparison of the survival curves between both groups will be carried out using the log-rank test. To adjust the primary objective to possible confounders such as age, sex, and service in that volunteer works, body mass index, etc.

Multiple regression will be performed using the Cox model to determine the adjusted primary endpoint hazard ratio.
For secondary outcomes such as the analysis at 30 days, the same statistical analysis expressed in RR and absolute risk will be used. Survival analysis will be used for the primary endpoint only.
An interim statistical analysis will be performed 30 days after the study starts to assess possible adverse effects and the efficacy of the intervention.
The study administrator will be the only one with access to the data. For the interim analysis and the final analysis, the administrator will export the data to Excel format to be analyzed by the study statistician blindly to the assignment of groups, participants, or results.

AEs, SAEs and SUSARs
By requiring the use of drugs, the participant will be exposed to risks inherent to the drug used, ranging from mild to severe or death. Any unexpected risks that may occur during the study will be immediately explained to the participants and the ethics committee. Any adverse event will be compiled and will not be disclosed under any condition to anyone other than the study administrator, treating physicians in case of severe care and the ethics committee. Besides, the results will be completely anonymous concerning the names of the participants. The results will be compiled and reported as combined collective data.

Patient and public involvement
Patients were not involved in the development of this research. However, the results of the study will be communicated to the study participants by sending the end product (article) to the provided email address.

ETHICS, DISSEMINATION AND SAFETY MONITORING
In case of damage, adverse events or complications derived from the study, participants will be assured attention by the staff of the National Institute of Medical Science and Nutrition "Salvador Zubirán" in an enclosure that ensures the safety of the participant, not subjecting volunteers to a higher risk of contamination. This care will be extended until adverse events are resolved. In case of no adverse events during the study, medical attention will be extended at the aforementioned institute until 15 days after the end of the study.
The protocol is approved by the local medical ethical review committee at the National Institute of Rehabilitation, Luis Guillermo Ibarra Ibarra with the internal number INRLGII/25/20. Definitions of Research Risk Regulation of the General Health Law on Research for Health (in Spanish, Reglamento de la Ley General de Salud en Materia de Investigación para la Salud) http://www.inr.gob.mx/Descargas/Investigacion/Reglamentos-LGS-Materia-Investigacion-Salud.pdf. ARTICLE 17; and by The Federal Commission for Protection against Sanitary Risk (in Spanish, Comisión Federal para la Protección contra Riesgos Sanitarios, COFEPRIS) approval number 203300410A0058/2020.
The study`s results will be published in journals of worldwide impact affiliated with the Journal Citation Reports (JCR). Likewise, the results of the study will be disseminated in national and international media, exposed in international and national congresses,

communicated to CONACYT, and recorded in Clinicaltrials.gov according to the study identifier number.
The help of non-profit organizations will be sought to disseminate the results of the investigation to interest groups.
The complete protocol will be published on Clinicaltrials.gov and the OSF - Center for Open Science platform https://osf.io/. Where a DOI will be assigned, and the amendments made to the original protocol will be assessed.
Amendments to the protocol may be made before the start of the study and during the study. Any amendment to the protocol will be clarified and posted on Clinicaltrials.gov under the same identifier as this study. Likewise, any amendment will be sent to the ethics committee of the same hospital.

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Contributors Study concept and design were conducted by author JGM. Advanced statistical input was given by author RJMP Critical revision of concept and design and intellectual input in the study protocol were done by authors, Study supervision and coordination were conducted by authors EHL and JGM Manuscript revision and editing was done by RAG and NML.

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Competing interest, the study is conducted by the department of Epidemiology at the National Institute of Rehabilitation Luis Guillermo Ibarra Ibarra, as stated above in the funding section, an unrestricted grant from SECTEI/061/2020was provided for the conduct of the trial.

Declaration of interests No one of the researchers presents a conflict of interest or has commercial agreements, or receives financial compensation from any commercial or pharmaceutical company.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not required

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ORCID ID

Julio Granados-Montiel https://orcid.org/0000-0002-0611-6421

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New prophylaxis regimen for SARS-CoV-2 infection in health professionals with low doses of Hydroxychloroquine and Bromhexine. A randomized, double-blind placebo clinical trial (ELEVATE Trial).

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New prophylaxis regimen for SARS-CoV-2 infection in health professionals with low doses of Hydroxychloroquine and Bromhexine: a randomized, double-blind placebo clinical trial (ELEVATE Trial).

Julio Granados-Montiel[1*], Eric Joseph Hazan Lasri[2], Rafael Franco Cendejas[3], Tatiana Chávez Heres[4], Phaedra Suriel Silva Bermúdez[1], Rocío Aguilar Gaytán[1], Natalia Manzano-León[5], Karla Méndez-Maldonado[1], Alejandro Alvarez-Arce[1], Raigam J. Martinez-Portilla[6]

[1] Tissue Engineering and Regenerative Medicine Unit. National Institute of Rehabilitation. Mexico City, Mexico.

[2] Division of Traumatology, Emergencies and Bone Infections. National Institute of Rehabilitation. Mexico City, Mexico.

[3] Infectology Laboratory. National Institute of Rehabilitation. Mexico City, Mexico.

[4] Service, Hospital Epidemiological Surveillance Unit. National Institute of Rehabilitation. Mexico City, Mexico.

[5] Basic Division Research, National Institute of Cancerology. Mexico City, Mexico.

[6] Barcelona Centre for Maternal-Fetal and Neonatal Medicine (Hospital Clínic and Hospital Sant Joan de Déu), Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Universitat de Barcelona, Centre for Biomedical Research on Rare Diseases (CIBER-ER), Barcelona, Spain.

*Corresponding author

Julio Granados-Montiel. M.D.& Ph.D.

Tissue Engineering and Regenerative Medicine Unit. National Institute of Rehabilitation

Av. México-Xochimilco 289. Col. Arenal de Guadalupe, Tlalpan. 14389, Mexico City, Mexico.

ORCID ID: 0000-0002-0611-6421

juliogram@gmail.com

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ABSTRACT

Introduction: SARS-CoV-2 infection in Mexico has caused ~1 million confirmed cases; around 20-25% of health workers will be infected by the virus at their workplace, with approximately 4.4% of mortality. High infectivity of SARS-CoV-2 is related with cell entry mechanism, through the angiotensin-converting enzyme (ACE) receptor. SARS-CoV-2 requires transmembrane protease serine 2 (TMPRSS2) to cleave its spike glycoprotein and ensure fusion of host cell and virus membrane. We propose studying prophylactic treatment with hydroxychloroquine (HCQ) and bromhexine (BHH), which have been shown to be effective in preventing SARS-CoV-2 infection progression when administered in early stages. The aim of this study is to assess the efficacy of HCQ and BHH as prophylactic treatments for SARS-CoV-2 infection in healthy health workers exposed to the virus.

Methods and analysis: Double-blind randomized clinical trial, with parallel allocation at a 1:1 ratio with placebo, of low doses of HCQ and BHH, for 60 days. Study groups will be defined as follows: 1) HCQ 200mg/d + BHH placebo; 2) BHH 8mg/8h + HCQ placebo; 3) HCQ 200mg/d + BHH 8mg/8h; and 4) HCQ placebo + BHH placebo. Primary endpoint will be efficacy of the interventions, determined by differences in the proportions of infected personnel. At least a 16% reduction in absolute risk is expected between the double intervention and double placebo groups; a minimum of 20% infection is expected in the placebo group. The sample size calculation estimated a total of 280 patients assigned: four groups of 70 participants each.

Ethics and dissemination: This protocol has been approved by the local Medical Ethics Committee (National Institute of Rehabilitation 'Luis Guillermo Ibarra Ibarra', approval number INRLGII/25/20) and by the Federal Commission for Protection against Sanitary Risks (COFEPRIS, approval number 203300410A0058/2020). The results of the study will be submitted for publication in peer-reviewed journals and disseminated through conferences.

Trial registration number: NCT04340349.

STRENGTHS AND LIMITATIONS OF THIS STUDY

Strengths

- This is a double-blind randomized single-centre clinical trial, involving low doses of hydroxychloroquine and bromhexine, adequately powered to provide clinically relevant information regarding prophylactic treatment for SARS-CoV-2 infection in health care personnel.
- This study will include 280 participants who are health workers exposed to SARS-CoV-2 patients with suspected or confirmed infection, with short term follow-up (60 days).
- A study of prophylactic treatment in this population is of great value and could provide the basis for protecting medical personnel around the world.
- Both drugs proposed for this study have minimal side effects and are commercially available worldwide; findings could be applied in a timely fashion in different regions.

Limitations

- None of the proposed drugs have proven to be effective as a treatment for symptomatic SARS-CoV-2 infected patients.

INTRODUCTION

On December 31, 2019, an outbreak of 27 pneumonia cases of unknown aetiology was reported in Wuhan, Hubei province, China[1]. A week later, the agent that caused the outbreak was identified as a new virus of the Coronaviridae family, being designated as a "severe acute respiratory syndrome coronavirus 2" (SARS-CoV-2) by the International Committee on Taxonomy of Viruses[2 3]. Affectation caused by this virus, coronavirus disease 2019 (COVID-19), has become a pandemic, implying the biggest health-related battle in recent years. In Mexico, up to December 2020, have been produced more than 1 million confirmed cases and ~130,000 deaths, according to WHO data[4]. The age group ranging between 30 and 79 years is the most highly affected, where 81% present mild symptoms, 14% severe and 5% critical, requiring intensive care unit management.

SARS-CoV-2 is a single-stranded RNA virion, member of the *Betacoronavirus* genus[5]. SARS-CoV-2 has an incubation period between 3 to 10 days, with different incubation periods related with different clinical symptoms[6 7]. It is transmitted through respiratory droplets from infected humans through contact with contaminated fomites and aerosols; on the other hand, asymptomatic patients in close contact can transmit the disease[8]. The mechanism through which the virus infects the respiratory cell is due to the angiotensin-converting enzyme protein 2 (ACE-2). This receptor is found in multiple tissues such as the oral cavity, brain, kidneys, intestine, and placenta[9-11].

Health personnel is not exempt from contracting the disease. In China, it was reported that 3.5-4.4% of the infected population belonged to this group, and 14.8% presented characteristics of severity or critical illness[7 12 13]. In Italy, around 20% of healthcare professionals became infected[14]; mean age of health workers who died was 55 years (range of 29-72 years) and mean period from hospital admission to death was 19 days, (range 1-47 days)[12].

Treatment of the SARS-Cov-2 infection has led different research groups to work on the development of vaccines. However, the use of vaccines can be a challenge. Early trials have shown minimal immune protection or long-term protection is low. On the other hand, because the virus is RNA and the mutation rate is high, we can expect new variants that reduce or nullify the effectiveness of vaccines. Therefore, it is not known if the vaccines that are now in the phase end of the clinical study and those that are administered will work with the same efficacy for the SARS-CoV-2 virus that gave rise to Covid-19. Therefore, it is important to develop a pharmacological strategy that allows the use of prophylactic drugs for the prevention of SARS-CoV-2 infection.

Chloroquine (CQ) and Hydroxychloroquine (HCQ) are known as an antimalarialagents; HCQ is a hydroxylated derivative from CQ. HCQ has been used in several viral infections, for example, as replication inhibitor for the dengue virus, decreasing *in vitro* virus infection and promoting activation of different immunological signal pathways[15]. It has also been used to treat patients infected with hepatitis C virus decreasing viral load, with minimal adverse effects reported[16]. HCQ has been reported to block viral infection by increasing the endosomal pH required for virus fusion to the cell, as well as interfere with SARS-CoV-2 cell receptors, through inhibition of ACE2 glycosylation receptor[17 18 19 20]. HCQ has immunomodulatory effects; it inhibits production and release of pro-inflammatory cytokines, that are associated with severe disease development[21 22]. Recently, it has been reported that HCQ works as a autophagy inhibitor, interfering with viral infection and replication[23]. There is recent evidence that HCQ could be used to treat COVID-19; studies in high-risk patients show that the use of HCQ was associated with a lower risk of intubation or death[24]. Finally, a recent study showed that pre-treatment with HCQ has shown a better effect on antiviral activity[19].

Another pharmacological option to treat SARS-CoV-2 infection is Bromhexine (BHH). BHH modifies the composition of mucus, increases ciliary clearance and decreases coughing, improving respiratory symptoms. It has also been reported to enhance the effects of some antibiotics[25]. The mechanism by which SARS-CoV-2 enters human cells depends on the ACE-2 receptor and the human transmembrane serine protease (TMPRSS2), on which BHH has a specific inhibitory effect[26 27]. BHH has been used to treat pneumonic damage in both lungs during early infection[28]. BHH turns out to be an ideal candidate for SARS-CoV-2 treatment, since it has few contraindications, and its side effects are minimal, demonstrating an extensive margin of pharmacological safety. BHH is widely available over the counter, and its low cost makes it an ideal therapeutic option.

According to a letter published in the New England Journal of Medicine, of 77,262 patients infected by SARS-CoV-2, 3387 (4.4%) were health workers[12]. Of these, 23 have died from this disease. The prevalence of infections in health personnel is alarming since health services in first world countries have been overwhelmed by this disease. In Italy, around 20% of health professionals had a SARS-CoV-2 infection[14]. Faced with a highly contagious disease, the care of health workers, who are first line of contact and on whom the health system of each country depends, is essential. This research regarding the use of HCQ and BHH in health personnel will allow us to determine and compare the effectiveness of both interventions, which is of vital importance to clarify whether these treatments may prevent the appearance of infection in this population. Describing for the first time that HCQ and/or BHH could function for disease prevention, would allow us to provide prophylaxis to health professionals worldwide. Therefore, the use of HCQ and BHH in healthy health personnel exposed in patients with confirmed or suspected SARS-CoV-2 will significantly reduce infection.

METHODS AND ANALYSIS

Study design

Double-blind randomized clinical trial, with parallel allocation at a 1:1 ratio with placebo, of low doses of HCQ and BHH, for 60 days, to determine the efficacy of low-dose HCQ and/or BHH for the prevention of SARS-CoV-2 infection in healthcare workers.

Participants

The study will be carried out at the "Instituto Nacional de Rehabilitación, Luis Guillermo Ibarra Ibarra" (INR-LGII). This institution is a tertiary hospital that at this time has not been designated as a COVID-19 centre. The Mexican government defined 3 phases to determine risk for SARS-CoV-2 infection: imported cases from outside Mexico; community infection and spread of the disease throughout the country (also known as Phase 3). In the latter, it is assumed that every person who enters a hospital is a potentially infected carrier; currently our centre is in Phase 3. Likewise, health personnel who work at the "Instituto Nacional de Ciencias Médicas y Nutrición, Salvador Zubirán" (INCMNSZ), which is a COVID-19 designated tertiary centre, and who meet inclusion criteria of the protocol will be invited to participate in the study.

Inclusion of participants will be assessed according to the eligibility criteria. **Table 1** shows the classification and characteristics of study variables. Continuous variables will be assessed for normality. Variables with a normal distribution will be compared using Student's *t*-test, non-parametric variables using the Mann-Whitney U-test. Categorical variables will be evaluated using the Chi-squared test.

Inclusion criteria

- Health personnel working at INR LGII or INCMNSZ who wish to participate in the study and sign the informed consent.
- Over 18 and under 60 years of age, both genders.

- Exposition or caring for patients with suspected or confirmed SARS-CoV-2 infection.
- Normal electrocardiogram.

Exclusion criteria

- Positive quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) test for SARS-CoV-2 at the time of inclusion.
- Panel of IgG or IgM antibodies positive for SARS-CoV-2 at the time of inclusion.
- Development of respiratory symptoms suspicious of SARS-CoV-2 infection during the first 7 days after treatment is initiated, confirmed by qRT-PCR.
- Health personnel with comorbidities such as diabetes, hypertension, autoimmune diseases (i.e., porphyria, psoriasis, systemic lupus erythematosus), obesity (defined as body mass index ≥30), cardiovascular diseases, respiratory diseases (such as asthma, chronic bronchitis, idiopathic pulmonary fibrosis).
- History of allergies to any hydroxychloroquine or bromhexine related compound or medication.
- Use of immunosuppressors for any reason.
- History of bone marrow transplant.
- Known glucose-6-phosphate dehydrogenase deficiency.
- Chronic kidney disease or glomerular filtration <20ml/min.
- Use of other drugs with reported pharmacological interactions (i.e., digitalis, flecainide, amiodarone, procainamide, or propafenone).
- History of long QT syndrome.
- Electrocardiogram with QTc>500 msec.
- Pregnant or breastfeeding personnel.

- Epilepsy.
- Known liver disease.

Elimination criteria

- Personnel who decide to leave the study for any reason not related to adverse events.
- Personnel with incomplete information on the primary outcome (qRT-PCR for SARS-CoV-2).
- Personnel who are relocated to work in another institution.
- Personnel who do not wish to perform consecutive sample analysis for SARS-CoV-2 at 30 and 60 days after the start of pharmacological treatment.

Table 1. Classification and characteristics of study variables.

Variable	Conceptual definition	Operational definition	Type
Age	Date at recruitment minus date of birth	Years of age	Quantitative
Gender	Male or female genotype of the person	Male/female	Qualitative nominal
Weight	How much the patient weighs at the time of study inclusion	Weight, kilograms	Continuous quantitative
Size	How tall is the patient from head to toe at the time of study inclusion	Height, centimetres	Continuous quantitative
Body mass index	The division between weight by height squared at the time of inclusion in the study	Units of Kg/cm^2	Continuous quantitative
Occupation	Remunerative work performed by the participant at the time of recruitment	Unemployed, informal, unskilled employee, micro-entrepreneur or saleswoman, administrative employee, professional, entrepreneur	Qualitative nominal

Civil status	Civil status of the individual	Married, single, widowed, divorced, common-law union	Qualitative nominal
Level of study	Years completed and approved at the time of study recruitment	No studies, primary, secondary, preparatory, technical career, undergraduate, postgraduate	Ordinal qualitative
Alcohol intake	Consumption of alcoholic beverages	Intake of alcoholic beverages	Qualitative nominal
Smoking habit	Habitual tobacco uses at the time of recruitment	Number of packs of cigarettes consumed per day.	Quantitative
Drug's use	Regular use of chemicals such as amphetamines, cocaine, marijuana, LSD, heroin, glass	Consumption of drugs	Qualitative nominal
Hypertension	Elevation of blood pressure >130/80	Positive/negative	Qualitative nominal
Asthma	Chronic inflammatory disease characterized by bronchial hyperactivity with recurrent episodes of bronchospasm	Positive/negative	Qualitative nominal
Diabetes	Group of metabolic diseases, which occurs when the pancreas does not produce enough insulin or when the body does not use the insulin it produces effectively	Positive/negative	Qualitative nominal
Obesity	Pathological state characterized by a general excess or excessive accumulation of fat in the body	Positive/negative	Qualitative nominal

SARS-CoV-2 pneumonia	A form of severe pneumonia caused by coronavirus	Positive/negative	Qualitative nominal
Death	Statistical term that describes the death of an individual	Positive/negative	Qualitative nominal
Intensive Care Unit	Special facility in a hospital area, which provides life support to critically ill patients, requiring intensive supervision and monitoring	Positive/negative	Qualitative nominal
Severe pneumonia	Defined by the American Thoracic Society Criteria requiring at least one main criterion (need for invasive mechanical ventilation and shock with need for vasopressors), or three minor criteria (respiratory rate> 30 bpm, Pa02 / FiO2 ratio <250, Infiltrates multilobars, confusion / disorientation, uremia [BUN> 20 mg / dL], leukopenia [<4,000], thrombocytopenia [<100,000 platelets / mm^3], hypothermia [core temperature <36ºC], or hypotension requiring aggressive fluid resuscitation	Positive/negative	Qualitative nominal
Pneumonia	Acute infection of the lung parenchyma, accompanied by bilateral infiltrates on chest X-ray	Positive/negative	Qualitative nominal
Confusion	Glasgow scale less than 15	Positive/negative	Qualitative nominal
Hypothermia	Body temperature less than 36 degrees Celsius	Positive/negative	Qualitative nominal
Thrombocytopenia	Total platelets less than 100,000 per mm^3.	Positive/negative	Qualitative nominal

Arterial hypotension	Systolic blood pressure less than 90 mmHg or mean arterial pressure less than 60 mmHg	Positive/negative	Qualitative nominal
Sepsis	Rapid SOFA score (qSOFA) with 2 of the following three clinical variables: Glasgow ≤13, systolic pressure ≤100 mm Hg, or respiratory rate ≥22 bpm	Positive/negative	Qualitative nominal
qRT-PCR for SARS-CoV-2	Molecular diagnosis for SARS-CoV-2 from viral RNA	Positive/negative	Qualitative nominal
Septic shock	Arterial hypotension that persists after resuscitation volume and that requires vasopressors to maintain MAP ≥65 mm Hg and lactate ≥2 mmol / L (18 mg / dL) in the absence of hypovolemia	Positive/negative	Qualitative nominal
Adverse events related to the use of Hydroxychloroquine	Indirect drug-related mortality, nausea, vomiting, abdominal pain, rash, itchy skin, hair loss, QT segment elongation on electrocardiogram, corneal opacity, cardiac arrhythmias, and heart failure	Positive/negative	Qualitative nominal
Adverse events related to the use of Bromhexine	Indirect drug-related mortality, nausea, vomiting, abdominal pain, rash, diarrhea.	Positive/negative	Qualitative nominal

Sample size calculation

According to the study by Remuzzi A et al.[14], the proportion of healthcare workers infected with SARS-CoV-2 and confirmed by RT-PCR was 20%. Taking this 20% as our null hypothesis, we calculate that the proportion of infections in the intervention group will be 4%. Using a two-tailed test, with a type I error of 0.05, a power of 80%, and taking into account a loss of 10% of participants for each group, we estimate that a total of 280 participants will be required, distributed in parallel groups (1:1) of 70 each. This number

of volunteers will allow us to find a difference of 16% between groups with a power of 80%. To ensure that desired simple size is reached, all health workers involved in managing patients suspected or infected by SARS-CoV-2 will be invited personally and by institutional email.

