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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).

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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

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

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16 In a letter to the editor in the New England Journal of Medicine, of 77,262 patients infected
17 by COVID-19, 3387 (4.4%) were from health workers. Of these, 23 have died from this
18 disease. The prevalence of infections in health personnel is alarming since the health
19 services in the first world countries have been overwhelmed by this disease. Each one of
20 the health personnel is fundamental and indispensable to reduce mortality and give
21 opportunity care to those patients who require it. In Italy, around 20% of health
22 professionals had a SARS-CoV-2 infection.[15] Faced with a highly contagious disease, the
23 care of health workers, who are first line of contact and on whom the health system of each
24 country depends, is essential. This research regarding the use of hydroxychloroquine and
25 bromhexine versus bromhexine in health personnel will allow us to determine and compare
26 the effectiveness of both interventions, which is of vital importance to clarify whether these
27 treatments are effective in preventing the appearance of infection in this population.
28 Describing for the first time that both hydroxychloroquine and bromhexine could function
29 as disease preventives, would allow us to provide prophylaxis to all-inclusive health
30 professionals in each country. Therefore the use of hydroxychloroquine+bromhexine in
31 prophylactic doses in healthy health personnel exposed to the first line of care in patients
32 confirmed or suspected with SARS-CoV-2 will significantly reduce infections. This drug
33 combination will be more effective compared with the exclusive use of bromhexine at
34 prophylactic doses in the same exposed population and will not have significant adverse
35 events such as mortality, nausea, vomiting, abdominal pain, diarrhea, rash, itchy skin, hair
36 loss, lengthening of the QT interval in the electrocardiogram (>500 msec), opacity corneal,
37 cardiac arrhythmias, heart failure or renal failure (defined as glomerular filtration
38 <20mL/min), when compared with the exclusive use of bromhexine at prophylactic doses.

43 44 **METHODS AND ANALYSIS**

45 **Study design**

46 Randomized, parallel-group, double-blind, placebo-controlled, 1:1 assignment to determine
47 the efficacy of low-dose hydroxychloroquine + bromhexine vs. low dose bromhexine, for
48 the prevention of SARS-CoV-2 infection in healthcare workers. The study will be carried
49 out at the “Instituto Nacional de Rehabilitación, Luis Guillermo Ibarra Ibarra” (INR LGII).
50 This institution is a third-level hospital that at the time of writing this protocol has not been
51 designated as a COVID-19 hospital. However, at this time that we are in phase 3, it is
52 assumed that every person who enters the hospital is a symptomatic or asymptomatic
53 carrier. Likewise, health personnel working at the “Instituto Nacional de Ciencias Médicas
54 y Nutrición, Salvador Zubirán” (INCMNSZ) and falls within the inclusion criteria of the
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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, PaO ₂ / FiO ₂ ratio < 250, Infiltrates	Presence of pneumonia	Qualitative nominal

	<p>multilobars, confusion / disorientation, uremia [BUN> 20 mg / dL], leukopenia [$<4,000$], thrombocytopenia [$<100,000$ platelets / mm^3], hypothermia [core temperature $<36^\circ\text{C}$], or hypotension requiring aggressive fluid resuscitation</p>		
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 ($BMI \geq 30$), 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 < 20 ml/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 sample 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 administered. 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.

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3 Informed consent will be obtained only by researcher A. If researcher A is not available,
4 the study administrator may obtain informed consent for participation. The informed
5 consent will contain the authorization to participate in the study and the authorization for
6 the taking of biological samples, electrocardiogram, and authorization to handle personal
7 information.
8

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

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

16 In order to protect the confidentiality of the participants, each one will be assigned a
17 participation number, and all biological samples, as well as medical history information,
18 will be identified by the participant's initials, date of birth and participant number. Part of
19 the confidentiality protection process will include data capture only by the researcher in
20 charge of data capture (researcher D), who will be the same for all participants and the
21 entire study. Secondly, the study administrator may also enter data into the database if
22 researcher D is unwell.
23

24 The study administrator will be blind to the allocation and results of the participants.
25 However, the administrator will be the only one who will be able to reveal the group and
26 treatment assignment in any of the cases: major adverse events such as cardiac arrhythmias,
27 heart failure, major following neurological abnormalities, atrial or ventricular fibrillation,
28 kidney failure, or any adverse event related to pharmacological treatment that endangers the
29 life or any organ of the participant's body. The objective of revealing the assignment by the
30 study administrator will be to be able to warn the participant of a timely treatment
31 according to the drugs ingested.
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35 **Participant timeline and intervention**

36 The inclusion of participants will be evaluated according to the eligibility criteria and by
37 invitation. Volunteers who wish to participate in the study will be summoned the next day
38 at a specialized office to carry out all the relevant studies to ensure the inclusion criteria.
39 These include a medical history, anthropometric measurements such as weight and body
40 mass index, electrocardiogram, hematic biometry, complete blood chemistry, and
41 serological test for antibodies and RT-PCT for SARS-CoV-2. Volunteers will be asked for
42 information to contact them once the serological results are obtained.
43