Interventions

Interventions will consist of low doses of HCQ 200 mg tablets every 24 hours for 60 days, and BHH 8 mg tablets every 8 hours for 60 days. Study groups will be defined as follows: 1) HCQ 200 mg every 24 hours plus BHH placebo; 2) BHH 8 mg every 8 hours plus HCQ placebo; 3) HCQ 200 mg every 24 hours plus BHH 8 mg every 8 hours; and 4) HCQ placebo plus BHH placebo. Fabrication of both drugs and placebos will be provided to our centre by a hired laboratory. Both drugs will be provided to participants directly at the hospital by a researcher blinded to group assignment process. To ensure that the intervention is carried out, each participant will be asked to keep a written record of the days and time the medication was administrated. This document will be reviewed weekly to verify that more than 50% adherence to treatment is maintained. Participants will be asked to record any symptoms related to the use of the medication, which will be reviewed by a researcher blinded to group assignment, weekly, or at the participants' request.

If any of the participants present symptoms of SARS-CoV-2 infection after the first 14 days from the beginning of the intervention or positive qRT-PCR is present, the drug will be discontinued. If the participant presents adverse events related to the drugs that are severe or intolerable, treatment will be suspended. If the participants report an adherence of less than 50% of the medication, the intervention will be discontinued. Use of drugs that interact with HCQ or BHH such as flecainide, digitalis, amiodarone, procainamide or propafenone will be prohibited. If a participant has to use these drugs during the study

period, they will be eliminated from the study. A free diet and outdoor activity will be allowed since these do not intervene with the implementation of the treatment or have interaction with the drugs used. Finally, incidence of adverse events such as nausea, vomiting, abdominal pain, rash, itchy skin, hair loss, lengthening of the QT interval in the electrocardiogram, corneal opacity, cardiac arrhythmias, heart failure and death will be determined.

Randomization and treatment allocation

Group randomization will be in a centralized and straightforward way using the Web program www.randomization.com. It will be carried out independently by a researcher blinded to inclusion criteria, delivery of medication, participant follow-up, results, statistical analysis, and writing of the final manuscript. Allocation will be established, for 280 participants in blocks of 70 assigned. The selection of health workers will be made regardless of the hospital shift, work schedule, or assigned area. If the desired sample size is not reached, the inclusion of personnel involved in the first line of care of other referral hospitals for patients with SARS-CoV-2 will be considered.

An independent researcher will allocate patients to the desired groups. Envelopes will be correctly sealed by the pharmacy department, and will contain HCQ, BHH or placebos as previously mentioned. In those who do not require HCQ or BHH, the drug will be replaced by tablets identical in colour and taste but lacking the active substance. In this way, drugs used in both groups will be indistinguishable.

Researcher A will recruit the participants and assess the inclusion criteria according to the serological, electrocardiographic, and biochemical results. Once included, volunteers will go to another office with researcher B, who will be blinded to the first procedure and the rest of the study. Researcher B will assign the groups independently, centrally, and through the use of the web program. This same researcher will

be the one who makes the packages indistinguishable to the person providing the drugs to the participant. Researcher C will provide treatment in a sealed envelope or box to the participant in the order of assignment, without knowing each participant's study group. This researcher will also be blinded to the rest of the results. Participants will be blinded to the treatment they will receive. The researchers performing follow-up, researchers for result assessment, and the researcher who performs the statistical analysis will be blinded.

Informed consent will be obtained only by researcher A. If researcher A is not available, the study administrator may obtain informed consent for participation. The informed consent will contain the authorization to participate in the study and the authorization for taking biological samples, electrocardiogram, and authorization to handle personal information. All participants will complete a written informed consent included on the first page of the questionnaire that requires permission to participate in the study. No candidate is required to participate in the study, and their participation is based on the agreement that they may withdraw at any time. All participants have the right to withdraw from the study if they feel uncomfortable answering a question or with a test to be performed. Also, no one, including the research team, will require a reason why the participant decides to leave the study.

In order to protect participant confidentiality, each one will be assigned a participation number, and all biological samples, as well as medical history information, will be identified by the participant's initials, date of birth and participant number. Part of the confidentiality protection process will include data capture only by the researcher in charge of data capture (researcher D), who will be the same for all participants and the entire study. Secondarily, the study administrator may also enter data into the database if researcher D is unavailable.

The study administrator will be blinded to allocation and results of the participants. However, the administrator will be the only one who will be able to reveal the group and treatment assignment in any of the cases: major adverse events such as cardiac arrhythmias, heart failure, major neurological abnormalities, atrial or ventricular fibrillation, kidney failure, or any adverse event related to pharmacological treatment that endangers the life or any organ of the participant's body. The objective of revealing the assignment by the study administrator will be to provide the participant of a timely treatment according to the drugs ingested.

Participant timeline and intervention

The inclusion of participants will be evaluated according to the eligibility criteria and by invitation. Volunteers who wish to participate in the study will be summoned the next day at a specialized office to carry out all the relevant studies to ensure the inclusion criteria. These include a medical history, anthropometric measurements such as weight and body mass index, electrocardiogram, hematic biometry, complete blood chemistry, and serological test for antibodies and qRT-PCR for SARS-CoV-2. Volunteers will be asked for information to contact them once the serological results are obtained.

Once the results are obtained (approximately 3 days), personnel eligible to participate in the study will be contacted. They will meet in a particular office to speak with a researcher who will be in charge of carrying out the eligibility criteria and medical history checklist. This researcher will be different from the one who makes the assignment, who delivers the medicine and the one who evaluates the results and performs the statistical analysis. The assignment of the group of each participant will be performed, and the participant will not know the group they have been assigned. This information will be known for the researcher in charge, unrelated to the delivery of the treatment, results, or inclusion of the participant in the study. After the assignment, the volunteers will receive the assigned treatment at the pharmacy using a code in a sealed

envelope assigned by the Web. Participants who meet the inclusion criteria and there is no reason for exclusion will proceed to the second phase of group assignment with researcher B, the next business day at a different time or office than researcher A.

The group of researchers in charge of monitoring the participants, who will be blinded to the group assignment at all times, will be in charge of assessing each participant´s adverse event and treatment adherence record weekly. These follow-up researchers will be available 24 hours a day throughout the week if participants experience undesirable adverse events that require urgent attention or that do not allow them to continue with drug treatment. If this situation happens, the researcher in charge of the follow-up will contact the study administrator to reveal to the treating physicians the treatment received by the participant.

At the end of the first 30 days, a new qRT-PCR will be requested from each participant. The same action will be carried out 60 days after the start of treatment for both groups. After 60 days, the treatment will be suspended and the results of the qRT-PCR samples for SARS-CoV-2 will be evaluated.

Outcome measures

This study compares the efficacy of the use of HCQ and/or BHH in prophylactic doses for 60 days in healthy health personnel exposed to the first line of care in confirmed patients with suspected infection by SARS-CoV-2.

Primary endpoint

The primary endpoint will be the proportion of health personnel infected by SARS-CoV-2 after seven days of inclusion and up 60 days after starting treatment, in all groups. The infection will be diagnosed

using qRT-PCR for SARS-CoV-2 after day 7 of treatment. The study period will be 60 days. The proportion of infected personnel will be evaluated using relative risk (RR) and absolute risk increase (ARI) with their respective 95% confidence intervals, in the established time. The disease-free period in the 60 days will also be evaluated by analysing the cumulative incidence of healthy patients, and the presence of confirmed infection by qRT-PCR of SARS-CoV-2 will be the outcome. The censoring variable will be the discontinuation of treatment either due to death, adverse events, or any elimination criteria. Since there is the possibility of false positives and negatives with qRT-PCR, we will perform qualitative measurements of IgM and IgG with the Elecsys® Anti-SARS-CoV-2 test from Roche laboratories.

Secondary endpoints

The secondary endpoint will be the proportion of health personnel infected by SARS-CoV-2 after seven days of inclusion and up 30 days after starting treatment, in all groups. The infection will be diagnosed using qRT-PCR for SARS-CoV-2 after day 7 of the start of treatment. The study period will be 30 days. The proportion of infected personnel will be evaluated using RR and ARI with their respective 95% confidence intervals, in the established time. The disease-free period in the 30 days will also be evaluated by analyzing the cumulative incidence of healthy patients, and the presence of confirmed infection by qRT-PCR of SARS-CoV-2 will be the outcome. The censoring variable will be the discontinuation of treatment either due to death, adverse events, or any elimination criteria.

Another secondary endpoint will be adverse events, defined as the presence of any of the following during the study period: death, nausea, vomiting, abdominal pain, diarrhoea, rash, itchy skin, hair loss, lengthening of the QT interval in the electrocardiogram (>500msec), corneal opacity, cardiac arrhythmias,

heart failure or kidney failure (renal clearance <20ml/min). The proportion of the compound of adverse events between the groups will be analysed using RR and ARI for 60 days with their respective 95% confidence intervals.

The efficacy of the treatment will be established as the proportion of volunteers infected with SARS-CoV-2. This difference should be sufficient to avoid overlapping of the 95% confidence intervals. It will be considered effective if the intervals do not overlap and ineffective if when comparing groups, they have a proportion of infected whose confidence intervals overlap. This type of evaluation will allow an adequate understanding of the efficacy of the treatment in both groups.

Handling and storage of data and documents

Before the start of the study, the researchers in charge of the recruitment, assignment, and delivery of drugs will be trained to perform the task assigned to them at least 3 days before the start of the study.

Researcher A will assess the eligibility criteria of potential participants and perform a detailed clinical examination to assess whether they can participate in the study. The data that will be collected initially will be the following:

- Medical history (includes personal data): study identifier number, history number, name, date of birth, gender, occupation, marital status, nationality, current residence, degree of studies (primary, secondary, upper secondary, bachelor`s degree, postgraduate), hospital service to which they belong and the number of hours worked per week.
- Personal history: alcohol intake (yes/no; how many glasses of beer or alcoholic beverages do you consume per week), smoking habit (yes/ no; and number of cigarettes per day), drug use (yes/no),

diet per week (dietary restrictions and number of meals per day) and number of hours of sleep per day.

- Gynaecological history (in women): Number of pregnancies, number of live children, menarche, menopause.
- History of respiratory disease, history of gastrointestinal disease, nephrological, neurological, haematological, cardiovascular, allergies.
- Genetic family history, such as hypertension, diabetes, heart disease, kidney disease.
- Physical examination: blood pressure, heart rate, respiratory rate, temperature, weight, height, body mass index, skin lesions, head and neck inspection, respiratory inspection (chest symmetry, lung expansion, palpation of the bases and preserved vertices, lung percussion, auscultation for lung murmur, breath sounds). Cardiovascular inspection (palpation of the fifth intercostal space, auscultation of heart sounds, pulses that are palpable and symmetrical), abdominal inspection (palpation, percussion and auscultation of peristaltic sounds), neurological evaluation (Glasgow, active motility, passive motility, reflex motility, cranial nerves, sensitivity).
- Hematic biometry: haematocrit, leukocytes, segmented (%), lymphocytes (%), monocytes (%), mean corpuscular volume, platelets.
- Blood chemistry: glycaemia, urea, creatinine, sodium, potassium, chlorine, aspartate transaminase, alanine transaminase, alkaline phosphatase, total bilirubin.
- Muscle enzymes.
- Clotting times: thrombin time, prothrombin time, international normalized ratio.
- Electrocardiogram: rhythm, heart rate, heart axis, evaluation of P wave, PR interval, duration of QRS, QT interval, time of T wave. The electrocardiogram will be performed using an instrument calibrated and validated for its use internationally, weekly.

- Molecular test results for IgG and IgM antibodies:
  - The FDA approved product called Cellex qSARS-CoV-2 IgG/IgM Rapid Test will be used for serological determination. The device cassette, sample, and buffer solution must be at room temperature. The sample (10 µL) is transferred to the center of the sample well. After the sample well is free of liquid, two drops of sample diluent are added. After fifteen to twenty minutes, read the test results. Results should not be read after twenty minutes.
  - A positive IgM result occurs when a coloured band appears on the M test line (M) and the control line (C) and indicates that IgM against SARS-CoV-2 is present.
  - A positive IgG result occurs when a coloured band appears on the G test line (G) and the control line (C) and indicates that IgG against SARS-CoV-2 is present.
  - A positive result for IgM and IgG occurs when coloured bands occur both M and G, as well as C.
  - A negative result occurs when a coloured band appears in C only and indicates that IgM and IgG antibodies against SARS-CoV-2 were not detected.
  - An invalid result occurs when a colour band is not produced in C, and the test must be repeated.
- Official qRT-PCR results (carried out by INCMNSZ)

All this information will be collected in a pre-established medical history questionnaire for each potential participant. The information obtained from the weekly assessment of adverse events, and the results of the qRT-PCR for SARS-CoV-2 at 30 and 60 days after starting treatment will be entered into an online database. In order to ensure the quality of the data collection, the database will be built in CASTOR, a database on the Web that allows entering all the pre-defined data for each participant, thus reducing human

error. This information will be stored on a server in the United States of America and can only be accessed by the study's administrator. The data may only be entered by a researcher in charge of collecting the data sheets and emptying them.

Monitoring and quality assurance

Every possible adverse event will be noted daily by the participants in the agenda that will be delivered to them. This agenda will be evaluated weekly by the researcher in charge of monitoring the participants (who will be blinded to group assignment). In case of unbearable adverse events for the participants or that put their health at risk, an open line will be available 24 hours a day with direct communication to the researcher in charge of monitoring the study to report any event that requires hospitalization or immediate evaluation at the hospital. All participants with adverse events that put their life or health at risk may be urgently assessed by personnel from both INCMNSZ or INR-LGII, if possible, by the same staff within which are part of the study. Patient follow-up investigator will immediately contact the study administrator to disclose the participant's assignment to treating physicians at that institution, but the assignment will never be disclosed to other investigators related to the study. All the study expenses and/or attention of collateral effects will be covered by the current cost of the financing SECTEI/061/2020.

Auditing will be carried out weekly, assessing adverse events, capturing data in the corresponding datasheets by the study administrator. Likewise, the data entered in the CASTOR web base will be valued to validate its quality. The paper data sheets must be kept in a special office dedicated to the study in folders separated by volunteers with the informed consent of each participant, the data of the medical history, laboratory results, eligibility criteria, adverse event sheet and results, molecular tests, as well as electrocardiogram. The letter of revocation of informed consent will also be protected if required. As part of the audit, an interim analysis will be carried out 30 days after the study starts to assess the possible

adverse effects and whether these outweigh the potential benefits of the intervention. In the adverse event outweigh the potential benefits, termination of the study will be assessed. The approval of the research ethics committee of the INR-LGII of Mexico has been obtained.

Statistical analysis

Data analysis will be carried out by intention to treat, which means that each participant will be analysed according to the group assigned regardless of whether they modified their treatment. The study variables will be divided according to the allocation group. The distribution of continuous variables will be assessed using the Shapiro Wilk test, Skewness and kurtosis. The variables of normal distribution will be compared using the Student's *t*-test, non-parametric distribution using the Mann-Whitney U test. Categorical variables will be evaluated using the Chi-squared test.

The primary objective will be expressed in number and proportion for each group. The RR will be obtained as the division between the proportion of primary outcomes in the intervention group(s) by the proportion of primary outcomes in the double placebo group. Adjusted risk ratios (aRR) will be obtained using a log-binomial regression, adjusting for age and gender as pre-specified confounding variables. It will be expressed as RR with its respective 95% confidence interval for the initial time, which is 60 days. Likewise, the result will be expressed as absolute risk, which will be derived from the proportion of the primary outcome in the intervention group minus the proportion of the primary outcome in the control group. Secondarily, the primary objective will be analysed with the non-parametric estimate of the survival and risk function using Kaplan-Meier curves for 60 days according to the allocation group. The primary endpoint will be SARS-CoV-2 infection within the 60-day period, and the silencing variable will be dropping out of the study for any reason. The comparison of the survival curves between both groups

will be carried out using the log-rank test. To adjust the primary objective to possible confounders such as age, gender, service in which the participant works, body mass index, etc. Multiple regression will be performed using the Cox model to determine the adjusted primary endpoint hazard ratio.

For secondary outcomes such as the analysis at 30 days, the same statistical analysis expressed in RR and absolute risk will be used. Survival analysis will be used for the primary endpoint only. An interim statistical analysis will be performed 30 days after the study starts to assess possible adverse effects and the efficacy of the intervention. The study administrator will be the only one with access to the data. For the interim analysis and the final analysis, the administrator will export the data to Excel format to be analysed by the study statistician blinded to the assignment of groups, participants, or results.

Adverse events, serious adverse events and suspected unexpected serious adverse reactions

By requiring the use of drugs, the participant will be exposed to risks inherent to the drug used, ranging from mild to severe or death. Any unexpected risks that may occur during the study will be immediately explained to the participants and the ethics committee. Any adverse event will be compiled and will not be disclosed under any condition to anyone other than the study administrator, treating physicians in case of severe events, and the ethics committee. The results will be completely anonymous concerning the names of the participants. The results will be compiled and reported as combined collective data.

Patient and public involvement

Patients were not involved in the development of this research. However, the results of the study will be communicated to the study participants by sending the end product (published article) to the provided email address.

ETHICS, DISSEMINATION AND SAFETY MONITORING

In case of adverse events or complications derived from the study, participants will be assured attention by the staff of the INCMNSZ in an enclosure that ensures the safety of the participant, not subjecting volunteers to a higher risk of contamination. This care will be extended until adverse events are resolved. In case of no adverse events during the study, medical attention will be extended at the aforementioned institute until 15 days after the end of the study.

This protocol has been approved by the local medical ethical review committee at the INR-LGII with the internal number INRLGII/25/20, and by the Federal Commission for Protection against Sanitary Risks (in Spanish, Comisión Federal para la Protección contra Riesgos Sanitarios, COFEPRIS), approval number 203300410A0058/2020.

The study results will be published in journals of worldwide impact affiliated with the Journal Citation Reports. Likewise, the results of the study will be disseminated in national and international media, exposed in international and national congresses, communicated to CONACYT, and recorded in Clinicaltrials.gov according to the study identifier number. The help of non-profit organizations will be sought to disseminate the results of the investigation to interest groups.

The complete protocol will be published on Clinicaltrials.gov and the OSF - Center for Open Science platform https://osf.io/. Where a DOI will be assigned, and the amendments made to the original protocol will be assessed.

Amendments to the protocol may be made before the start of the study and during the study. Any amendment to the protocol will be clarified and posted on Clinicaltrials.gov under the same identifier as this study. Likewise, any amendment will be sent to the ethics committee of the same hospital.

AUTHOR CONTRIBUTIONS

JGM is the lead study investigator, developed the study concepts and design, and wrote the manuscript by adapting the original study protocol for publication, subsequent reviews and amendments. EJHL, KMM and AAA contributed to the development and refining of the protocol, writing of manuscript and subsequent review. RJMP provided advanced methodological and statistical input, and contributed to the study design and subsequent amendments. RFC, TCH, PSSB, RAG and NML reviewed, commented and informed methodology, development and writing of the protocol.

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COMPETING INTERESTS STATEMENT

None of the authors have conflict of interests, commercial agreements, or receive financial fees or compensation from any commercial or pharmaceutical company.

ETHICS APPROVAL

This protocol has been approved by the local medical ethical review committee at the INR-LGII with the internal number INRLGII/25/20. Definitions of Research Risk Regulation of the General Health Law on

Research for Health (in Spanish, Reglamento de la Ley General de Salud en Materia de Investigación para la Salud) http://www.inr.gob.mx/Descargas/Investigacion/Reglamentos-LGS-Materia-Investigacion-Salud.pdf. ARTICLE 17; and by Federal Commission for Protection against Sanitary Risks (in Spanish, Comisión Federal para la Protección contra Riesgos Sanitarios, COFEPRIS), approval number 203300410A0058/2020.

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ORCID ID

Julio Granados-Montiel https://orcid.org/0000-0002-0611-64

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

Section/item	ItemNo	Description	Page/Line
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1/1-3
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	52/4
	2b	All items from the World Health Organization Trial Registration Data Set	-
Protocol version	3	Date and version identifier	-
Funding	4	Sources and types of financial, material, and other support	27/516-517
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1/4-16 27/507-513
	5b	Name and contact information for the trial sponsor	1/19-23
	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	27/507-513
	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)	-
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	3/30-46
	6b	Explanation for choice of comparators	7/133-139
Objectives	7	Specific objectives or hypotheses	7/137-139
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)	8/143-145

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	8/143-145
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)	8-10/161-195
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	14/209-217
	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)	14/222-228
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	14/222-228
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	-
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	18-20/312-351
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)	17-18/282-310
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	13/199-207
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	8/157, 197

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	.
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	15/234-239
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	15/242
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	15/247-256
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	17/274278
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	20/353, 407-414
	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	
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	22/428-437
Statistical methods	20a	Statistical methods for analyzing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	24/440-445
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	24/454-460
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomized analysis), and any statistical methods to handle missing data (eg, multiple imputation)	-
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	-
	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	-
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	25/470-475
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	23/428-429
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	26-27/488-491, 524-530

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)	26/493-501
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	16/258-263
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if 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	16/267-272
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	27/515-521
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	26/493-496

BMJ Open

New prophylaxis regimen for SARS-CoV-2 infection in health professionals with low doses of Hydroxychloroquine and Bromhexine. A randomized, double-blind placebo clinical trial (ELEVATE Trial).

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New prophylaxis regimen for SARS-CoV-2 infection in health professionals with low doses of Hydroxychloroquine and Bromhexine: a randomized, double-blind placebo clinical trial (ELEVATE Trial).

Julio Granados-Montiel[1]*, Eric Joseph Hazan Lasri[2], Rafael Franco Cendejas[3], Tatiana Chávez Heres[4], Phaedra Suriel Silva Bermúdez[1], Rocío Aguilar Gaytán[1], Natalia Manzano-León[5], Karla Méndez-Maldonado[1], Alejandro Alvarez-Arce[1], Raigam J. Martinez-Portilla[6].

[1] Tissue Engineering and Regenerative Medicine Unit. National Institute of Rehabilitation. Mexico City, Mexico.

[2] Division of Traumatology, Emergencies and Bone Infections. National Institute of Rehabilitation. Mexico City, Mexico.

[3] Infectology Laboratory. National Institute of Rehabilitation. Mexico City, Mexico.

[4] Service, Hospital Epidemiological Surveillance Unit. National Institute of Rehabilitation. Mexico City, Mexico.

[5] Basic Division Research, National Institute of Cancerology. Mexico City, Mexico.

[6] Clinical research division. National Institute of Perinatology

*Corresponding author

Julio Granados-Montiel. M.D.& Ph.D.

Tissue Engineering and Regenerative Medicine Unit. National Institute of Rehabilitation

Av. México-Xochimilco 289. Col. Arenal de Guadalupe, Tlalpan. 14389, Mexico City, Mexico.

ORCID ID: 0000-0002-0611-6421

juliogram@gmail.com

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ABSTRACT

Introduction: SARS-CoV-2 infection in Mexico has caused ~1 million confirmed cases; around 20-25% of health workers will be infected by the virus at their workplace, with approximately 4.4% of mortality. High infectivity of SARS-CoV-2 is related with cell entry mechanism, through the angiotensin-converting enzyme (ACE) receptor. SARS-CoV-2 requires transmembrane protease serine 2 (TMPRSS2) to cleave its spike glycoprotein and ensure fusion of host cell and virus membrane. We propose studying prophylactic treatment with hydroxychloroquine (HCQ) and bromhexine (BHH), which have been shown to be effective in preventing SARS-CoV-2 infection progression when administered in early stages. The aim of this study is to assess the efficacy of HCQ and BHH as prophylactic treatments for SARS-CoV-2 infection in healthy health workers exposed to the virus.

Methods and analysis: Double-blind randomized clinical trial, with parallel allocation at a 1:1 ratio with placebo, of low doses of HCQ plus BHH, for 60 days. Study groups will be defined as follows: 1) HCQ 200mg/d + BHH 8mg/8h vs 2) HCQ placebo plus

BHH placebo. Primary endpoint will be efficacy of both interventions for the prevention of SARS-CoV-2 infection, determined by the risk ratio (RR) of infected personnel and the absolute risk. At least a 16% reduction in absolute risk is expected between the intervention and placebo groups; a minimum of 20% infection is expected in the placebo group. The sample size calculation estimated a total of 140 patients assigned: two groups of 70 participants each.

Ethics and dissemination: This protocol has been approved by the local Medical Ethics Committee (National Institute of Rehabilitation 'Luis Guillermo Ibarra Ibarra', approval number INRLGII/25/20) and by the Federal Commission for Protection against Sanitary Risks (COFEPRIS, approval number 203300410A0058/2020). The results of the study will be submitted for publication in peer-reviewed journals and disseminated through conferences.

Trial registration number 2a: NCT04340349.

2b: NA

Protocol version: #3

STRENGTHS AND LIMITATIONS OF THIS STUDY

Strengths

- This is a double-blind randomized single-centre clinical trial, involving low doses of hydroxychloroquine and

bromhexine, adequately powered to provide clinically relevant information regarding prophylactic treatment for SARS-CoV-2 infection in health care personnel.

- This study will include 140 participants who are health workers exposed to SARS-CoV-2 patients with suspected or confirmed infection, with short term follow-up (60 days).
- A study of prophylactic treatment in this population is of great value and could provide the basis for protecting medical personnel around the world.
- Bromhexine have minimal side effects and are commercially available worldwide; findings could be applied in a timely fashion in different regions.

Limitations

- Long-term use of hydroxychloroquine can cause heart rhythm problems
- For the moment, people who are not candidates to receive the vaccine, due to chronic diseases or severe allergies, will not be included

INTRODUCTION

In Mexico, up to February 2021, have been produced more than 1.9 millions confirmed cases and ~170,000 deaths have arisen[1]. The age group ranging between 30 and 79 years is the most highly affected, where 81% present mild symptoms, 14%

severe and 5% critical, requiring intensive care unit management.

SARS-CoV-2 is a single-stranded RNA virion, member of the *Betacoronavirus* genus [2]. SARS-CoV-2 has an incubation period between 3 to 10 days, with different incubation periods related with different clinical symptoms [3,4] . It is transmitted through respiratory droplets from infected humans and through contact with contaminated fomites and aerosols; moreover, even asymptomatic persons in close contact can transmit the disease [5]. The mechanism through which the virus infects the respiratory cell is due to the angiotensin-converting enzyme protein 2 (ACE-2). This receptor is found in multiple tissues such as the oral cavity, brain, kidneys, intestine, and placenta [6–8].

Health personnel is not exempt from contracting the disease. In China, it was reported that 3.5-4.4% of the infected population belonged to this group, and 14.8% presented characteristics of severity or critical illness [4,9,10]. Italy, around 20% of healthcare professionals became infected [11] ; mean age of health workers who died was 55 years (range of 29-72 years) and mean period from hospital admission to death was 19 days, (range 1-47 days) [9].

Treatment of the SARS-Cov-2 infection has led different research groups to work on the development of vaccines.

However, the use of vaccines can be a challenge. Early trials have shown minimal immune protection or long-term protection is low. On the other hand, because the virus is RNA and the mutation rate is high, we can expect new variants that reduce or nullify the effectiveness of vaccines. Therefore, it is not known if the vaccines that are now in the phase end of the clinical study and those that are administered will work with the same efficacy for the SARS-CoV-2 virus that gave rise to Covid-19. On the other hand, around the world there are groups of people who are against vaccination, or people that have severe allergies, as well as populations that will take much longer to reach the moment when they can acquire the vaccine, so it is extremely necessary that people who do not vaccinate by choice, by disease or by the lack of the vaccine, have an alternative to avoid infection and avoid the spread of the virus.

Therefore, it is important to develop a pharmacological strategy that allows the use of prophylactic drugs for the prevention of SARS-CoV-2 infection.

Chloroquine (CQ) and Hydroxychloroquine (HCQ) are known as an antimalarial agent; HCQ is a hydroxylated derivative from CQ. CQ and HCQ have gained attention as possible therapies in Covid-19 disease. In overdose, both drugs can cause severe, potentially life-threatening effects as visual disturbances,

corneal opacities, irreversible retinopathy can occur with cumulative doses exceeding 100 grams. When lower daily doses (250 mg are used) retinopathy may not occur after many years of treatment [12]. It has been reported in Cynomolgus Macaques that the maximum concentration of HCQ was 293.33ng/mL in blood and 36.90 ng/mL in plasma after single dose of 3 mg/kg [13]. HCQ has been used in several viral infections, for example, as replication inhibitor for the dengue virus, decreasing in vitro virus infection and promoting activation of different immunological signal pathways [14]. It has also been used to treat patients infected with hepatitis C virus decreasing viral load, with minimal adverse effects reported [15]. HCQ has been reported to block viral infection by increasing the endosomal pH required for virus fusion to the cell, as well as interfere with SARS-CoV-2 cell receptors, through inhibition of ACE2 glycosylation receptor [16–19]. HCQ has immunomodulatory effects; it inhibits production and release of pro-inflammatory cytokines, that are associated with severe disease development [20,21] . Recently, it has been reported that HCQ works as a autophagy inhibitor, interfering with viral infection and replication [22]. There is recent evidence that HCQ could be used to treat COVID-19; studies in high-risk patients show that the use of HCQ was associated with a lower risk of intubation or death [23]. Recent study showed that pre-treatment with HCQ

has shown a better effect on antiviral activity [18] and it has been reported that loading doses of 1600 mg HCQ followed by 600 mg daily doses are needed have a relevant effect to SARS-CoV-2 inhibition within 72 hours in 60% of COVID-19 patients [24]. Finally, a study where evaluated the antiviral mechanisms of CQ and the adverse effects, repositioned the CQ to have more efficacy when used as a prophylactic treatment rather than as a therapeutic [25]. On the other hand, CQ and HCQ has been reported to have various adverse effects, the CQ being the most toxic in overdose. However, it was recently published that *in vivo* trials are lacking to determine whether this drug is useful as a prophylactic treatment against SARS-Cov-2 [26]. Based on the above, do more studies will be important to determine its effectiveness at low doses (<250 mg) as a prophylactic treatment.

Another pharmacological option to treat SARS-CoV-2 infection is Bromhexine (BHH). BHH modifies the composition of mucus, increases ciliary clearance and decreases coughing, improving respiratory symptoms. It has also been reported to enhance the effects of some antibiotics [27]. The mechanism by which SARS-CoV-2 enters human cells depends on the ACE-2 receptor and the human transmembrane serine protease (TMPRSS2), on which BHH has a specific inhibitory effect [28,29]. BHH has been used to treat pneumonic damage in both lungs during early infection

[30]. BHH turns out to be an ideal candidate for SARS-CoV-2 treatment, since it has few contraindications, and its side effects are minimal, demonstrating an extensive margin of pharmacological safety. BHH is widely available over the counter, and its low cost makes it an ideal therapeutic option. According to a letter published in the New England Journal of Medicine, of 77,262 patients infected by SARS-CoV-2, 3387 (4.4%) were health workers [9]. Of these, 23 have died from this disease. The prevalence of infections in health personnel is alarming since health services in first world countries have been overwhelmed by this disease. In Italy, around 20% of health professionals had a SARS-CoV-2 infection [11]. Faced with a highly contagious disease, the care of health workers, who are first line of contact and on whom the health system of each country depends, is essential. This research regarding the use of HCQ and BHH in health personnel will allow us to determine and compare the effectiveness of both interventions, which is of vital importance to clarify whether these treatments may prevent the appearance of infection in this population. Describing for the first time that HCQ plus BHH could function for disease prevention, would allow us to provide prophylaxis to health professionals worldwide. Therefore, the use of HCQ and BHH in healthy health personnel

exposed to patients with confirmed or suspected SARS-CoV-2 will significantly reduce infection.

METHODS AND ANALYSIS

Study design

Double-blind randomized clinical trial, with parallel allocation at a 1:1 ratio with HCQ + BHH vs placebo for both drugs for 60 days, to determine the efficacy of the combined drugs for the prevention of SARS-CoV-2 infection in healthcare workers.

Participants

The study will be carried out at the "Instituto Nacional de Rehabilitación, Luis Guillermo Ibarra Ibarra" (INR-LGII). This institution is a tertiary hospital that at this time has not been designated as a COVID-19 centre. The Mexican government defined 3 phases to determine risk for SARS-CoV-2 infection: imported cases from outside Mexico; community infection and spread of the disease throughout the country (also known as

Phase 3). In the latter, it is assumed that every person who enters a hospital is a potentially infected carrier; currently our centre is in Phase 3. Likewise, health personnel who work at the "Instituto Nacional de Ciencias Médicas y Nutrición, Salvador Zubirán" (INCMNSZ), which is a COVID-19 designated tertiary centre, and who meet inclusion criteria of the protocol will be invited to participate in the study.

Inclusion of participants will be assessed according to the eligibility criteria. Table 1 shows the classification and characteristics of study variables. Continuous variables will be assessed for normality. Variables with a normal distribution will be compared using Student's t-test, non-parametric variables using the Mann-Whitney U-test. Categorical variables will be evaluated using the Chi-squared test.

Inclusion criteria

- Health personnel working at INR LGII or INCMNSZ who wish to participate in the study and sign the informed consent.
- Over 18 and under 60 years of age, both genders.
- Contacting with suspected or confirmed SARS-CoV-2 infection.
- Normal electrocardiogram.

Exclusion criteria

- Positive quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) test for SARS-CoV-2 at the time of inclusion.
- Panel of IgG or IgM antibodies positive for SARS-CoV-2 at the time of inclusion.
- Development of respiratory symptoms suspicious of SARS-CoV-2 infection during the first 7 days after treatment is initiated, confirmed by qRT-PCR and IgG or IgM antibodies postiver for SARS-CoV-2.
- Health personnel with comorbidities such as diabetes, hypertension, autoimmune diseases (i.e., porphyria, psoriasis, systemic lupus erythematosus), obesity (defined as body mass index ≥30), cardiovascular diseases, respiratory diseases (such as asthma, chronic bronchitis, idiopathic pulmonary fibrosis).
- History of allergies to any hydroxychloroquine or bromhexine related compound or medication.
- Use of immunosuppressors for any reason.
- History of bone marrow transplant.
- Known glucose-6-phosphate dehydrogenase deficiency.
- Chronic kidney disease or glomerular filtration <20ml/min.

- Use of other drugs with reported pharmacological interactions (i.e., digitalis, flecainide, amiodarone, procainamide, or propafenone).
- History of long QT syndrome.
- Electrocardiogram with QTc>500 msec.
- Pregnant or breastfeeding personnel.
- Epilepsy.
- Known liver disease.
- Personnel who have received the Covid-19 vaccine

Elimination criteria

- Personnel who decide to leave the study for any reason not related to adverse events.
- Personnel with incomplete information on the primary outcome (qRT-PCR for SARS-CoV-2).
- Personnel who are relocated to work in another institution.
- Personnel who do not wish to participate in the study

Table 1. Classification and characteristics of study variables.

Variable	Conceptual definition	Operational definition	Type
Age	Date at recruitment minus date of birth	Years of age	Quantitative
Gender	Male or female genotype of the person	Male/female	Qualitative nominal

Weight	How much the patient weighs at the time of study inclusion	Weight, kilograms	Continuous quantitative
Size	How tall is the patient from head to toe at the time of study inclusion	Height, centimetres	Continuous quantitative
Body mass index	The division between weight by height squared at the time of inclusion in the study	Units of Kg/cm^2	Continuous quantitative
Occupation	Remunerative work performed by the participant at the time of recruitment	Unemployed, informal, unskilled employee, micro-entrepreneur or saleswoman, administrative employee, professional, entrepreneur	Qualitative nominal
Civil status	Civil status of the individual	Married, single, widowed, divorced, common-law union	Qualitative nominal
Level of study	Years completed and approved at the time of study recruitment	No studies, primary, secondary, preparatory, technical career, undergraduate, postgraduate	Ordinal qualitative
Alcohol intake	Consumption of alcoholic beverages	Intake of alcoholic beverages	Qualitative nominal

Smoking habit	Habitual tobacco uses at the time of recruitment	Number of packs of cigarettes consumed per day.	Quantitative
Drug's use	Regular use of chemicals such as amphetamines, cocaine, marijuana, LSD, heroin, glass	Consumption of drugs	Qualitative nominal
Hypertension	Elevation of blood pressure >130/80	Positive/negative	Qualitative nominal
Asthma	Chronic inflammatory disease characterized by bronchial hyperactivity with recurrent episodes of bronchospasm	Positive/negative	Qualitative nominal
Diabetes	Group of metabolic diseases, which occurs when the pancreas does not produce enough insulin or when the body does not use the insulin it produces effectively	Positive/negative	Qualitative nominal
Obesity	Pathological state characterized by a general excess or excessive accumulation of fat in the body	Positive/negative	Qualitative nominal
SARS-CoV-2 pneumonia	A form of severe pneumonia caused by coronavirus	Positive/negative	Qualitative nominal
Death	Statistical term that describes the death of an individual	Positive/negative	Qualitative nominal
Intensive Care Unit	Special facility in a hospital area, which provides life support to critically ill	Positive/negative	Qualitative nominal

	patients, requiring intensive supervision and monitoring		
Severe pneumonia	Defined by the American Thoracic Society Criteria requiring at least one main criterion (need for invasive mechanical ventilation and shock with need for vasopressors), or three minor criteria (respiratory rate> 30 bpm, Pa02 / FiO2 ratio <250, Infiltrates multilobars, confusion / disorientation, uremia [BUN> 20 mg / dL], leukopenia [<4,000], thrombocytopenia [<100,000 platelets / mm^3], hypothermia [core temperature <36ºC], or hypotension requiring aggressive fluid resuscitation	Positive/negative	Qualitative nominal
Pneumonia	Acute infection of the lung parenchyma, accompanied by bilateral infiltrates on chest X-ray	Positive/negative	Qualitative nominal
Confusion	Glasgow scale less than 15	Positive/negative	Qualitative nominal
Hypothermia	Body temperature less than 36 degrees Celsius	Positive/negative	Qualitative nominal
Thrombocytopenia	Total platelets less than 100,000 per mm^3.	Positive/negative	Qualitative nominal

Arterial hypotension	Systolic blood pressure less than 90 mmHg or mean arterial pressure less than 60 mmHg	Positive/negative	Qualitative nominal
Sepsis	Rapid SOFA score (qSOFA) with 2 of the following three clinical variables: Glasgow ≤13, systolic pressure ≤100 mm Hg, or respiratory rate ≥22 bpm	Positive/negative	Qualitative nominal
qRT-PCR for SARS-CoV-2	Molecular diagnosis for SARS-CoV-2 from viral RNA	Positive/negative	Qualitative nominal
Septic shock	Arterial hypotension that persists after resuscitation volume and that requires vasopressors to maintain MAP ≥65 mm Hg and lactate ≥2 mmol / L (18 mg / dL) in the absence of hypovolemia	Positive/negative	Qualitative nominal
Adverse events related to the use of Hydroxychloroquine	Indirect drug-related mortality, nausea, vomiting, abdominal pain, rash, itchy skin, hair loss, QT segment elongation on electrocardiogram, corneal opacity, cardiac arrhythmias, and heart failure	Positive/negative	Qualitative nominal
Adverse events related to the use of Bromhexine	Indirect drug-related mortality, nausea, vomiting, abdominal pain, rash, diarrhea.	Positive/negative	Qualitative nominal

Sample size calculation

According to the study by Remuzzi A et al. [11], the proportion of healthcare workers infected with SARS-CoV-2 and confirmed by RT-PCR was 20%. Taking this 20% as our null hypothesis, we estimate that the proportion of infections in the intervention group will be 4%. Using a two-tailed test, with a type I error of 0.05, a power of 90%, and taking into account a loss of 10% of participants for each group, we estimate that a total of 214 participants will be required, distributed in parallel groups (1:1) of 107 each. This number of volunteers will allow us to find a difference of 16% between groups with a power of 90% and an attrition of 20%. To ensure that desired simple size is reached, all health workers involved in managing patients suspected or infected by SARS-CoV-2 will be invited personally and by institutional email.

Interventions

Interventions will consist of low doses of HCQ 200 mg tablets every 24 hours for 60 days plus BHH 8 mg tablets every 8 hours for 60 days. Study groups will be defined as follows: 1) HCQ plus BHH vs placebo for both drugs. Fabrication of both drugs and placebos will be provided to our centre by a hired laboratory. Both drugs will be provided to participants directly at the hospital by a researcher blinded to group assignment process. To ensure that the intervention is carried

out, each participant will be asked to keep a written record of the days and time the medication was administrated. This document will be reviewed weekly to verify that more than 50% adherence to treatment is maintained. Participants will be asked to record any symptoms related to the use of the medication, which will be reviewed by a researcher blinded to group assignment, weekly, or at the participants' request.