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

6 The group of researchers in charge of monitoring the participants, who will be blind to the
7 group assignment at all times, will be in charge of assessing each participant's adverse
8 event and treatment adherence record weekly. These follow-up researchers will be available
9 24 hours a day throughout the week if participants experience undesirable adverse events
10 that require urgent attention or that do not allow them to continue with drug treatment. If
11 this latter situation happens, the researcher in charge of the follow-up will contact the study
12 administrator to reveal to the treating physicians the treatment received by the participant.
13 At the end of the first 30 days, a new RT-PCR will be requested from each participant. The
14 same action will be carried out 60 days after the start of treatment for both groups. After 60
15 days, the treatment will be suspended and the results of the RT-PCR samples for SARS-
16 CoV-2 will be evaluated.
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22 **Outcome measures**

23 This study compares the efficacy of the use of hydroxychloroquine plus bromhexine vs. the
24 exclusive use of bromhexine in prophylactic doses every 24 hours for 60 days in healthy
25 health personnel exposed to the first line of care in confirmed patients with suspected
26 infection by SARS-CoV-2.
27

28 **Primary endpoint**

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

45 **Secondary endpoints**

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

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

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

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

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

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

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

31 **Statistical analysis**

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

40
41 The primary objective will be expressed in number and proportion for each group. The
42 relative risk will be obtained as the division between the proportion of primary outcomes in
43 the intervention group by the proportion of primary outcomes in the control group. It will
44 be expressed as RR with its respective 95% confidence interval for the initial time, which is
45 60 days. Likewise, the result will be expressed as absolute risk, which will be derived from
46 the proportion of the primary outcome in the intervention group minus the proportion of the
47 primary outcome in the control group. Secondly, the primary objective will be analyzed
48 with the non-parametric estimate of the survival and risk function using Kaplan-Meier
49 curves for 60 days according to the allocation group. The primary endpoint will be SARS-
50 CoV-2 infection within the 60-day period, and the silencing variable will be dropping out
51 of the study for any reason. The comparison of the survival curves between both groups
52 will be carried out using the log-rank test. To adjust the primary objective to possible
53 confounders such as age, sex, and service in that volunteer works, body mass index, etc.

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3 Multiple regression will be performed using the Cox model to determine the adjusted
4 primary endpoint hazard ratio.

5 For secondary outcomes such as the analysis at 30 days, the same statistical analysis
6 expressed in RR and absolute risk will be used. Survival analysis will be used for the
7 primary endpoint only.

8 An interim statistical analysis will be performed 30 days after the study starts to assess
9 possible adverse effects and the efficacy of the intervention.

10 The study administrator will be the only one with access to the data. For the interim
11 analysis and the final analysis, the administrator will export the data to Excel format to be
12 analyzed by the study statistician blindly to the assignment of groups, participants, or
13 results.
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16 17 **AEs, SAEs and SUSARs**

18 By requiring the use of drugs, the participant will be exposed to risks inherent to the drug
19 used, ranging from mild to severe or death. Any unexpected risks that may occur during the
20 study will be immediately explained to the participants and the ethics committee. Any
21 adverse event will be compiled and will not be disclosed under any condition to anyone
22 other than the study administrator, treating physicians in case of severe care and the ethics
23 committee. Besides, the results will be completely anonymous concerning the names of the
24 participants. The results will be compiled and reported as combined collective data.
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28 29 **Patient and public involvement**

30 Patients were not involved in the development of this research. However, the results of the
31 study will be communicated to the study participants by sending the end product (article) to
32 the provided email address.
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34 35 **ETHICS, DISSEMINATION AND SAFETY MONITORING**

36 In case of damage, adverse events or complications derived from the study, participants
37 will be assured attention by the staff of the National Institute of Medical Science and
38 Nutrition "Salvador Zubirán" in an enclosure that ensures the safety of the participant, not
39 subjecting volunteers to a higher risk of contamination. This care will be extended until
40 adverse events are resolved. In case of no adverse events during the study, medical
41 attention will be extended at the aforementioned institute until 15 days after the end of the
42 study.
43

44 The protocol is approved by the local medical ethical review committee at the National
45 Institute of Rehabilitation, Luis Guillermo Ibarra Ibarra with the internal number
46 INRLGII/25/20. Definitions of Research Risk Regulation of the General Health Law on
47 Research for Health (in Spanish, Reglamento de la Ley General de Salud en Materia de
48 Investigación para la Salud) [http://www.inr.gob.mx/Descargas/Investigacion/Reglamentos-](http://www.inr.gob.mx/Descargas/Investigacion/Reglamentos-LGS-Materia-Investigacion-Salud.pdf)
49 [LGS-Materia-Investigacion-Salud.pdf](http://www.inr.gob.mx/Descargas/Investigacion/Reglamentos-LGS-Materia-Investigacion-Salud.pdf). ARTICLE 17; and by The Federal Commission for
50 Protection against Sanitary Risk (in Spanish, Comisión Federal para la Protección contra
51 Riesgos Sanitarios, COFEPRIS) approval number 203300410A0058/2020.