If any of the participants present symptoms of SARS-CoV-2 infection after the first 14 days from the beginning of the intervention or positive qRT-PCR is present, the drug will not be discontinued. If the participant presents adverse events related to the drugs that are severe or intolerable, treatment will not be suspended. If the participants report an adherence of less than 50% of the medication, the intervention will not be discontinued to avoid imbalances between groups. Use of drugs that interact with HCQ or BHH such as flecainide, digitalis, amiodarone, procainamide or propafenone will be prohibited. If a participant has to use these drugs during the study period, they will be eliminated from the study. A free diet and outdoor activity will be allowed since these do not intervene with the implementation of the treatment or have interaction with the drugs used. Finally, incidence of adverse events such as nausea, vomiting, abdominal pain, rash, itchy

skin, hair loss, lengthening of the QT interval in the electrocardiogram, corneal opacity, cardiac arrhythmias, heart failure and death will be determined.

Randomization and treatment allocation

Group randomization will be in a centralized and straightforward way using the Web program www.randomization.com. It will be carried out independently by a researcher blinded to inclusion criteria, delivery of medication, participant follow-up, results, statistical analysis, and writing of the final manuscript. Allocation will be established, for 214 participants in blocks of 107 assigned. The selection of health workers will be made regardless of the hospital shift, work schedule, or assigned area. If the desired sample size is not reached, the inclusion of personnel involved in the first line of care of other referral hospitals for patients with SARS-CoV-2 will be considered.

An independent researcher will allocate patients to the desired groups. Envelopes will be correctly sealed by the pharmacy department and will contain HCQ plus BHH or placebos as previously mentioned. In those who do not require HCQ and BHH, the drug will be replaced by tablets identical in colour and taste but lacking the active substance. In this way, drugs used in both groups will be indistinguishable.

Researcher A will recruit the participants and assess the inclusion criteria according to the serological, electrocardiographic, biochemical results and clinical investigation. Once included, volunteers will go to another office with researcher B, who will be blinded to the first procedure and the rest of the study. Researcher B will assign the groups independently, centrally, and through the use of the web program. This same researcher will be the one who makes the packages indistinguishable to the person providing the drugs to the participant. Researcher C will provide treatment in a sealed envelope or box to the participant in the order of assignment, without knowing each participant's study group. This researcher will also be blinded to the rest of the results. Participants will be blinded to the treatment they will receive. The researchers performing follow-up, researchers for result assessment, and the researcher who performs the statistical analysis will be blinded.

Informed consent will be obtained only by researcher A. If researcher A is not available, the study administrator may obtain informed consent for participation. The informed consent will contain the authorization to participate in the study and the authorization for taking biological samples,

electrocardiogram, and authorization to handle personal information. All participants will complete a written informed consent included on the first page of the questionnaire that requires permission to participate in the study. No candidate is required to participate in the study, and their participation is based on the agreement that they may withdraw at any time. All participants have the right to withdraw from the study if they feel uncomfortable answering a question or with a test to be performed. Also, no one, including the research team, will require a reason why the participant decides to leave the study.

In order to protect participant confidentiality, each one will be assigned a participation number, and all biological samples, as well as medical history information, will be identified by the participant's initials and participant number. Part of the confidentiality protection process will include data capture only by the researcher in charge of data capture (researcher D), who will be the same for all participants and the entire study. Secondarily, the study administrator may also enter data into the database if researcher D is unavailable.

The study administrator will be blinded to allocation and results of the participants. However, the administrator will be the only one who will be able to reveal the group and

treatment assignment in any of the cases: major adverse events such as cardiac arrhythmias, heart failure, major neurological abnormalities, atrial or ventricular fibrillation, kidney failure, or any adverse event related to pharmacological treatment that endangers the life or any organ of the participant's body. The objective of revealing the assignment by the study administrator will be to provide the participant of a timely treatment according to the drugs ingested.

Participant timeline and intervention

The inclusion of participants will be evaluated according to the eligibility criteria and by invitation. Volunteers who wish to participate in the study will be summoned the next day at a specialized office to carry out all the relevant studies to ensure the inclusion criteria. These include a medical history, anthropometric measurements such as weight and body mass index, electrocardiogram (at days 30, 60 and 90 or whe the participant requests it if they have any discomfort), hematic biometry, complete blood chemistry, and serological test for antibodies and qRT-PCR for SARS-CoV-2. Volunteers will be asked for information to contact them once the serological results are obtained.

Once the results are obtained (approximately 3 days), personnel eligible to participate in the study will be contacted. They

will meet in a particular office to speak with a researcher who will be in charge of carrying out the eligibility criteria and medical history checklist. This researcher will be different from the one who makes the assignment, who delivers the medicine and the one who evaluates the results and performs the statistical analysis. The assignment of the group of each participant will be performed, and the participant will not know the group they have been assigned. This information will be known for the researcher in charge, unrelated to the delivery of the treatment, results, or inclusion of the participant in the study. After the assignment, the volunteers will receive the assigned treatment at the pharmacy using a code in a sealed envelope assigned by the Web. Participants who meet the inclusion criteria and there is no reason for exclusion will proceed to the second phase of group assignment with researcher B, the next business day at a different time or office than researcher A.

The group of researchers in charge of monitoring the participants, who will be blinded to the group assignment at all times, will be in charge of assessing each participant´s adverse event and treatment adherence record weekly. These follow-up researchers will be available 24 hours a day throughout the week if participants experience undesirable

adverse events that require urgent attention or that do not allow them to continue with drug treatment. If this situation happens, the researcher in charge of the follow-up will contact the study administrator to reveal to the treating physicians the treatment received by the participant. Health evaluation of all participants will be performed at day 30, day 60 and day 90, this includes electrocardiogram analysis, blood chemistry analysis, antibody test, qRT-PCR, or at request of the participant due to adverse clinical symptoms.

At the end of the first 60 days, a new qRT-PCR will be requested from each participant. All participants who present symptoms after the first 7 days of initiation of the intervention, will be considered as a positive individual for the analysis and will not be excluded from the study. The same action will be carried out 90 days after the start of treatment for both groups. After 60 days, the treatment will be suspended and the results of the qRT-PCR samples for SARS-CoV-2 will be evaluated. After finishing the intervention (60 days), all participants will be followed-up 30 more days with a new qRT-PCR at day 90 after initiation of the intervention and 30 days after the end of the intervention, to assess the efficacy or the treatment during the follow-up period.

Outcome measures

This study compares the efficacy of the use of HCQ plus BHH (as a conjoined treatment) in prophylactic doses for 60 days in healthy health personnel exposed to the first line of care in confirmed patients with suspected infection by SARS-CoV-2.

Primary endpoint

The primary endpoint will be the proportion of health personnel infected by SARS-CoV-2 at 60 days after starting treatment, in all groups. The infection will be diagnosed using qRT-PCR for SARS-CoV-2 and IgM and IgG antibodies anti-SARS-CoV-2 after day 7 of treatment. All participants presenting symptoms with positive qRT-PCR after 7 days of initiation of the intervention, will be considered positive and will be included in the analysis. The study period will be 90 days (60 days for the primary end point plus 30 days of follow-up). The proportion of infected personnel will be evaluated using relative risk (RR) and absolute risk increase (ARI) with their respective 95% confidence intervals, in the established time. The disease-free period in the 60 days will also be evaluated by analysing the cumulative incidence of healthy personnel, and the presence of confirmed infection by qRT-PCR of SARS-CoV-2 and IgM and IgG antibodies anti-SARS-CoV-2 will be the outcome. The censoring variable will be the discontinuation of treatment either due to death, adverse events, or any

elimination criteria. Since there is the possibility of false positives and negatives with qRT-PCR, we will perform qualitative measurements of IgM and IgG with the Elecsys® Anti-SARS-CoV-2 test from Roche laboratories.

Secondary endpoints

The secondary endpoint will be the proportion of health personnel infected 90 days after starting treatment in all groups. The infection will be diagnosed using qRT-PCR for SARS-CoV-2 and IgM and IgG antibodies anti-SARS-CoV-2 after day 7 of the start of treatment. The study period will be 90 days. The proportion of infected personnel will be evaluated using RR and ARI with their respective 95% confidence intervals, in the established time. The disease-free period in the 90 days will also be evaluated by analysing the cumulative incidence of healthy personnel, and the presence of confirmed infection by qRT-PCR of SARS-CoV-2 will be the outcome. The censoring variable will be the discontinuation of treatment either due to death, adverse events, or any elimination criteria.

Also, secondary outcomes will be, in case of a positive SARS-CoV-2 result, the need for oxygen use, admission to the intensive care unit (ICU), presence of pneumonia by computer tomography scan (CT), death, severe pneumonia defined by the

American Thoracic Association, time from hospitalization to recovery in days.

Another secondary endpoint will be adverse events, defined as the presence of any of the following during the study period: death, nausea, vomiting, abdominal pain, diarrhea, rash, itchy skin, hair loss, lengthening of the QT interval in the electrocardiogram (>500msec), corneal opacity, cardiac arrhythmias, heart failure or kidney failure (renal clearance <20ml/min). The proportion of the compound of adverse events between the groups will be analyed using RR and ARI for 60 days with their respective 95% confidence intervals.

The efficacy of the treatment will be established as the proportion of volunteers infected with SARS-CoV-2. This difference should be sufficient to avoid overlapping of the 95% confidence intervals. It will be considered effective if the intervals do not overlap and ineffective if when comparing groups, they have a proportion of infected whose confidence intervals overlap. This type of evaluation will allow an adequate understanding of the efficacy of the treatment in both groups.

Handling and storage of data and documents

Before the start of the study, the researchers in charge of the recruitment, assignment, and delivery of drugs will be trained to perform the task assigned to them at least 3 days before the start of the study.

Researcher A will assess the eligibility criteria of potential participants and perform a detailed clinical examination to assess whether they can participate in the study. The data that will be collected initially will be the following:

- Medical history (includes personal data): study identifier number, history number, name, date of birth, gender, occupation, marital status, nationality, current residence, degree of studies (primary, secondary, upper secondary, bachelor`s degree, postgraduate), hospital service to which they belong and the number of hours worked per week.
- Personal history: alcohol intake (yes/no; how many glasses of beer or alcoholic beverages do you consume per week), smoking habit (yes/ no; and number of cigarettes per day), drug use (yes/no), diet per week (dietary restrictions and number of meals per day) and number of hours of sleep per day.

- Gynaecological history (in women): Number of pregnancies, number of live children, menarche, menopause.
- History of respiratory disease, history of gastrointestinal disease, nephrological, neurological, haematological, cardiovascular, allergies.
- Genetic family history, such as hypertension, diabetes, heart disease, kidney disease.
- Physical examination: blood pressure, heart rate, respiratory rate, temperature, weight, height, body mass index, skin lesions, head and neck inspection, respiratory inspection (chest symmetry, lung expansion, palpation of the bases and preserved vertices, lung percussion, auscultation for lung murmur, breath sounds). Cardiovascular inspection (palpation of the fifth intercostal space, auscultation of heart sounds, pulses that are palpable and symmetrical), abdominal inspection (palpation, percussion and auscultation of peristaltic sounds), neurological evaluation (Glasgow, active motility, passive motility, reflex motility, cranial nerves, sensitivity).
- Hematic biometry: haematocrit, leukocytes, segmented (%), lymphocytes (%), monocytes (%), mean corpuscular volume, platelets.

- Blood chemistry: glycaemia, urea, creatinine, sodium, potassium, chlorine, aspartate transaminase, alanine transaminase, alkaline phosphatase, total bilirubin.
- Muscle enzymes.
- Clotting times: thrombin time, prothrombin time, international normalized ratio.
- Electrocardiogram: rhythm, heart rate, heart axis, evaluation of P wave, PR interval, duration of QRS, QT interval, time of T wave. The electrocardiogram will be performed using an instrument calibrated and validated for its use internationally, weekly.

- Molecular test results for IgG and IgM antibodies:

    - The FDA approved product called Cellex qSARS-CoV-2 IgG/IgM Rapid Test will be used for serological determination. The device cassette, sample, and buffer solution must be at room temperature. The sample (10 µL) is transferred to the center of the sample well. After the sample well is free of liquid, two drops of sample diluent are added. After fifteen to twenty minutes, read the test results. Results should not be read after twenty minutes.

- A positive IgM result occurs when a coloured band appears on the M test line (M) and the control line (C) and indicates that IgM against SARS-CoV-2 is present.
- A positive IgG result occurs when a coloured band appears on the G test line (G) and the control line (C) and indicates that IgG against SARS-CoV-2 is present.
- A positive result for IgM and IgG occurs when coloured bands occur both M and G, as well as C.
- A negative result occurs when a coloured band appears in C only and indicates that IgM and IgG antibodies against SARS-CoV-2 were not detected.
- An invalid result occurs when a colour band is not produced in C, and the test must be repeated.

▶ Official qRT-PCR results (carried out by INCMNSZ)

All this information will be collected in a pre-established medical history questionnaire for each potential participant. The information obtained from the weekly assessment of adverse events, and the results of the qRT-PCR for SARS-CoV-2 at 60 and 90 days (60 for the primary end point plus 30 more days of follow-up) after starting treatment will be entered into an online database. In order to ensure the quality of the data

collection, the database will be built in CASTOR, a database on the Web that allows entering all the pre-defined data for each participant, thus reducing human error. This information will be stored on a server in the United States of America and can only be accessed by the study's administrator. The data may only be entered by a researcher in charge of collecting the data sheets and emptying them.

Monitoring and quality assurance

Every possible adverse event will be noted daily by the participants in the agenda that will be delivered to them. This agenda will be evaluated weekly by the researcher in charge of monitoring the participants (who will be blinded to group assignment). In case of unbearable adverse events for the participants or that put their health at risk, an open line will be available 24 hours a day with direct communication to the researcher in charge of monitoring the study to report any event that requires hospitalization or immediate evaluation at the hospital. All participants with adverse events that put their life or health at risk may be urgently assessed by personnel from both INCMNSZ and INR-LGII, if possible, by the staff involved into the study. Patient follow-up investigator will immediately contact the study administrator to disclose the participant's assignment to treating physicians at that

institution, but the assignment will never be disclosed to other investigators related to the study. All the study expenses and/or attention of collateral effects will be covered by the current cost of the financing SECTEI/061/2020.

Auditing will be carried out weekly, assessing adverse events, capturing data in the corresponding datasheets by the study administrator. Likewise, the data entered in the CASTOR web base will be valued to validate its quality. The paper data sheets must be kept in a special office dedicated to the study in folders separated by volunteers with the informed consent of each participant, the data of the medical history, laboratory results, eligibility criteria, adverse event sheet and results, molecular tests, as well as electrocardiogram. The letter of revocation of informed consent will also be protected if required. As part of the audit, an interim analysis will be carried out 30 days after the study starts to assess the possible adverse effects and whether these outweigh the potential benefits of the intervention. In the adverse event outweigh the potential benefits, termination of the study will be assessed. The approval of the research ethics committee of the INR-LGII of Mexico has been obtained.

Statistical analysis

Data analysis will be carried out by intention to treat, which means that each participant will be analysed according to the group assigned regardless of whether they modified their treatment. The study variables will be divided according to the allocation group. The statistical analysis will be carried out by evaluation the difference between the different groups of HCQ plus BHH versus placebos. Missing data will be handled by multiple imputation analysis when missing at random. Deaths will be censored.

The primary objective will be expressed in number and proportion for each group. The RR will be obtained as the division between the proportion of primary outcomes in the intervention group(s) by the proportion of primary outcomes in the double placebo group. Adjusted risk ratios (aRR) will be obtained using a log-binomial regression, adjusting for age and gender as pre-specified confounding variables. It will be expressed as RR with its respective 95% confidence interval for the initial time, which is 60 days. Likewise, the result will be expressed as absolute risk, which will be derived from the proportion of the primary outcome in the intervention group minus the proportion of the primary outcome in the control group. Secondarily, the primary objective will be analysed with the non-parametric estimate of the survival and risk function

using Kaplan-Meier curves for 60 days according to the allocation group. The primary endpoint will be SARS-CoV-2 infection within the 60-day period, and the silencing variable will be dropping out of the study for any reason. The comparison of the survival curves between both groups will be carried out using the log-rank test. To adjust the primary objective to possible confounders such as age, gender, service in which the participant works, body mass index, etc. Multiple regression will be performed using the Cox model to determine the adjusted primary endpoint hazard ratio.

For secondary outcomes such as the analysis at 90 days, the same statistical analysis expressed in RR and absolute risk will be used. Survival analysis will be used for the primary endpoint only. An interim statistical analysis will be performed 30 days after the study starts to assess possible adverse effects and the efficacy of the intervention. The study administrator will be the only one with access to the data. For the interim analysis and the final analysis, the administrator will export the data to Excel format to be analysed by the study statistician blinded to the assignment of groups, participants, or results.

Adverse events, serious adverse events and suspected unexpected serious adverse reactions

By requiring the use of drugs, the participant will be exposed to risks inherent to the drug used, ranging from mild to severe or death. Any unexpected risks that may occur during the study will be immediately explained to the participants and the ethics committee. Any adverse event will be compiled and will not be disclosed under any condition to anyone other than the study administrator, treating physicians in case of severe events, and the ethics committee. The results will be completely anonymous concerning the names of the participants. The results will be compiled and reported as combined collective data.

Patient and public involvement

Patients were not involved in the development of this research. However, the results of the study will be communicated to the study participants by sending the end product (published article) to the provided email address.

ETHICS, DISSEMINATION AND SAFETY MONITORING

In case of adverse events or complications derived from the study, participants will be assured attention by the staff of the INCMNSZ in an enclosure that ensures the safety of the

participant, not subjecting volunteers to a higher risk of contamination. This care will be extended until adverse events are resolved. In case of no adverse events during the study, medical attention will be extended at the aforementioned institute until 15 days after the end of the study.

This protocol has been approved by the local medical ethical review committee at the INR-LGII with the internal number INRLGII/25/20, and by the Federal Commission for Protection against Sanitary Risks (in Spanish, Comisión Federal para la Protección contra Riesgos Sanitarios, COFEPRIS), approval number 203300410A0058/2020.

The study results will be published in journals of worldwide impact affiliated with the Journal Citation Reports. Likewise, the results of the study will be disseminated in national and international media, exposed in international and national congresses, communicated to CONACYT, and recorded in Clinicaltrials.gov according to the study identifier number. The help of non-profit organizations will be sought to disseminate the results of the investigation to interest groups.

The complete protocol will be published on Clinicaltrials.gov and the OSF - Center for Open Science platform https://osf.io/.

Where a DOI will be assigned, and the amendments made to the original protocol will be assessed.

Amendments to the protocol may be made before the start of the study and during the study. Any amendment to the protocol will be clarified and posted on Clinicaltrials.gov under the same identifier as this study. Likewise, any amendment will be sent to the ethics committee of the same hospital.

AUTHOR CONTRIBUTIONS

JGM is the lead study investigator, developed the study concepts and design, and wrote the manuscript by adapting the original study protocol for publication, subsequent reviews and amendments. EJHL, KMM and AAA contributed to the development and refining of the protocol, writing of manuscript and subsequent review. RJMP provided advanced methodological and statistical input, and contributed to the study design and subsequent amendments. RFC, TCH, PSSB, RAG and NML reviewed, commented and informed methodology, development and writing of the protocol.

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COMPETING INTERESTS STATEMENT

None of the authors have conflict of interests, commercial agreements, or receive financial fees or compensation from any commercial or pharmaceutical company.

ETHICS APPROVAL

This protocol has been approved by the local medical ethical review committee at the INR-LGII with the internal number INRLGII/25/20. Definitions of Research Risk Regulation of the General Health Law on Research for Health (in Spanish, Reglamento de la Ley General de Salud en Materia de Investigación para la Salud) http://www.inr.gob.mx/Descargas/Investigacion/Reglamentos-LGS-Materia-Investigacion-Salud.pdf. ARTICLE 17; and by Federal Commission for Protection against Sanitary Risks (in Spanish, Comisión Federal para la Protección contra Riesgos Sanitarios, COFEPRIS), approval number 203300410A0058/2020.

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ORCID ID

Julio Granados-Montiel https://orcid.org/0000-0002-0611-64

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

Section/item	ItemNo	Description	Page/Line
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1/1-3
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	4/72
	2b	All items from the World Health Organization Trial Registration Data Set	NA
Protocol version	3	Date and version identifier	4/74
Funding	4	Sources and types of financial, material, and other support	32/623625
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1/5-17 31/614-620
	5b	Name and contact information for the trial sponsor	1/19-23
	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	31/614-620
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	NA
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	5-8/94-179
	6b	Explanation for choice of comparators	8/170-179
Objectives	7	Specific objectives or hypotheses	8/170-179
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)	9/187-189

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	9/187-202
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)	9-11/203-244
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	16/261-284
	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)	17/277-279
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	16/265-268
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	NA
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	21-23 /383-430
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)	19-21/340-380
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	15/249-258
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	10/208-244

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	17/287-290
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	17/290-294
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	17/295-297
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	18/301-328
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	NA
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	26/499-508
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants	NA

		who discontinue or deviate from intervention protocols	
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	26/500-505
Statistical methods	20a	Statistical methods for analyzing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	28/539-542
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	28/552-554
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomized analysis), and any statistical methods to handle missing data (eg, multiple imputation)	28/541-542
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	NA
	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	29/563-567
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	29/571-576
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	27-28/524-534
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	30/585-595
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant	31/596-601

		parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	18/313-316
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NA
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	19/323-328
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	32/627-628
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	18/307-311

BMJ Open

New prophylaxis regimen for SARS-CoV-2 infection in health professionals with low doses of Hydroxychloroquine and Bromhexine. A randomized, double-blind placebo clinical trial (ELEVATE Trial).

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New prophylaxis regimen for SARS-CoV-2 infection in health professionals with low doses of Hydroxychloroquine and Bromhexine: a randomized, double-blind placebo clinical trial (ELEVATE Trial).

Julio Granados-Montiel[1]*, Eric Joseph Hazan Lasri[2], Rafael Franco Cendejas[3], Tatiana Chávez Heres[4], Phaedra Suriel Silva Bermúdez[1], Rocío Aguilar Gaytán[1], Natalia Manzano-León[5], Karla Méndez-Maldonado[1], Alejandro Alvarez-Arce[1], Raigam J. Martinez-Portilla[6].

[1] Tissue Engineering and Regenerative Medicine Unit. National Institute of Rehabilitation. Mexico City, Mexico.

[2] Division of Traumatology, Emergencies and Bone Infections. National Institute of Rehabilitation. Mexico City, Mexico.

[3] Infectology Laboratory. National Institute of Rehabilitation. Mexico City, Mexico.

[4] Service, Hospital Epidemiological Surveillance Unit. National Institute of Rehabilitation. Mexico City, Mexico.

[5] Basic Division Research, National Institute of Cancerology. Mexico City, Mexico.

[6] Clinical research division. National Institute of Perinatology

*Corresponding author

Julio Granados-Montiel. M.D.& Ph.D.

Tissue Engineering and Regenerative Medicine Unit. National Institute of Rehabilitation

Av. México-Xochimilco 289. Col. Arenal de Guadalupe, Tlalpan. 14389, Mexico City, Mexico.

ORCID ID: 0000-0002-0611-6421

juliogram@gmail.com

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ABSTRACT

Introduction: SARS-CoV-2 infection in Mexico has caused ~1 million confirmed cases; around 20-25% of health workers will be infected by the virus at their workplace, with approximately 4.4% of mortality. High infectivity of SARS-CoV-2 is related with cell entry mechanism, through the angiotensin-converting enzyme (ACE) receptor. SARS-CoV-2 requires transmembrane protease serine 2 (TMPRSS2) to cleave its spike glycoprotein and ensure fusion of host cell and virus membrane. We propose studying prophylactic treatment with hydroxychloroquine (HCQ) and bromhexine (BHH), which have been shown to be effective in preventing SARS-CoV-2 infection progression when administered in early stages. The aim of this study is to assess the efficacy of HCQ and BHH as prophylactic treatments for SARS-CoV-2 infection in healthy health workers exposed to the virus.

Methods and analysis: Double-blind randomized clinical trial, with parallel allocation at a 1:1 ratio with placebo, of low doses of HCQ plus BHH, for 60 days. Study groups will be defined as follows: 1) HCQ 200mg/d + BHH 8mg/8h vs 2) HCQ placebo plus

BHH placebo. Primary endpoint will be efficacy of both interventions for the prevention of SARS-CoV-2 infection, determined by the risk ratio (RR) of infected personnel and the absolute risk. At least a 16% reduction in absolute risk is expected between the intervention and placebo groups; a minimum of 20% infection is expected in the placebo group. The sample size calculation estimated a total of 214patients assigned: two groups of 107 participants each.

Ethics and dissemination: This protocol has been approved by the local Medical Ethics Committee (National Institute of Rehabilitation 'Luis Guillermo Ibarra Ibarra', approval number INRLGII/25/20) and by the Federal Commission for Protection against Sanitary Risks (COFEPRIS, approval number 203300410A0058/2020). The results of the study will be submitted for publication in peer-reviewed journals and disseminated through conferences.

Trial registration number 2a: NCT04340349.

2b: NA

Protocol version: #4

STRENGTHS AND LIMITATIONS OF THIS STUDY

Strengths

- This is a double-blind randomized single-centre clinical trial, involving low doses of hydroxychloroquine and

bromhexine, adequately powered to provide clinically relevant information regarding prophylactic treatment for SARS-CoV-2 infection in health care personnel.

- Bromhexine have minimal side effects, has been used in the early infection with SARS-CoV-2 showing satisfactory results and are commercially available worldwide. Findings could be applied in a timely fashion in different regions.

Limitations

- Long-term use of hydroxychloroquine can cause heart rhythm problems
- For the moment, people who are not candidates to receive the vaccine, due to chronic diseases or severe allergies, will not be included.
- Hydroxychloroquine has not been shown to have a prophylactic effect; however, we believe that the use of HCQ in conjunction with BHH may inhibit SARS-Cov-2 infection.

INTRODUCTION

In Mexico, up to February 2021, have been produced more than 1.9 millions confirmed cases and ~170,000 deaths have arisen[1]. The age group ranging between 30 and 79 years is the most highly affected, where 81% present mild symptoms, 14%

severe and 5% critical, requiring intensive care unit management.

SARS-CoV-2 is a single-stranded RNA virion, member of the *Betacoronavirus* genus [2]. SARS-CoV-2 has an incubation period between 3 to 10 days, with different incubation periods related with different clinical symptoms [3,4] . It is transmitted through respiratory droplets from infected humans and through contact with contaminated fomites and aerosols; moreover, even asymptomatic persons in close contact can transmit the disease [5]. The mechanism through which the virus infects the respiratory cell is due to the angiotensin-converting enzyme protein 2 (ACE-2). This receptor is found in multiple tissues such as the oral cavity, brain, kidneys, intestine, and placenta [6–8].

Health personnel is not exempt from contracting the disease. In China, it was reported that 3.5-4.4% of the infected population belonged to this group, and 14.8% presented characteristics of severity or critical illness [4,9,10]. Italy, around 20% of healthcare professionals became infected [11]; mean age of health workers who died was 55 years (range of 29-72 years) and mean period from hospital admission to death was 19 days, (range 1-47 days) [9].

Treatment of the SARS-Cov-2 infection has led different research groups to work on the development of vaccines.

However, the use of vaccines can be a challenge. The first trials have shown that the immune protection is not 100% and the protection is only temporary, so it will be necessary to carry out the vaccination periodically. On the other hand, because the virus is RNA and the mutation rate is high, we can expect new variants that reduce or nullify the effectiveness of the vaccines, this depends on the origin of vaccine, if it is made with viral vectors (such as from CanSino or AstraZeneca), if it is mRNA(such as from Moderna or Pfizer-BioNTech) or if it is inactivated virus (Sinovac). Mainly because the development of vaccine that can be efficient for the new variants could be delayed and this could once again increase the number of people who acquire the SARS-CoV-2 virus. On the other hand, around the world there are groups of people who are against vaccination, or people that have severe allergies, as well as populations that will take much longer to reach the moment when they can acquire the vaccine, so it is extremely necessary that people who do not vaccinate by choice, by disease or by the lack of the vaccine, have an alternative to avoid infection and avoid the spread of the virus.

Therefore, it is important to develop a pharmacological strategy that allows the use of prophylactic drugs for the prevention of SARS-CoV-2 infection.

Chloroquine (CQ) and Hydroxychloroquine (HCQ) are known as an antimalarial agent; HCQ is a hydroxylated derivative from CQ. CQ and HCQ have gained attention as possible therapies in Covid-19 disease. In overdose, both drugs can cause severe, potentially life-threatening effects as visual disturbances, corneal opacities, irreversible retinopathy can occur with cumulative doses exceeding 100 grams. When lower daily doses (250 mg are used) retinopathy may not occur after many years of treatment [12]. The above indicates that the use of HCQ at low doses, to avoid SARS-CoV-2 infection, does not produce toxicity and could be used as a prophylactic treatment. HCQ has been used in several viral infections, for example, as replication inhibitor for the dengue virus, decreasing in vitro virus infection and promoting activation of different immunological signal pathways [13]. It has also been used to treat patients infected with hepatitis C virus decreasing viral load, with minimal adverse effects reported [14]. HCQ has been reported to block viral infection by increasing the endosomal pH required for virus fusion to the cell, as well as interfere with SARS-CoV-2 cell receptors, through inhibition of ACE2 glycosylation receptor [15–18]. HCQ has immunomodulatory effects; it inhibits production and release of pro-inflammatory cytokines, that are associated with severe disease development [19,20] . Recently, it has been reported that HCQ works as a

autophagy inhibitor, interfering with viral infection and replication [21]. There is recent evidence that HCQ could be used to treat COVID-19; studies in high-risk patients show that the use of HCQ was associated with a lower risk of intubation or death [22]. Recent study showed that pre-treatment with HCQ has shown a better effect on antiviral activity [17] and it has been reported that loading doses of 1600 mg HCQ followed by 600 mg daily doses are needed have a relevant effect to SARS-CoV-2 inhibition within 72 hours in 60% of COVID-19 patients [23]. Finally, a study where evaluated the antiviral mechanisms of CQ and the adverse effects, repositioned the CQ to have more efficacy when used as a prophylactic treatment rather than as a therapeutic [24]. On the other hand, CQ and HCQ has been reported to have various adverse effects, the CQ being the most toxic in overdose. However, it was recently published that *in vivo* trials are lacking to determine whether this drug is useful as a prophylactic treatment against SARS-Cov-2 [25]. Based on the above, do more studies will be important to determine its effectiveness at low doses (<250 mg) as a prophylactic treatment.

Another pharmacological option to treat SARS-CoV-2 infection is Bromhexine (BHH). BHH modifies the composition of mucus, increases ciliary clearance and decreases coughing, improving respiratory symptoms. It has also been reported to enhance the

effects of some antibiotics [26]. The mechanism by which SARS-CoV-2 enters human cells depends on the ACE-2 receptor and the human transmembrane serine protease (TMPRSS2), on which BHH has a specific inhibitory effect [27,28]. BHH has been used to treat pneumonic damage in both lungs during early infection [29]. BHH turns out to be an ideal candidate for SARS-CoV-2 treatment, since it has few contraindications, and its side effects are minimal, demonstrating an extensive margin of pharmacological safety. BHH is widely available over the counter, and its low cost makes it an ideal therapeutic option. According to a letter published in the New England Journal of Medicine, of 77,262 patients infected by SARS-CoV-2, 3387 (4.4%) were health workers [9]. Of these, 23 have died from this disease. The prevalence of infections in health personnel is alarming since health services in first world countries have been overwhelmed by this disease. In Italy, around 20% of health professionals had a SARS-CoV-2 infection [11]. Faced with a highly contagious disease, the care of health workers, who are first line of contact and on whom the health system of each country depends, is essential. This research regarding the use of HCQ and BHH in health personnel will allow us to determine and compare the effectiveness of both interventions, which is of vital importance to clarify whether these treatments may prevent the appearance of infection in this

population. Describing for the first time that HCQ plus BHH could function for disease prevention, would allow us to provide prophylaxis to health professionals worldwide. Therefore, the use of HCQ and BHH in healthy health personnel exposed to patients with confirmed or suspected SARS-CoV-2 will significantly reduce infection.

METHODS AND ANALYSIS

Study design

Double-blind randomized clinical trial, with parallel allocation at a 1:1 ratio with HCQ + BHH vs placebo for both drugs for 60 days, to determine the efficacy of the combined drugs for the prevention of SARS-CoV-2 infection in healthcare workers.

Participants

The study will be carried out at the "Instituto Nacional de Rehabilitación, Luis Guillermo Ibarra Ibarra" (INR-LGII). This institution is a tertiary hospital that at this time has not

been designated as a COVID-19 centre. The Mexican government defined 3 phases to determine risk for SARS-CoV-2 infection: imported cases from outside Mexico; community infection and spread of the disease throughout the country (also known as Phase 3). In the latter, it is assumed that every person who enters a hospital is a potentially infected carrier; currently our centre is in Phase 3. Likewise, health personnel who work at the "Instituto Nacional de Ciencias Médicas y Nutrición, Salvador Zubirán" (INCMNSZ), which is a COVID-19 designated tertiary centre, and who meet inclusion criteria of the protocol will be invited to participate in the study.

Inclusion of participants will be assessed according to the eligibility criteria. Table 1 shows the classification and characteristics of study variables. Continuous variables will be assessed for normality. Variables with a normal distribution will be compared using Student's t-test, non-parametric variables using the Mann-Whitney U-test. Categorical variables will be evaluated using the Chi-squared test.

Inclusion criteria

- Health personnel working at INR LGII or INCMNSZ who wish to participate in the study and sign the informed consent.
- Over 18 and under 60 years of age, both genders.
- Contacting with suspected or confirmed SARS-CoV-2 infection.

- Normal electrocardiogram.

Exclusion criteria

- Positive quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) test for SARS-CoV-2 at the time of inclusion.
- Panel of IgG or IgM antibodies positive for SARS-CoV-2 at the time of inclusion.
- Development of respiratory symptoms suspicious of SARS-CoV-2 infection during the first 7 days after treatment is initiated, confirmed by qRT-PCR and IgG or IgM antibodies postiver for SARS-CoV-2.
- Health personnel with comorbidities such as diabetes, hypertension, autoimmune diseases (i.e., porphyria, psoriasis, systemic lupus erythematosus), obesity (defined as body mass index ≥30), cardiovascular diseases, respiratory diseases (such as asthma, chronic bronchitis, idiopathic pulmonary fibrosis).
- History of allergies to any hydroxychloroquine or bromhexine related compound or medication.
- Use of immunosuppressors for any reason.
- History of bone marrow transplant.
- Known glucose-6-phosphate dehydrogenase deficiency.

- Chronic kidney disease or glomerular filtration <20ml/min.
- Use of other drugs with reported pharmacological interactions (i.e., digitalis, flecainide, amiodarone, procainamide, or propafenone).
- History of long QT syndrome.
- Electrocardiogram with QTc>500 msec.
- Pregnant or breastfeeding personnel.
- Epilepsy.
- Known liver disease.
- Personnel who have received the Covid-19 vaccine

Elimination criteria

- Personnel who decide to leave the study for any reason not related to adverse events.
- Personnel with incomplete information on the primary outcome (qRT-PCR for SARS-CoV-2).
- Personnel who are relocated to work in another institution.
- Personnel who do not wish to participate in the study

Table 1. Classification and characteristics of study variables.

Variable	Conceptual definition	Operational definition	Type
Age	Date at recruitment minus date of birth	Years of age	Quantitative

Gender	Male or female genotype of the person	Male/female	Qualitative nominal
Weight	How much the patient weighs at the time of study inclusion	Weight, kilograms	Continuous quantitative
Size	How tall is the patient from head to toe at the time of study inclusion	Height, centimetres	Continuous quantitative
Body mass index	The division between weight by height squared at the time of inclusion in the study	Units of Kg/cm^2	Continuous quantitative
Occupation	Remunerative work performed by the participant at the time of recruitment	Unemployed, informal, unskilled employee, micro-entrepreneur or saleswoman, administrative employee, professional, entrepreneur	Qualitative nominal
Civil status	Civil status of the individual	Married, single, widowed, divorced, common-law union	Qualitative nominal
Level of study	Years completed and approved at the time of study recruitment	No studies, primary, secondary, preparatory, technical career, undergraduate, postgraduate	Ordinal qualitative

Alcohol intake	Consumption of alcoholic beverages	Intake of alcoholic beverages	Qualitative nominal
Smoking habit	Habitual tobacco uses at the time of recruitment	Number of packs of cigarettes consumed per day.	Quantitative
Drug's use	Regular use of chemicals such as amphetamines, cocaine, marijuana, LSD, heroin, glass	Consumption of drugs	Qualitative nominal
Hypertension	Elevation of blood pressure >130/80	Positive/negative	Qualitative nominal
Asthma	Chronic inflammatory disease characterized by bronchial hyperactivity with recurrent episodes of bronchospasm	Positive/negative	Qualitative nominal
Diabetes	Group of metabolic diseases, which occurs when the pancreas does not produce enough insulin or when the body does not use the insulin it produces effectively	Positive/negative	Qualitative nominal
Obesity	Pathological state characterized by a general excess or excessive accumulation of fat in the body	Positive/negative	Qualitative nominal
SARS-CoV-2 pneumonia	A form of severe pneumonia caused by coronavirus	Positive/negative	Qualitative nominal
Death	Statistical term that describes the death of an individual	Positive/negative	Qualitative nominal

Intensive Care Unit	Special facility in a hospital area, which provides life support to critically ill patients, requiring intensive supervision and monitoring	Positive/negative	Qualitative nominal
Severe pneumonia	Defined by the American Thoracic Society Criteria requiring at least one main criterion (need for invasive mechanical ventilation and shock with need for vasopressors), or three minor criteria (respiratory rate> 30 bpm, Pa02 / FiO2 ratio <250, Infiltrates multilobars, confusion / disorientation, uremia [BUN> 20 mg / dL], leukopenia [<4,000], thrombocytopenia [<100,000 platelets / mm^3], hypothermia [core temperature <36ºC], or hypotension requiring aggressive fluid resuscitation	Positive/negative	Qualitative nominal
Pneumonia	Acute infection of the lung parenchyma, accompanied by bilateral infiltrates on chest X-ray	Positive/negative	Qualitative nominal
Confusion	Glasgow scale less than 15	Positive/negative	Qualitative nominal
Hypothermia	Body temperature less than 36 degrees Celsius	Positive/negative	Qualitative nominal
Thrombocytopenia	Total platelets less than 100,000 per mm^3.	Positive/negative	Qualitative nominal

Arterial hypotension	Systolic blood pressure less than 90 mmHg or mean arterial pressure less than 60 mmHg	Positive/negative	Qualitative nominal
Sepsis	Rapid SOFA score (qSOFA) with 2 of the following three clinical variables: Glasgow ≤13, systolic pressure ≤100 mm Hg, or respiratory rate ≥22 bpm	Positive/negative	Qualitative nominal
qRT-PCR for SARS-CoV-2	Molecular diagnosis for SARS-CoV-2 from viral RNA	Positive/negative	Qualitative nominal
Septic shock	Arterial hypotension that persists after resuscitation volume and that requires vasopressors to maintain MAP ≥65 mm Hg and lactate ≥2 mmol / L (18 mg / dL) in the absence of hypovolemia	Positive/negative	Qualitative nominal
Adverse events related to the use of Hydroxychloroquine	Indirect drug-related mortality, nausea, vomiting, abdominal pain, rash, itchy skin, hair loss, QT segment elongation on electrocardiogram, corneal opacity, cardiac arrhythmias, and heart failure	Positive/negative	Qualitative nominal
Adverse events related to the use of Bromhexine	Indirect drug-related mortality, nausea, vomiting, abdominal pain, rash, diarrhea.	Positive/negative	Qualitative nominal

Sample size calculation

According to the study by Remuzzi A et al. [11], the proportion of healthcare workers infected with SARS-CoV-2 and confirmed by RT-PCR was 20%. Taking this 20% as our null hypothesis, we estimate that the proportion of infections in the intervention group will be 4%. Using a two-tailed test, with a type I error of 0.05, a power of 90%, and taking into account a loss of 10% of participants for each group, we estimate that a total of 214 participants will be required, distributed in parallel groups (1:1) of 107 each. This number of volunteers will allow us to find a difference of 16% between groups with a power of 90% and an attrition of 20%. To ensure that desired simple size is reached, all health workers involved in managing patients suspected or infected by SARS-CoV-2 will be invited personally and by institutional email.

Interventions

Interventions will consist of low doses of HCQ 200 mg tablets every 24 hours for 60 days plus BHH 8 mg tablets every 8 hours for 60 days. Study groups will be defined as follows: 1) HCQ plus BHH vs placebo for both drugs. Fabrication of both drugs and placebos will be provided to our centre by a hired laboratory. Both drugs will be provided to participants directly at the hospital by a researcher blinded to group assignment process. To ensure that the intervention is carried

out, each participant will be asked to keep a written record of the days and time the medication was administrated. This document will be reviewed weekly to verify that more than 50% adherence to treatment is maintained. Participants will be asked to record any symptoms related to the use of the medication, which will be reviewed by a researcher blinded to group assignment, weekly, or at the participants' request.

If any of the participants present symptoms of SARS-CoV-2 infection after the first 14 days from the beginning of the intervention or positive qRT-PCR is present, the drug will not be discontinued. If the participant presents adverse events related to the drugs that are severe or intolerable, treatment will be suspended. If the participants report an adherence of less than 50% of the medication, the intervention will not be discontinued to avoid imbalances between groups. Use of drugs that interact with HCQ or BHH such as flecainide, digitalis, amiodarone, procainamide or propafenone will be prohibited. If a participant has to use these drugs during the study period, they will be eliminated from the study. A free diet and outdoor activity will be allowed since these do not intervene with the implementation of the treatment or have interaction with the drugs used. Finally, incidence of adverse events such as nausea, vomiting, abdominal pain, rash, itchy skin, hair loss,

lengthening of the QT interval in the electrocardiogram, corneal opacity, cardiac arrhythmias, heart failure and death will be determined.

Randomization and treatment allocation

Group randomization will be in a centralized and straightforward way using the Web program www.randomization.com. It will be carried out independently by a researcher blinded to inclusion criteria, delivery of medication, participant follow-up, results, statistical analysis, and writing of the final manuscript. Allocation will be established, for 214 participants in blocks of 107 assigned. The selection of health workers will be made regardless of the hospital shift, work schedule, or assigned area. If the desired sample size is not reached, the inclusion of personnel involved in the first line of care of other referral hospitals for patients with SARS-CoV-2 will be considered.

An independent researcher will allocate patients to the desired groups. Envelopes will be correctly sealed by the pharmacy department and will contain HCQ plus BHH or placebos as previously mentioned. In those who do not require HCQ and BHH, the drug will be replaced by tablets identical in colour and taste but lacking the active substance. In this way, drugs used in both groups will be indistinguishable.

Researcher A will recruit the participants and assess the inclusion criteria according to the serological, electrocardiographic, biochemical results and clinical investigation. Once included, volunteers will go to another office with researcher B, who will be blinded to the first procedure and the rest of the study. Researcher B will assign the groups independently, centrally, and through the use of the web program. This same researcher will be the one who makes the packages indistinguishable to the person providing the drugs to the participant. Researcher C will provide treatment in a sealed envelope or box to the participant in the order of assignment, without knowing each participant's study group. This researcher will also be blinded to the rest of the results. Participants will be blinded to the treatment they will receive. The researchers performing follow-up, researchers for result assessment, and the researcher who performs the statistical analysis will be blinded.

Informed consent will be obtained only by researcher A. If researcher A is not available, the study administrator may obtain informed consent for participation. The informed consent will contain the authorization to participate in the study and the authorization for taking biological samples,

electrocardiogram, and authorization to handle personal information. All participants will complete a written informed consent included on the first page of the questionnaire that requires permission to participate in the study. No candidate is required to participate in the study, and their participation is based on the agreement that they may withdraw at any time. All participants have the right to withdraw from the study if they feel uncomfortable answering a question or with a test to be performed. Also, no one, including the research team, will require a reason why the participant decides to leave the study.

In order to protect participant confidentiality, each one will be assigned a participation number, and all biological samples, as well as medical history information, will be identified by the participant's initials and participant number. Part of the confidentiality protection process will include data capture only by the researcher in charge of data capture (researcher D), who will be the same for all participants and the entire study. Secondarily, the study administrator may also enter data into the database if researcher D is unavailable.

The study administrator will be blinded to allocation and results of the participants. However, the administrator will be the only one who will be able to reveal the group and

treatment assignment in any of the cases: major adverse events such as cardiac arrhythmias, heart failure, major neurological abnormalities, atrial or ventricular fibrillation, kidney failure, or any adverse event related to pharmacological treatment that endangers the life or any organ of the participant's body. The objective of revealing the assignment by the study administrator will be to provide the participant of a timely treatment according to the drugs ingested.

Participant timeline and intervention

The inclusion of participants will be evaluated according to the eligibility criteria and by invitation. Volunteers who wish to participate in the study will be summoned the next day at a specialized office to carry out all the relevant studies to ensure the inclusion criteria. These include a medical history, anthropometric measurements such as weight and body mass index, electrocardiogram (every week until the end of the study or when the participant requests it if they have any discomfort), hematic biometry, complete blood chemistry, and serological test for antibodies and qRT-PCR for SARS-CoV-2. Volunteers will be asked for information to contact them once the serological results are obtained.

Once the results are obtained (approximately 3 days), personnel eligible to participate in the study will be contacted. They

will meet in a particular office to speak with a researcher who will be in charge of carrying out the eligibility criteria and medical history checklist. This researcher will be different from the one who makes the assignment, who delivers the medicine and the one who evaluates the results and performs the statistical analysis. The assignment of the group of each participant will be performed, and the participant will not know the group they have been assigned. This information will be known for the researcher in charge, unrelated to the delivery of the treatment, results, or inclusion of the participant in the study. After the assignment, the volunteers will receive the assigned treatment at the pharmacy using a code in a sealed envelope assigned by the Web. Participants who meet the inclusion criteria and there is no reason for exclusion will proceed to the second phase of group assignment with researcher B, the next business day at a different time or office than researcher A.

The group of researchers in charge of monitoring the participants, who will be blinded to the group assignment at all times, will be in charge of assessing each participant´s adverse event and treatment adherence record weekly. These follow-up researchers will be available 24 hours a day throughout the week if participants experience undesirable

adverse events that require urgent attention or that do not allow them to continue with drug treatment. If this situation happens, the researcher in charge of the follow-up will contact the study administrator to reveal to the treating physicians the treatment received by the participant. Health evaluation of all participants will be performed at day 30, day 60 and day 90, this includes electrocardiogram analysis, blood chemistry analysis, antibody test, qRT-PCR, or at request of the participant due to adverse clinical symptoms.

At the end of the first 60 days, a new qRT-PCR will be requested from each participant. All participants who present symptoms after the first 7 days of initiation of the intervention, will be considered as a positive individual for the analysis and will not be excluded from the study. The same action will be carried out 90 days after the start of treatment for both groups. After 60 days, the treatment will be suspended and the results of the qRT-PCR samples for SARS-CoV-2 will be evaluated. After finishing the intervention (60 days), all participants will be followed-up 30 more days with a new qRT-PCR at day 90 after initiation of the intervention and 30 days after the end of the intervention, to assess the efficacy or the treatment during the follow-up period.

Outcome measures

This study compares the efficacy of the use of HCQ plus BHH (as a conjoined treatment) in prophylactic doses for 60 days in healthy health personnel exposed to the first line of care in confirmed patients with suspected infection by SARS-CoV-2.

Primary endpoint

The primary endpoint will be the proportion of health personnel infected by SARS-CoV-2 at 60 days after starting treatment, in both groups. The infection will be diagnosed using qRT-PCR for relative expression of the mRNA SARS-CoV-2 and the measure of IgM and IgG antibodies anti-SARS-CoV-2 after day 7 of treatment using rapid test Cellex qSARS-CoV-2 IgG/IgM. All participants presenting symptoms with positive qRT-PCR after 7 days of initiation of the intervention, will be considered positive and will be included in the analysis. The study period will be 90 days (60 days for the primary end point plus 30 days of follow-up). The proportion of infected personnel will be evaluated using relative risk (RR) and absolute risk increase (ARI) with their respective 95% confidence intervals, in the established time. The disease-free period in the 60 days will also be evaluated by analysing the cumulative incidence of healthy personnel, and the presence of confirmed infection by qRT-PCR of SARS-CoV-2 and IgM and IgG antibodies anti-SARS-CoV-2 will be the outcome. The censoring variable will be the

discontinuation of treatment either due to death, adverse events, or any elimination criteria. Since there is the possibility of false positives and negatives with qRT-PCR, we will perform qualitative measurements of IgM and IgG with the Cellex qSARS-CoV-2 IgG/IgM Rapid test from test from which is authorized by FDA. The test can be used on serum, plasma, or whole blood samples. The clinical sensitivity of the assay was 93.8% and the clinical specificity was 96%[30].

Secondary endpoints

The secondary endpoint will be the proportion of health personnel infected 90 days after starting treatment in both groups. The infection will be diagnosed using qRT-PCR for relative expression of the mRNA of SARS-CoV-2 and the measure of IgM and IgG antibodies anti-SARS-CoV-2 after day 7 of the start of treatment using rapid test Cellex qSARS-CoV-2 IgG/IgM. The study period will be 90 days. The proportion of infected personnel will be evaluated using RR and ARI with their respective 95% confidence intervals, in the established time. The disease-free period in the 90 days will also be evaluated by analysing the cumulative incidence of healthy personnel, and the presence of confirmed infection by qRT-PCR of SARS-CoV-2 will be the outcome. The censoring variable will be the

discontinuation of treatment either due to death, adverse events, or any elimination criteria.

Also, secondary outcomes will be, in case of a positive SARS-CoV-2 result, the need for oxygen use, admission to the intensive care unit (ICU), presence of pneumonia by computer tomography scan (CT), death, severe pneumonia defined by the American Thoracic Association, time from hospitalization to recovery in days.

Another secondary endpoint will be adverse events, defined as the presence of any of the following during the study period: death, nausea, vomiting, abdominal pain, diarrhea, rash, itchy skin, hair loss, lengthening of the QT interval in the electrocardiogram (>500msec), corneal opacity, cardiac arrhythmias, heart failure or kidney failure (renal clearance <20ml/min). The proportion of the compound of adverse events between the groups will be analsyed using RR and ARI for 60 days with their respective 95% confidence intervals.

The efficacy of the treatment will be established as the proportion of volunteers infected with SARS-CoV-2. This difference should be sufficient to avoid overlapping of the 95% confidence intervals. It will be considered effective if the intervals do not overlap and ineffective if when comparing groups, they have a proportion of infected whose confidence

intervals overlap. This type of evaluation will allow an adequate understanding of the efficacy of the treatment in both groups.

Handling and storage of data and documents

Before the start of the study, the researchers in charge of the recruitment, assignment, and delivery of drugs will be trained to perform the task assigned to them at least 3 days before the start of the study.

Researcher A will assess the eligibility criteria of potential participants and perform a detailed clinical examination to assess whether they can participate in the study. The data that will be collected initially will be the following:

- Medical history (includes personal data): study identifier number, history number, name, date of birth, gender, occupation, marital status, nationality, current residence, degree of studies (primary, secondary, upper secondary, bachelor`s degree, postgraduate), hospital service to which they belong and the number of hours worked per week.

- Personal history: alcohol intake (yes/no; how many glasses of beer or alcoholic beverages do you consume per week), smoking habit (yes/ no; and number of cigarettes per day), drug use (yes/no), diet per week (dietary restrictions and number of meals per day) and number of hours of sleep per day.
- Gynaecological history (in women): Number of pregnancies, number of live children, menarche, menopause.
- History of respiratory disease, history of gastrointestinal disease, nephrological, neurological, haematological, cardiovascular, allergies.
- Genetic family history, such as hypertension, diabetes, heart disease, kidney disease.
- Physical examination: blood pressure, heart rate, respiratory rate, temperature, weight, height, body mass index, skin lesions, head and neck inspection, respiratory inspection (chest symmetry, lung expansion, palpation of the bases and preserved vertices, lung percussion, auscultation for lung murmur, breath sounds). Cardiovascular inspection (palpation of the fifth intercostal space, auscultation of heart sounds, pulses that are palpable and symmetrical), abdominal inspection (palpation, percussion and auscultation of peristaltic sounds), neurological evaluation (Glasgow, active

motility, passive motility, reflex motility, cranial nerves, sensitivity).

- Hematic biometry: haematocrit, leukocytes, segmented (%), lymphocytes (%), monocytes (%), mean corpuscular volume, platelets.
- Blood chemistry: glycaemia, urea, creatinine, sodium, potassium, chlorine, aspartate transaminase, alanine transaminase, alkaline phosphatase, total bilirubin.
- Muscle enzymes.
- Clotting times: thrombin time, prothrombin time, international normalized ratio.
- Electrocardiogram: rhythm, heart rate, heart axis, evaluation of P wave, PR interval, duration of QRS, QT interval, time of T wave. The electrocardiogram will be performed using an instrument calibrated and validated for its use internationally, weekly.

- Molecular test results for IgG and IgM antibodies:

    - The FDA approved product called Cellex qSARS-CoV-2 IgG/IgM Rapid Test will be used for serological determination. The device cassette, sample, and buffer solution must be at room temperature. The sample (10 µL) is transferred to the center of the

sample well. After the sample well is free of liquid, two drops of sample diluent are added. After fifteen to twenty minutes, read the test results. Results should not be read after twenty minutes.

- A positive IgM result occurs when a coloured band appears on the M test line (M) and the control line (C) and indicates that IgM against SARS-CoV-2 is present.
- A positive IgG result occurs when a coloured band appears on the G test line (G) and the control line (C) and indicates that IgG against SARS-CoV-2 is present.
- A positive result for IgM and IgG occurs when coloured bands occur both M and G, as well as C.
- A negative result occurs when a coloured band appears in C only and indicates that IgM and IgG antibodies against SARS-CoV-2 were not detected.
- An invalid result occurs when a colour band is not produced in C, and the test must be repeated.

▶ Official qRT-PCR results (carried out by INCMNSZ)

All this information will be collected in a pre-established medical history questionnaire for each potential participant. The information obtained from the weekly assessment of adverse

events, and the results of the qRT-PCR for SARS-CoV-2 at 60 and 90 days (60 for the primary end point plus 30 more days of follow-up) after starting treatment will be entered into an online database. In order to ensure the quality of the data collection, the database will be built in CASTOR, a database on the Web that allows entering all the pre-defined data for each participant, thus reducing human error. This information will be stored on a server in the United States of America and can only be accessed by the study's administrator. The data may only be entered by a researcher in charge of collecting the data sheets and emptying them.

Monitoring and quality assurance

Every possible adverse event will be noted daily by the participants in the agenda that will be delivered to them. This agenda will be evaluated weekly by the researcher in charge of monitoring the participants (who will be blinded to group assignment). In case of unbearable adverse events for the participants or that put their health at risk, an open line will be available 24 hours a day with direct communication to the researcher in charge of monitoring the study to report any event that requires hospitalization or immediate evaluation at the hospital. All participants with adverse events that put their life or health at risk may be urgently assessed by

personnel from both INCMNSZ and INR-LGII, if possible, by the staff involved into the study. Patient follow-up investigator will immediately contact the study administrator to disclose the participant's assignment to treating physicians at that institution, but the assignment will never be disclosed to other investigators related to the study. All the study expenses and/or attention of collateral effects will be covered by the current cost of the financing SECTEI/061/2020.

Auditing will be carried out weekly, assessing adverse events, capturing data in the corresponding datasheets by the study administrator. Likewise, the data entered in the CASTOR web base will be valued to validate its quality. The paper data sheets must be kept in a special office dedicated to the study in folders separated by volunteers with the informed consent of each participant, the data of the medical history, laboratory results, eligibility criteria, adverse event sheet and results, molecular tests, as well as electrocardiogram. The letter of revocation of informed consent will also be protected if required. As part of the audit, an interim analysis will be carried out 30 days after the study starts to assess the possible adverse effects and whether these outweigh the potential benefits of the intervention. In the adverse event outweigh the potential benefits, termination of the study

will be assessed. The approval of the research ethics committee of the INR-LGII of Mexico has been obtained.

Statistical analysis

Data analysis will be carried out by intention to treat, which means that each participant will be analysed according to the group assigned regardless of whether they modified their treatment. The study variables will be divided according to the allocation group. The statistical analysis will be carried out by evaluation the difference between the different groups of HCQ plus BHH versus placebos. Missing data will be handled by multiple imputation analysis when missing at random. Deaths will be censored.

The primary objective will be expressed in number and proportion for each group. The RR will be obtained as the division between the proportion of primary outcomes in the intervention group(s) by the proportion of primary outcomes in the double placebo group. Adjusted risk ratios (aRR) will be obtained using a log-binomial regression, adjusting for age and gender as pre-specified confounding variables. It will be expressed as RR with its respective 95% confidence interval for the initial time, which is 60 days. Likewise, the result will be expressed as absolute risk, which will be derived from

the proportion of the primary outcome in the intervention group minus the proportion of the primary outcome in the control group. Secondarily, the primary objective will be analysed with the non-parametric estimate of the survival and risk function using Kaplan-Meier curves for 60 days according to the allocation group. The primary endpoint will be SARS-CoV-2 infection within the 60-day period, and the silencing variable will be dropping out of the study for any reason. The comparison of the survival curves between both groups will be carried out using the log-rank test. Risk ratio will be used for treatment effect. A log-binomial regression adjusted by age, gender, service in which the participant works, body mass index, will be used.

For secondary outcomes such as the analysis at 90 days, the same statistical analysis expressed in RR and absolute risk will be used. Survival analysis will be used for the primary endpoint only. An interim statistical analysis will be performed 30 days after the study starts to assess possible adverse effects and the efficacy of the intervention. The study administrator will be the only one with access to the data. For the interim analysis and the final analysis, the administrator will export the data to Excel format to be

analysed by the study statistician blinded to the assignment of groups, participants, or results.

Adverse events, serious adverse events and suspected unexpected serious adverse reactions

By requiring the use of drugs, the participant will be exposed to risks inherent to the drug used, ranging from mild to severe or death. Any unexpected risks that may occur during the study will be immediately explained to the participants and the ethics committee. Any adverse event will be compiled and will not be disclosed under any condition to anyone other than the study administrator, treating physicians in case of severe events, and the ethics committee. The results will be completely anonymous concerning the names of the participants. The results will be compiled and reported as combined collective data.

Patient and public involvement

Patients were not involved in the development of this research. However, the results of the study will be communicated to the study participants by sending the end product (published article) to the provided email address.

ETHICS, DISSEMINATION AND SAFETY MONITORING

In case of adverse events or complications derived from the study, participants will be assured attention by the staff of the INCMNSZ in an enclosure that ensures the safety of the participant, not subjecting volunteers to a higher risk of contamination. This care will be extended until adverse events are resolved. In case of no adverse events during the study, medical attention will be extended at the aforementioned institute until 15 days after the end of the study.

This protocol has been approved by the local medical ethical review committee at the INR-LGII with the internal number INRLGII/25/20, and by the Federal Commission for Protection against Sanitary Risks (in Spanish, Comisión Federal para la Protección contra Riesgos Sanitarios, COFEPRIS), approval number 203300410A0058/2020.

The study results will be published in journals of worldwide impact affiliated with the Journal Citation Reports. Likewise, the results of the study will be disseminated in national and international media, exposed in international and national congresses, communicated to CONACYT, and recorded in Clinicaltrials.gov according to the study identifier number. The help of non-profit organizations will be sought to disseminate the results of the investigation to interest groups.

The complete protocol will be published on Clinicaltrials.gov and the OSF - Center for Open Science platform https://osf.io/. Where a DOI will be assigned, and the amendments made to the original protocol will be assessed.

Amendments to the protocol may be made before the start of the study and during the study. Any amendment to the protocol will be clarified and posted on Clinicaltrials.gov under the same identifier as this study. Likewise, any amendment will be sent to the ethics committee of the same hospital.

AUTHOR CONTRIBUTIONS

JGM is the lead study investigator, developed the study concepts and design, and wrote the manuscript by adapting the original study protocol for publication, subsequent reviews and amendments. EJHL, KMM and AAA contributed to the development and refining of the protocol, writing of manuscript and subsequent review. RJMP provided advanced methodological and statistical input, and contributed to the study design and subsequent amendments. RFC, TCH, PSSB, RAG and NML reviewed, commented and informed methodology, development and writing of the protocol.

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COMPETING INTERESTS STATEMENT

None of the authors have conflict of interests, commercial agreements, or receive financial fees or compensation from any commercial or pharmaceutical company.

ETHICS APPROVAL

This protocol has been approved by the local medical ethical review committee at the INR-LGII with the internal number INRLGII/25/20. Definitions of Research Risk Regulation of the General Health Law on Research for Health (in Spanish, Reglamento de la Ley General de Salud en Materia de Investigación para la Salud) http://www.inr.gob.mx/Descargas/Investigacion/Reglamentos-LGS-Materia-Investigacion-Salud.pdf. ARTICLE 17; and by Federal Commission for Protection against Sanitary Risks (in Spanish, Comisión Federal para la Protección contra Riesgos Sanitarios, COFEPRIS), approval number 203300410A0058/2020.

PROVENANCE AND PEER REVIEW

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ORCID ID

Julio Granados-Montiel https://orcid.org/0000-0002-0611-64

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

Section/item	ItemNo	Description	Page/Line
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1/1-3
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	4/72
	2b	All items from the World Health Organization Trial Registration Data Set	NA
Protocol version	3	Date and version identifier	4/74
Funding	4	Sources and types of financial, material, and other support	32/623625
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1/5-17 31/614-620
	5b	Name and contact information for the trial sponsor	1/19-23
	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	31/614-620
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	NA
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	6-9/98-187
	6b	Explanation for choice of comparators	9/180-187
Objectives	7	Specific objectives or hypotheses	9/183-187
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)	10/191-193

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	10/197-211
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)	11-12/212-248
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	17/265-288
	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)	17/278-281
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	17/269-274
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	NA
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	22-24 /392-432
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)	20-22/344-384
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	16/253-260

Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	10-16/207-250
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	18/291-295
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	18-19/299-303
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	19/305-315
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	19/307-315
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	NA
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	27/504-510

	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	NA
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	27/508-513
Statistical methods	20a	Statistical methods for analyzing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	29/542-547
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	29/560-563
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomized analysis), and any statistical methods to handle missing data (eg, multiple imputation)	29/546-547
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	NA
	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	30/567-571
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	30/575-581
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	28-29/529-539
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	31/589-599

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)	32/600-605
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	19/317-318
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NA
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	20/327-332
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	33/631-632
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	28/510-513

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New prophylaxis regimen for SARS-CoV-2 infection in health professionals with low doses of Hydroxychloroquine and Bromhexine. A randomized, double-blind placebo clinical trial (ELEVATE Trial).

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New prophylaxis regimen for SARS-CoV-2 infection in health professionals with low doses of Hydroxychloroquine and Bromhexine: a randomized, double-blind placebo clinical trial (ELEVATE Trial).

Julio Granados-Montiel[1]*, Eric Joseph Hazan Lasri[2], Rafael Franco Cendejas[3], Tatiana Chávez Heres[4], Phaedra Suriel Silva Bermúdez[1], Rocío Aguilar Gaytán[1], Natalia Manzano-León[5], Karla Méndez-Maldonado[1], Alejandro Alvarez-Arce[1], Raigam J. Martinez-Portilla[6].

[1] Tissue Engineering and Regenerative Medicine Unit. National Institute of Rehabilitation. Mexico City, Mexico.

[2] Division of Traumatology, Emergencies and Bone Infections. National Institute of Rehabilitation. Mexico City, Mexico.

[3] Infectology Laboratory. National Institute of Rehabilitation. Mexico City, Mexico.

[4] Service, Hospital Epidemiological Surveillance Unit. National Institute of Rehabilitation. Mexico City, Mexico.

[5] Basic Division Research, National Institute of Cancerology. Mexico City, Mexico.

[6] Clinical research division. National Institute of Perinatology

*Corresponding author

Julio Granados-Montiel. M.D.& Ph.D.

Tissue Engineering and Regenerative Medicine Unit. National Institute of Rehabilitation

Av. México-Xochimilco 289. Col. Arenal de Guadalupe, Tlalpan. 14389, Mexico City, Mexico.

ORCID ID: 0000-0002-0611-6421

juliogram@gmail.com

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ABSTRACT

Introduction: SARS-CoV-2 infection in Mexico has caused ~2.5 million confirmed cases; around 20-25% of health workers will be infected by the virus at their workplace, with approximately 4.4% of mortality. High infectivity of SARS-CoV-2 is related with cell entry mechanism, through the angiotensin-converting enzyme (ACE) receptor. SARS-CoV-2 requires transmembrane protease serine 2 (TMPRSS2) to cleave its spike glycoprotein and ensure fusion of host cell and virus membrane. We propose studying prophylactic treatment with hydroxychloroquine (HCQ) and bromhexine (BHH), which have been shown to be effective in preventing SARS-CoV-2 infection progression when administered in early stages. The aim of this study is to assess the efficacy of HCQ and BHH as prophylactic treatments for SARS-CoV-2 infection in healthy health workers exposed to the virus.

Methods and analysis: Double-blind randomized clinical trial, with parallel allocation at a 1:1 ratio with placebo, of low doses of HCQ plus BHH, for 60 days. Study groups will be defined as follows: 1) HCQ 200mg/d + BHH 8mg/8h vs 2) HCQ placebo plus BHH placebo. Primary endpoint will be efficacy of both interventions for the prevention of SARS-CoV-2 infection, determined by the risk ratio (RR) of infected personnel and the absolute risk. At least a 16% reduction in absolute risk is expected between the intervention and placebo

groups; a minimum of 20% infection is expected in the placebo group. The sample size calculation estimated a total of 214patients assigned: two groups of 107 participants each.

Ethics and dissemination: This protocol has been approved by the local Medical Ethics Committee (National Institute of Rehabilitation 'Luis Guillermo Ibarra Ibarra', approval number INRLGII/25/20) and by the Federal Commission for Protection against Sanitary Risks (COFEPRIS, approval number 203300410A0058/2020). The results of the study will be submitted for publication in peer-reviewed journals and disseminated through conferences.

Trial registration number 2a: NCT04340349.

2b: NA

Protocol version: #4

STRENGTHS AND LIMITATIONS OF THIS STUDY

Strengths

- This is a double-blind randomized single-centre clinical trial, involving low doses of hydroxychloroquine and bromhexine, adequately powered to provide clinically

relevant information regarding prophylactic treatment for SARS-CoV-2 infection in health care personnel.

- Bromhexine has minimal side effects and is commercially available worldwide so, positive results could be applied in a timely fashion in different regions.

Limitations

- Long-term use of hydroxychloroquine can cause heart rhythm problems
- For the moment, people who are not candidates to receive the vaccine, due severe allergies, will not be included.
- Hydroxychloroquine has not been shown to be effective in monotherapy or with azithromycin, but adjunctive BHH could be an effective combination to inhibit SARS-Cov-2 infection.

INTRODUCTION

In Mexico, up to June 2021, have been produced more than 2.5 millions confirmed cases and ~232,000 deaths have arisen[1]. The age group ranging between 30 and 79 years is the most

highly affected, where 81% present mild symptoms, 14% severe and 5% critical, requiring intensive care unit management.

SARS-CoV-2 is a single-stranded RNA virion, member of the *Betacoronavirus* genus [2]. SARS-CoV-2 has an incubation period between 3 to 10 days, with different incubation periods related with different clinical symptoms [3,4] . It is transmitted through respiratory droplets from infected humans and through contact with contaminated fomites and aerosols; moreover, even asymptomatic persons in close contact can transmit the disease [5]. The mechanism through which the virus infects the respiratory cell is due to the angiotensin-converting enzyme protein 2 (ACE-2) receptor. This receptor is found in multiple tissues such as the oral cavity, brain, kidneys, gut, and placenta [6–8].

Health personnel is not exempt from contracting the disease. In China, it was reported that 3.5-4.4% of the infected population belonged to this group, and 14.8% presented characteristics of severity or critical illness [4,9,10]. Italy, around 20% of healthcare professionals became infected [11]; mean age of health workers who died was 55 years (range of 29-72 years) and mean period from hospital admission to death was 19 days, (range 1-47 days) [9].

Treatment of the SARS-Cov-2 infection has led different research groups to work on the development of vaccines. However, the use of vaccines can be a challenge. The first trials have shown that the immune protection is not 100% and protection may wane over time so periodic vaccination or booster shots for new variants may be needed . On the other hand, because the virus is RNA and the mutation rate is high, we can expect new variants that reduce or nullify the effectiveness of the vaccines, this depends on the origin of vaccine, if it is made with viral vectors (such as from CanSino or AstraZeneca), if it is mRNA (such as from Moderna or Pfizer-BioNTech) or if it is inactivated virus (Sinovac). Mainly because the development of vaccine that can be efficient for the new variants could be delayed and this could once again increase the number of people who acquire the SARS-CoV-2 virus. On the other hand, around the world there are groups of people who are against vaccination, or people that have severe allergies, as well as populations that will take much longer to reach the moment when they can acquire the vaccine, so it is extremely necessary that people who do not vaccinate by choice, by disease or by the lack of the vaccine, have an alternative to avoid infection and avoid the spread of the virus.

Therefore, it is important to develop a pharmacological strategy that allows the use of prophylactic drugs for the prevention of SARS-CoV-2 infection.

Chloroquine (CQ) and Hydroxychloroquine (HCQ) are known as an antimalarial agent; HCQ is a hydroxylated derivative from CQ. CQ and HCQ have gained attention as possible therapies in Covid-19 disease. In overdose, both drugs can cause severe, potentially life-threatening effects as visual disturbances, corneal opacities, irreversible retinopathy can occur with cumulative doses exceeding 100 grams. When lower daily doses (250 mg are used) retinopathy may not occur after many years of treatment [12]. The above indicates that the use of HCQ at low doses to avoid SARS-CoV-2 infection, has a low possibility of being toxic and could be used as a prophylactic treatment. HCQ has been used in several viral infections, for example, as replication inhibitor for the dengue virus, decreasing in vitro virus infection and promoting activation of different immunological signal pathways [13]. It has also been used to treat patients infected with hepatitis C virus decreasing viral load, with minimal adverse effects reported [14]. HCQ has been reported to block viral infection by increasing the endosomal pH required for virus fusion to the cell, as well as interfering with cellular SARS-CoV-2 cell receptors, through inhibition of receptor glycosylation by ACE2

[15–18]. HCQ has immunomodulatory effects; it inhibits production and release of pro-inflammatory cytokines, that are associated with severe disease development [19,20] . Recently, it has been reported that HCQ works as a autophagy inhibitor, interfering with viral infection and replication [21]. There is recent evidence that HCQ could be used to treat COVID-19; studies in high-risk patients show that the use of HCQ was associated with a lower risk of intubation or death [22]. Recent study showed that pre-treatment with HCQ has shown a better effect on antiviral activity [17] and it has been reported that loading doses of 1600 mg HCQ followed by 600 mg daily doses are needed have a relevant effect to SARS-CoV-2 inhibition within 72 hours in 60% of COVID-19 patients [23]. Finally, a study where evaluated the antiviral mechanisms of CQ and the adverse effects, repositioned the CQ to have more efficacy when used as a prophylactic treatment rather than as a therapeutic [24]. On the other hand, CQ and HCQ has been reported to have various adverse effects, the CQ being the most toxic in overdose. However, it was recently published that *in vivo* trials are lacking to determine whether this drug is useful as a prophylactic treatment against SARS-Cov-2 [25]. Based on the above, do more studies will be important to determine its effectiveness at low doses (<250 mg) as a prophylactic treatment.

Another pharmacological option to treat SARS-CoV-2 infection is Bromhexine (BHH). BHH modifies the composition of mucus, increases ciliary clearance and decreases coughing, improving respiratory symptoms. It has also been reported to enhance the effects of some antibiotics [26]. The mechanism by which SARS-CoV-2 enters human cells depends on the ACE-2 receptor and the human transmembrane serine protease (TMPRSS2), on which BHH has a specific inhibitory effect [27,28]. BHH has been used to treat pneumonic damage in both lungs during early infection [29]. BHH turns out to be an ideal candidate for SARS-CoV-2 treatment, since it has few contraindications, and its side effects are minimal, demonstrating an extensive margin of pharmacological safety. BHH is widely available over the counter, and its low cost makes it an ideal therapeutic option.

According to a letter published in the New England Journal of Medicine, of 77,262 patients infected by SARS-CoV-2, 3387 (4.4%) were health workers [9]. Of these, 23 have died from this disease. The prevalence of infections in health personnel is alarming since health services in first world countries have been overwhelmed by this disease. In Italy, around 20% of health professionals had a SARS-CoV-2 infection [11]. Faced with a highly contagious disease, the care of health workers, who are first line of contact and on whom the health system of each

country depends, is essential. This research regarding the use of HCQ and BHH in health personnel will allow us to determine and compare the effectiveness of both interventions, which is of vital importance to clarify whether these treatments may prevent the appearance of infection in this population. Describing for the first time that HCQ plus BHH could function for disease prevention, would allow us to provide prophylaxis to health professionals worldwide. Therefore, the use of HCQ and BHH in healthy health personnel exposed to patients with confirmed or suspected SARS-CoV-2 will significantly reduce infection.

METHODS AND ANALYSIS

Study design

Double-blind randomized clinical trial, with parallel allocation at a 1:1 ratio with HCQ + BHH vs placebo for both drugs for 60 days, to determine the efficacy of the combined drugs for the prevention of SARS-CoV-2 infection in healthcare workers.

Participants

The study will be carried out at the "Instituto Nacional de Rehabilitación, Luis Guillermo Ibarra Ibarra" (INR-LGII). This institution is a tertiary hospital that at this time has not been designated as a COVID-19 centre. The Mexican government defined 3 phases to determine risk for SARS-CoV-2 infection: imported cases from outside Mexico; community infection and spread of the disease throughout the country (also known as Phase 3). In the latter, it is assumed that every person who enters a hospital is a potentially infected carrier; currently our centre is in Phase 3. Likewise, health personnel who work at the "Instituto Nacional de Ciencias Médicas y Nutrición, Salvador Zubirán" (INCMNSZ), which is a COVID-19

designated tertiary centre, and who meet inclusion criteria of the protocol will be invited to participate in the study.

Inclusion of participants will be assessed according to the eligibility criteria. Table 1 shows the classification and characteristics of study variables. Continuous variables will be assessed for normality. Variables with a normal distribution will be compared using Student's t-test, non-parametric variables using the Mann-Whitney U-test. Categorical variables will be evaluated using the Chi-squared test.

Inclusion criteria

- Health personnel working at INR LGII or INCMNSZ who wish to participate in the study and sign the informed consent.
- Over 18 and under 60 years of age, both genders.
- Contacting with suspected or confirmed SARS-CoV-2 infection.
- Normal electrocardiogram.

Exclusion criteria

- Positive quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) test for SARS-CoV-2 at the time of inclusion.
- Panel of IgG or IgM antibodies positive for SARS-CoV-2 at the time of inclusion.
- Development of respiratory symptoms suspicious of SARS-CoV-2 infection during the first 7 days after treatment is initiated, confirmed by qRT-PCR and IgG or IgM antibodies positive for SARS-CoV-2.
- History of allergies to any hydroxychloroquine or bromhexine related compound or medication.
- Use of immunosuppressors for any reason.
- History of bone marrow transplant.
- Known glucose-6-phosphate dehydrogenase deficiency.
- Chronic kidney disease or glomerular filtration <20ml/min.
- Use of other drugs with reported pharmacological interactions (i.e., digitalis, flecainide, amiodarone, procainamide, or propafenone).
- History of long QT syndrome.
- Electrocardiogram with QTc>500 msec.

- Pregnant or breastfeeding personnel.
- Epilepsy.
- Known liver disease.
- Personnel who have received the Covid-19 vaccine

Elimination criteria

- Personnel who decide to leave the study for any reason not related to adverse events.
- Personnel with incomplete information on the primary outcome (qRT-PCR for SARS-CoV-2).
- Personnel who are relocated to work in another institution.
- Personnel who do not wish to participate in the study

Table 1. Classification and characteristics of study variables.

Variable	Conceptual definition	Operational definition	Type
Age	Date at recruitment minus date of birth	Years of age	Quantitative
Gender	Male or female genotype of the person	Male/female	Qualitative nominal
Weight	How much the patient weighs at the time of study inclusion	Weight, kilograms	Continuous quantitative

Size	How tall is the patient from head to toe at the time of study inclusion	Height, centimetres	Continuous quantitative
Body mass index	The division between weight by height squared at the time of inclusion in the study	Units of Kg/cm^2	Continuous quantitative
Occupation	Remunerative work performed by the participant at the time of recruitment	Unemployed, informal, unskilled employee, micro-entrepreneur or saleswoman, administrative employee, professional, entrepreneur	Qualitative nominal
Civil status	Civil status of the individual	Married, single, widowed, divorced, common-law union	Qualitative nominal
Level of study	Years completed and approved at the time of study recruitment	No studies, primary, secondary, preparatory, technical career, undergraduate, postgraduate	Ordinal qualitative
Alcohol intake	Consumption of alcoholic beverages	Intake of alcoholic beverages	Qualitative nominal
Smoking habit	Habitual tobacco uses at the time of recruitment	Number of packs of cigarettes consumed per day.	Quantitative

Drug's use	Regular use of chemicals such as amphetamines, cocaine, marijuana, LSD, heroin, glass	Consumption of drugs	Qualitative nominal
Hypertension	Elevation of blood pressure >130/80	Positive/negative	Qualitative nominal
Asthma	Chronic inflammatory disease characterized by bronchial hyperactivity with recurrent episodes of bronchospasm	Positive/negative	Qualitative nominal
Diabetes	Group of metabolic diseases, which occurs when the pancreas does not produce enough insulin or when the body does not use the insulin it produces effectively	Positive/negative	Qualitative nominal
Obesity	Pathological state characterized by a general excess or excessive accumulation of fat in the body	Positive/negative	Qualitative nominal
SARS-CoV-2 pneumonia	A form of severe pneumonia caused by coronavirus	Positive/negative	Qualitative nominal
Death	Statistical term that describes the death of an individual	Positive/negative	Qualitative nominal
Intensive Care Unit	Special facility in a hospital area, which provides life support to critically ill patients, requiring intensive supervision and monitoring	Positive/negative	Qualitative nominal

Severe pneumonia	Defined by the American Thoracic Society Criteria requiring at least one main criterion (need for invasive mechanical ventilation and shock with need for vasopressors), or three minor criteria (respiratory rate> 30 bpm, Pa02 / FiO2 ratio <250, Infiltrates multilobars, confusion / disorientation, uremia [BUN> 20 mg / dL], leukopenia [<4,000], thrombocytopenia [<100,000 platelets / mm^3], hypothermia [core temperature <36ºC], or hypotension requiring aggressive fluid resuscitation	Positive/negative	Qualitative nominal
Pneumonia	Acute infection of the lung parenchyma, accompanied by bilateral infiltrates on chest X-ray	Positive/negative	Qualitative nominal
Confusion	Glasgow scale less than 15	Positive/negative	Qualitative nominal
Hypothermia	Body temperature less than 36 degrees Celsius	Positive/negative	Qualitative nominal
Thrombocytopenia	Total platelets less than 100,000 per mm^3.	Positive/negative	Qualitative nominal
Arterial hypotension	Systolic blood pressure less than 90 mmHg or mean arterial pressure less than 60 mmHg	Positive/negative	Qualitative nominal

Sepsis	Rapid SOFA score (qSOFA) with 2 of the following three clinical variables: Glasgow ≤13, systolic pressure ≤100 mm Hg, or respiratory rate ≥22 bpm	Positive/negative	Qualitative nominal
qRT-PCR for SARS-CoV-2	Molecular diagnosis for SARS-CoV-2 from viral RNA	Positive/negative	Qualitative nominal
Septic shock	Arterial hypotension that persists after resuscitation volume and that requires vasopressors to maintain MAP ≥65 mm Hg and lactate ≥2 mmol / L (18 mg / dL) in the absence of hypovolemia	Positive/negative	Qualitative nominal
Adverse events related to the use of Hydroxychloroquine	Indirect drug-related mortality, nausea, vomiting, abdominal pain, rash, itchy skin, hair loss, QT segment elongation on electrocardiogram, corneal opacity, cardiac arrhythmias, and heart failure	Positive/negative	Qualitative nominal
Adverse events related to the use of Bromhexine	Indirect drug-related mortality, nausea, vomiting, abdominal pain, rash, diarrhoea.	Positive/negative	Qualitative nominal

Sample size calculation

According to the study by Remuzzi A et al. [11], the proportion of healthcare workers infected with SARS-CoV-2 and confirmed by RT-PCR was 20%. Taking this 20% as our

null hypothesis, we estimate that the proportion of infections in the intervention group will be 4%. Using a two-tailed test, with a type I error of 0.05, a power of 90%, and taking into account a loss of 10% of participants for each group, we estimate that a total of 214 participants will be required, distributed in parallel groups (1:1) of 107 each. This number of volunteers will allow us to find a difference of 16% between groups with a power of 90% and an attrition of 20%. To ensure that desired simple size is reached, all health workers involved in managing patients suspected or infected by SARS-CoV-2 will be invited personally and by institutional email.

Interventions

Interventions will consist of low doses of HCQ 200 mg tablets every 24 hours for 60 days plus BHH 8 mg tablets every 8 hours for 60 days. Study groups will be defined as follows: 1) HCQ plus BHH vs placebo for both drugs. Fabrication of both drugs and placebos will be provided to our centre by a hired laboratory. Both drugs will be provided to participants directly at the hospital by a researcher blinded to group assignment process. To ensure that the intervention is carried out, each participant will be asked to keep a written record of the

days and time the medication was administrated. This document will be reviewed weekly to verify that more than 50% adherence to treatment is maintained. Participants will be asked to record any symptoms related to the use of the medication, which will be reviewed by a researcher blinded to group assignment, weekly, or at the participants' request.

If any of the participants present symptoms of SARS-CoV-2 infection after the first 14 days from the beginning of the intervention or positive qRT-PCR is present, the drug will not be discontinued. If the participant presents adverse events related to the drugs that are severe or intolerable, treatment will be suspended. If the participants report an adherence of less than 50% of the medication, the intervention will not be discontinued to avoid imbalances between groups. Use of drugs that interact with HCQ or BHH such as flecainide, digitalis, amiodarone, procainamide or propafenone will be prohibited. If a participant has to use these drugs during the study period, they will be eliminated from the study. A free diet and outdoor activity will be allowed since these do not intervene with the implementation of the treatment or have interaction with the drugs used. Finally, incidence of adverse events such as nausea, vomiting, abdominal pain, rash, itchy skin, hair loss, lengthening of the QT interval in the

electrocardiogram, corneal opacity, cardiac arrhythmias, heart failure and death will be determined.

Randomization and treatment allocation

Group randomization will be in a centralized and straightforward way using the Web program www.randomization.com. It will be carried out independently by a researcher blinded to inclusion criteria, delivery of medication, participant follow-up, results, statistical analysis, and writing of the final manuscript. Allocation will be established, for 214 participants in blocks of 107 assigned. The selection of health workers will be made regardless of the hospital shift, work schedule, or assigned area. If the desired sample size is not reached, the inclusion of personnel involved in the first line of care of other referral hospitals for patients with SARS-CoV-2 will be considered.

An independent researcher will allocate patients to the desired groups. Envelopes will be correctly sealed by the pharmacy department and will contain HCQ plus BHH or placebos as previously mentioned. In those who do not require HCQ and BHH, the drug will be

replaced by tablets identical in colour and taste but lacking the active substance. In this way, drugs used in both groups will be indistinguishable.

Researcher A will recruit the participants and assess the inclusion criteria according to the serological, electrocardiographic, biochemical results and clinical investigation. Once included, volunteers will go to another office with researcher B, who will be blinded to the first procedure and the rest of the study. Researcher B will assign the groups independently, centrally, and through the use of the web program. This same researcher will be the one who makes the packages indistinguishable to the person providing the drugs to the participant. Researcher C will provide treatment in a sealed envelope or box to the participant in the order of assignment, without knowing each participant's study group. This researcher will also be blinded to the rest of the results. Participants will be blinded to the treatment they will receive. The researchers performing follow-up, researchers for result assessment, and the researcher who performs the statistical analysis will be blinded.

Informed consent will be obtained only by researcher A. If researcher A is not available, the study administrator may obtain informed consent for participation. The informed consent will contain the authorization to participate in the study and the authorization for taking biological samples, electrocardiogram, and authorization to handle personal information. All participants will complete a written informed consent included on the first page of the questionnaire that requires permission to participate in the study. No candidate is required to participate in the study, and their participation is based on the agreement that they may withdraw at any time. All participants have the right to withdraw from the study if they feel uncomfortable answering a question or with a test to be performed. Also, no one, including the research team, will require a reason why the participant decides to leave the study.

In order to protect participant confidentiality, each one will be assigned a participation number, and all biological samples, as well as medical history information, will be identified by the participant's initials and participant number. Part of the confidentiality protection process will include data capture only by the researcher in charge of data capture (researcher D), who will be the same for all participants and the entire study. Secondarily, the study administrator may also enter data into the database if researcher D is unavailable.

The study administrator will be blinded to allocation and results of the participants. However, the administrator will be the only one who will be able to reveal the group and treatment assignment in any of the cases: major adverse events such as cardiac arrhythmias, heart failure, major neurological abnormalities, atrial or ventricular fibrillation, kidney failure, or any adverse event related to pharmacological treatment that endangers the life or any organ of the participant's body. The objective of revealing the assignment by the study administrator will be to provide the participant of a timely treatment according to the drugs ingested.

Participant timeline and intervention

The inclusion of participants will be evaluated according to the eligibility criteria and by invitation. Volunteers who wish to participate in the study will be scheduled the next day at a specialized office to carry out all the relevant studies to ensure the inclusion criteria. These include a medical history, anthropometric measurements such as weight and body mass index, electrocardiogram (every week until the end of the study or when the participant

requests it if they have any discomfort), complete blood count (cbc), complete blood chemistry, and serological test for antibodies and qRT-PCR for SARS-CoV-2. Volunteers will be asked for information to contact them once the serological results are obtained.

Once the results are obtained (approximately 3 days), personnel eligible to participate in the study will be contacted. They will meet in a particular office to speak with a researcher who will be in charge of carrying out the eligibility criteria and medical history checklist. This researcher will be different from the one who makes the assignment, who delivers the medicine and the one who evaluates the results and performs the statistical analysis. The assignment of the group of each participant will be performed, and the participant will not know the group they have been assigned. This information will be known for the researcher in charge, unrelated to the delivery of the treatment, results, or inclusion of the participant in the study. After the assignment, the volunteers will receive the assigned treatment at the pharmacy using a code in a sealed envelope assigned by the Web. Participants who meet the inclusion criteria and there is no reason for exclusion will proceed to the second phase of group assignment with researcher B, the next business day at a different time or office than researcher A.

The group of researchers in charge of monitoring the participants, who will be blinded to the group assignment at all times, will be in charge of assessing each participant´s adverse event and treatment adherence record weekly. These follow-up researchers will be available 24 hours a day throughout the week if participants experience undesirable adverse events that require urgent attention or that do not allow them to continue with drug treatment. If this situation happens, the researcher in charge of the follow-up will contact the study administrator to reveal to the treating physicians the treatment received by the participant. Health evaluation of all participants will be performed at day 30, day 60 and day 90, this includes electrocardiogram analysis, blood chemistry analysis, antibody test, qRT-PCR, or at request of the participant due to adverse clinical symptoms.

At the end of the first 60 days, a new qRT-PCR will be requested from each participant. All participants who present symptoms after the first 7 days of initiation of the intervention, will be considered as a positive individual for the analysis and will not be excluded from the study. The same action will be carried out 90 days after the start of treatment for both groups. After 60 days, the treatment will be suspended and the results of the qRT-PCR samples for

SARS-CoV-2 will be evaluated. After finishing the intervention (60 days), all participants will be followed-up 30 more days with a new qRT-PCR at day 90 after initiation of the intervention and 30 days after the end of the intervention, to assess the efficacy or the treatment during the follow-up period.

Outcome measures

This study compares the efficacy of the use of HCQ plus BHH (as a conjoined treatment) in prophylactic doses for 60 days in healthy health personnel exposed to the first line of care in confirmed patients with suspected infection by SARS-CoV-2.

Primary endpoint

The primary endpoint will be the proportion of health personnel infected by SARS-CoV-2 at 60 days after starting treatment, in both groups. The infection will be diagnosed using qRT-PCR for relative expression of the mRNA SARS-CoV-2 and the measure of IgM and IgG antibodies anti-SARS-CoV-2 after day 7 of treatment using rapid test Cellex qSARS-CoV-2 IgG/IgM. All participants presenting symptoms with positive qRT-PCR after 7 days of

initiation of the intervention, will be considered positive and will be included in the analysis. The study period will be 90 days (60 days for the primary end point plus 30 days of follow-up). The proportion of infected personnel will be evaluated using relative risk (RR) and absolute risk increase (ARI) with their respective 95% confidence intervals, in the established time. The disease-free period in the 60 days will also be evaluated, analysing the cumulative incidence of healthy personnel, presence of infection will be confirmed by qRT-PCR for SARS-CoV-2 and by presence of IgM and IgG antibodies for SARS-CoV-2. The censoring variable will be interruption of treatment either due to death, adverse events, or any elimination criteria. Since there is the possibility of false positives and negatives with qRT-PCR, we will perform qualitative measurements of IgM and IgG with the Cellex qSARS-CoV-2 IgG/IgM Rapid test which is authorized by FDA. The test can be used on serum, plasma, or whole blood samples. The clinical sensitivity of the assay was 93.8% and the clinical specificity was 96%[30].

Secondary endpoints

The secondary endpoint will be the proportion of health personnel infected 90 days after starting treatment in both groups. The infection will be diagnosed using qRT-PCR for relative expression of the mRNA of SARS-CoV-2 and the measure of IgM and IgG antibodies anti-SARS-CoV-2 after day 7 of the start of treatment using rapid test Cellex qSARS-CoV-2 IgG/IgM. The study period will be 90 days. The proportion of infected personnel will be evaluated using RR and ARI with their respective 95% confidence intervals, in the established time. The disease-free period in the 90 days will also be evaluated by analysing the cumulative incidence of healthy personnel, and the presence of confirmed infection by qRT-PCR of SARS-CoV-2 will be the outcome. The censoring variable will be the discontinuation of treatment either due to death, adverse events, or any elimination criteria.

Also, secondary outcomes will be, in case of a positive SARS-CoV-2 result, the need for oxygen use, admission to the intensive care unit (ICU), presence of pneumonia by computer tomography scan (CT), death, severe pneumonia defined by the American Thoracic Association, time from hospitalization to recovery in days.

Another secondary endpoint will be adverse events, defined as the presence of any of the following during the study period: death, nausea, vomiting, abdominal pain, diarrhoea, rash,

itchy skin, hair loss, lengthening of the QT interval in the electrocardiogram (>500msec), corneal opacity, cardiac arrhythmias, heart failure or kidney failure (renal clearance <20ml/min). The proportion of the compound of adverse events between the groups will be analysed using RR and ARI for 60 days with their respective 95% confidence intervals.

The efficacy of the treatment will be established as the proportion of volunteers infected with SARS-CoV-2. This difference should be sufficient to avoid overlapping of the 95% confidence intervals. It will be considered effective if the intervals do not overlap and ineffective if when comparing groups, they have a proportion of infected whose confidence intervals overlap. This type of evaluation will allow an adequate understanding of the efficacy of the treatment in both groups.

Handling and storage of data and documents

Before the start of the study, the researchers in charge of the recruitment, assignment, and delivery of drugs will be trained to perform the task assigned to them at least 3 days before the start of the study.

Researcher A will assess the eligibility criteria of potential participants and perform a detailed clinical examination to assess whether they can participate in the study. The data that will be collected initially will be the following:

- Medical history (includes personal data): study identifier number, history number, name, date of birth, gender, occupation, marital status, nationality, current residence, degree of studies (primary, secondary, upper secondary, bachelor degree, postgraduate), hospital service to which they belong and the number of hours worked per week.
- Personal history: alcohol intake (yes/no; how many glasses of beer or alcoholic beverages do you consume per week), smoking habit (yes/ no; and number of cigarettes per day), drug use (yes/no), diet per week (dietary restrictions and number of meals per day) and number of hours of sleep per day.
- Gynaecological history (in women): Number of pregnancies, number of live children, menarche, menopause.

- History of respiratory disease, history of gastrointestinal disease, nephrological, neurological, haematological, cardiovascular, allergies.
- Genetic family history, such as hypertension, diabetes, heart disease, kidney disease.
- Physical examination: blood pressure, heart rate, respiratory rate, temperature, weight, height, body mass index, skin lesions, head and neck inspection, respiratory inspection (chest symmetry, lung expansion, palpation of the bases and preserved vertices, lung percussion, auscultation for lung murmur, breath sounds). Cardiovascular inspection (palpation of the fifth intercostal space, auscultation of heart sounds, pulses that are palpable and symmetrical), abdominal inspection (palpation, percussion and auscultation of peristaltic sounds), neurological evaluation (Glasgow, active motility, passive motility, reflex motility, cranial nerves, sensitivity).
- Complete blood count: haematocrit, leukocytes, segmented (%), lymphocytes (%), monocytes (%), mean corpuscular volume, platelets.
- Blood chemistry: glycaemia, urea, creatinine, sodium, potassium, chlorine, aspartate transaminase, alanine transaminase, alkaline phosphatase, total bilirubin.

- Muscle enzymes (creatine kinase)
- Clotting times: thrombin time, prothrombin time, international normalized ratio.
- Electrocardiogram: rhythm, heart rate, heart axis, evaluation of P wave, PR interval, duration of QRS, QT interval, time of T wave. The electrocardiogram will be performed using an instrument calibrated and validated for its use internationally, weekly.
- Molecular test results for IgG and IgM antibodies:
    - The FDA approved product called Cellex qSARS-CoV-2 IgG/IgM Rapid Test will be used for serological determination. The device cassette, sample, and buffer solution must be at room temperature. The sample (10 µL) is transferred to the centre of the sample well. After the sample well is free of liquid, two drops of sample diluent are added. After fifteen to twenty minutes, read the test results. Results should not be read after twenty minutes.
    - A positive IgM result occurs when a coloured band appears on the M test line (M) and the control line (C) and indicates that IgM against SARS-CoV-2 is present.

- A positive IgG result occurs when a coloured band appears on the G test line (G) and the control line (C) and indicates that IgG against SARS-CoV-2 is present.
- A positive result for IgM and IgG occurs when coloured bands occur both M and G, as well as C.
- A negative result occurs when a coloured band appears in C only and indicates that IgM and IgG antibodies against SARS-CoV-2 were not detected.
- An invalid result occurs when a colour band is not produced in C, and the test must be repeated.

▶ Official qRT-PCR results

All this information will be collected in a pre-established medical history questionnaire for each potential participant. The information obtained from the weekly assessment of adverse events, and the results of the qRT-PCR for SARS-CoV-2 at 60 and 90 days (60 for the primary end point plus 30 more days of follow-up) after starting treatment will be entered into an online database. In order to ensure the quality of the data collection, the database will be built in CASTOR, a database on the Web that allows entering all the pre-defined data for

each participant, thus reducing human error. This information will be stored on a server in the United States of America and can only be accessed by the study's administrator. The data may only be entered by a researcher in charge of collecting the data sheets and emptying them.

Monitoring and quality assurance

Every possible adverse event will be noted daily by the participants in the agenda that will be delivered to them. This agenda will be evaluated weekly by the researcher in charge of monitoring the participants (who will be blinded to group assignment). In case of unbearable adverse events for the participants or that put their health at risk, an open line will be available 24 hours a day with direct communication to the researcher in charge of monitoring the study to report any event that requires hospitalization or immediate evaluation at the hospital. All participants with adverse events that put their life or health at risk may be urgently assessed by personnel from both INCMNSZ and INR-LGII, if possible, by the staff involved into the study. Patient follow-up investigator will immediately contact the study administrator to disclose the participant's assignment to treating physicians at that institution, but the assignment will never be disclosed to other investigators related to the study. All the study

expenses and/or attention of collateral effects will be covered by the current cost of the financing SECTEI/061/2020.

Auditing will be carried out weekly, assessing adverse events, capturing data in the corresponding datasheets by the study administrator. Likewise, the data entered in the CASTOR web base will be valued to validate its quality. The paper data sheets must be kept in a special office dedicated to the study in folders separated by volunteers with the informed consent of each participant, the data of the medical history, laboratory results, eligibility criteria, adverse event sheet and results, molecular tests, as well as electrocardiogram. The letter of revocation of informed consent will also be protected if required. As part of the audit, an interim analysis will be carried out 30 days after the study starts to assess the possible adverse effects and whether these outweigh the potential benefits of the intervention. In the adverse event outweigh the potential benefits, termination of the study will be assessed. The approval of the research ethics committee of the INR-LGII of Mexico has been obtained.

Statistical analysis

Data analysis will be carried out by intention to treat, which means that each participant will be analysed according to the group assigned regardless of whether they modified their treatment. The study variables will be divided according to the allocation group. The statistical analysis will be carried out by evaluation the difference between the different groups of HCQ plus BHH versus placebos. Missing data will be handled by multiple imputation analysis when missing at random. Deaths will be censored.

The primary objective will be expressed in number and proportion for each group. The RR will be obtained as the division between the proportion of primary outcomes in the intervention group(s) by the proportion of primary outcomes in the double placebo group. Adjusted risk ratios (aRR) will be obtained using a log-binomial regression, adjusting for age and gender as pre-specified confounding variables. It will be expressed as RR with its respective 95% confidence interval for the initial time, which is 60 days. Likewise, the result will be expressed as absolute risk, which will be derived from the proportion of the primary outcome in the intervention group minus the proportion of the primary outcome in the control group. Secondarily, the primary objective will be analysed with the non-parametric estimate

of the survival and risk function using Kaplan-Meier curves for 60 days according to the allocation group. The primary endpoint will be SARS-CoV-2 infection within the 60-day period, and the silencing variable will be dropping out of the study for any reason. The comparison of the survival curves between both groups will be carried out using the log-rank test. Risk ratio will be used for treatment effect. A log-binomial regression adjusted by age, gender, service in which the participant works, body mass index, will be used.

For secondary outcomes such as the analysis at 90 days, the same statistical analysis expressed in RR and absolute risk will be used. Survival analysis will be used for the primary endpoint only. An interim statistical analysis will be performed 30 days after the study starts to assess possible adverse effects and the efficacy of the intervention. The study administrator will be the only one with access to the data. For the interim analysis and the final analysis, the administrator will export the data to Excel format to be analysed by the study statistician blinded to the assignment of groups, participants, or results.

Adverse events, serious adverse events and suspected unexpected serious adverse reactions

By requiring the use of drugs, the participant will be exposed to risks inherent to the drug used, ranging from mild to severe or death. Any unexpected risks that may occur during the study will be immediately explained to the participants and the ethics committee. Any adverse event will be compiled and will not be disclosed under any condition to anyone other than the study administrator, treating physicians in case of severe events, and the ethics committee. The results will be completely anonymous concerning the names of the participants. The results will be compiled and reported as combined collective data.

Patient and public involvement

Patients were not involved in the development of this research. However, the results of the study will be communicated to the study participants by sending the end product (published article) to the provided email address.

ETHICS, DISSEMINATION AND SAFETY MONITORING

In case of adverse events or complications derived from the study, participants will be assured attention by the staff of the INCMNSZ in an enclosure that ensures the safety of the participant, not subjecting volunteers to a higher risk of contamination. This care will be extended until adverse events are resolved. In case of no adverse events during the study, medical attention will be extended at the aforementioned institute until 15 days after the end of the study.

This protocol has been approved by the local medical ethical review committee at the INR-LGII with the internal number INRLGII/25/20, and by the Federal Commission for Protection against Sanitary Risks (in Spanish, Comisión Federal para la Protección contra Riesgos Sanitarios, COFEPRIS), approval number 203300410A0058/2020.

The study results will be published in journals of worldwide impact affiliated with the Journal Citation Reports. Likewise, the results of the study will be disseminated in national and international media, exposed in international and national congresses, communicated to CONACYT, and recorded in Clinicaltrials.gov according to the study identifier number. The

help of non-profit organizations will be sought to disseminate the results of the investigation to interest groups.

The complete protocol will be published on Clinicaltrials.gov and the OSF - Center for Open Science platform https://osf.io/. Where a DOI will be assigned, and the amendments made to the original protocol will be assessed.

Amendments to the protocol may be made before the start of the study and during the study. Any amendment to the protocol will be clarified and posted on Clinicaltrials.gov under the same identifier as this study. Likewise, any amendment will be sent to the ethics committee of the same hospital.

AUTHOR CONTRIBUTIONS

JGM is the lead study investigator, developed the study concepts and design, and wrote the manuscript by adapting the original study protocol for publication, subsequent reviews and amendments. EJHL, KMM and AAA contributed to the development and refining of the protocol, writing of manuscript and subsequent review. RJMP provided advanced

methodological and statistical input, and contributed to the study design and subsequent amendments. RFC, TCH, PSSB, RAG and NML reviewed, commented and informed methodology, development and writing of the protocol.

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COMPETING INTERESTS STATEMENT

None of the authors have conflict of interests, commercial agreements, or receive financial fees or compensation from any commercial or pharmaceutical company.

ETHICS APPROVAL

This protocol has been approved by the local medical ethical review committee at the INR-LGII with the internal number INRLGII/25/20. Definitions of Research Risk Regulation of

the General Health Law on Research for Health (in Spanish, Reglamento de la Ley General de Salud en Materia de Investigación para la Salud) http://www.inr.gob.mx/Descargas/Investigacion/Reglamentos-LGS-Materia-Investigacion-Salud.pdf. ARTICLE 17; and by Federal Commission for Protection against Sanitary Risks (in Spanish, Comisión Federal para la Protección contra Riesgos Sanitarios, COFEPRIS), approval number 203300410A0058/2020.

PROVENANCE AND PEER REVIEW

Not commissioned; externally peer reviewed.

ORCID ID

Julio Granados-Montiel https://orcid.org/0000-0002-0611-64

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

Section/item	ItemNo	Description	Page/Line
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1/1-3
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	4/72
	2b	All items from the World Health Organization Trial Registration Data Set	NA
Protocol version	3	Date and version identifier	4/74
Funding	4	Sources and types of financial, material, and other support	32/623625
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1/5-17 31/614-620
	5b	Name and contact information for the trial sponsor	1/19-23
	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	31/614-620
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	NA
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	6-9/98-187
	6b	Explanation for choice of comparators	9/180-187
Objectives	7	Specific objectives or hypotheses	9/183-187
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)	10/191-193

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	10/197-211
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)	11-12/212-248
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	17/265-288
	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)	17/278-281
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	17/269-274
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	NA
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	22-24 /392-432
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)	20-22/344-384
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	16/253-260

Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	10-16/207-250
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	18/291-295
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	18-19/299-303
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	19/305-315
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	19/307-315
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	NA
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	27/504-510

	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	NA
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	27/508-513
Statistical methods	20a	Statistical methods for analyzing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	29/542-547
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	29/560-563
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomized analysis), and any statistical methods to handle missing data (eg, multiple imputation)	29/546-547
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	NA
	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	30/567-571
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	30/575-581
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	28-29/529-539
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	31/589-599

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)	32/600-605
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	19/317-318
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NA
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	20/327-332
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	33/631-632
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	28/510-513