52 The study's results will be published in journals of worldwide impact affiliated with the
53 Journal Citation Reports (JCR). Likewise, the results of the study will be disseminated in
54 national and international media, exposed in international and national congresses,
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3 communicated to CONACYT, and recorded in Clinicaltrials.gov according to the study
4 identifier number.

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

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

11 Amendments to the protocol may be made before the start of the study and during the
12 study. Any amendment to the protocol will be clarified and posted on Clinicaltrials.gov
13 under the same identifier as this study. Likewise, any amendment will be sent to the ethics
14 committee of the same hospital.
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16
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18

19 Contributors Study concept and design were conducted by author JGM. Advanced
20 statistical input was given by author RJMP Critical revision of concept and design and
21 intellectual input in the study protocol were done by authors, Study supervision and
22 coordination were conducted by authors EHL and JGM Manuscript revision and editing
23 was done by RAG and NML.
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26

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32 **Competing interest**, the study is conducted by the department of Epidemiology at the
33 National Institute of Rehabilitation Luis Guillermo Ibarra Ibarra, as stated above in the
34 funding section, an unrestricted grant from SECTEI/061/2020 was provided for the conduct
35 of the trial.
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37

38 **Declaration of interests** No one of the researchers presents a conflict of interest or has
39 commercial agreements, or receives financial compensation from any commercial or
40 pharmaceutical company.
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43 **Patient and public involvement** Patients and/or the public were not involved in the design,
44 or conduct, or reporting, or dissemination plans of this research.
45

46 **Patient consent for publication** Not required
47

48 **Provenance and peer review**, externally peer reviewed
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51 **Open access**
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53 **ORCID ID**
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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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3 1 **New prophylaxis regimen for SARS-CoV-2 infection in health professionals with low doses of**
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5 2 **Hydroxychloroquine and Bromhexine: a randomized, double-blind placebo clinical trial**
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8 3 **(ELEVATE Trial).**
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29 ABSTRACT

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

39 **Methods and analysis:** Double-blind randomized clinical trial, with parallel allocation at a 1:1 ratio with
40 placebo, of low doses of HCQ and BHH, for 60 days. Study groups will be defined as follows: 1) HCQ
41 200mg/d + BHH placebo; 2) BHH 8mg/8h + HCQ placebo; 3) HCQ 200mg/d + BHH 8mg/8h; and 4)
42 HCQ placebo + BHH placebo. Primary endpoint will be efficacy of the interventions, determined by
43 differences in the proportions of infected personnel. At least a 16% reduction in absolute risk is expected
44 between the double intervention and double placebo groups; a minimum of 20% infection is expected in
45 the placebo group. The sample size calculation estimated a total of 280 patients assigned: four groups of
46 70 participants each.

47 **Ethics and dissemination:** This protocol has been approved by the local Medical Ethics Committee
48 (National Institute of Rehabilitation 'Luis Guillermo Ibarra Ibarra', approval number INRLGII/25/20) and
49 by the Federal Commission for Protection against Sanitary Risks (COFEPRIS, approval number
50 203300410A0058/2020). The results of the study will be submitted for publication in peer-reviewed
51 journals and disseminated through conferences.

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3 52 **Trial registration number:** NCT04340349.
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7 8 54 **STRENGTHS AND LIMITATIONS OF THIS STUDY**

9 10 55 *Strengths*

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13 56 ▶ This is a double-blind randomized single-centre clinical trial, involving low doses of
14
15 57 hydroxychloroquine and bromhexine, adequately powered to provide clinically relevant
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17 58 information regarding prophylactic treatment for SARS-CoV-2 infection in health care personnel.
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20 59 ▶ This study will include 280 participants who are health workers exposed to SARS-CoV-2 patients
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22 60 with suspected or confirmed infection, with short term follow-up (60 days).
- 23
24 61 ▶ A study of prophylactic treatment in this population is of great value and could provide the basis
25
26 62 for protecting medical personnel around the world.
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29 63 ▶ Both drugs proposed for this study have minimal side effects and are commercially available
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31 64 worldwide; findings could be applied in a timely fashion in different regions.

32 33 65 *Limitations*

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36 66 ▶ None of the proposed drugs have proven to be effective as a treatment for symptomatic SARS-
37
38 67 CoV-2 infected patients.

74 INTRODUCTION

75 On December 31, 2019, an outbreak of 27 pneumonia cases of unknown aetiology was reported in Wuhan,
76 Hubei province, China¹. A week later, the agent that caused the outbreak was identified as a new virus of
77 the Coronaviridae family, being designated as a “severe acute respiratory syndrome coronavirus 2”
78 (SARS-CoV-2) by the International Committee on Taxonomy of Viruses^{2 3}. Affection caused by this
79 virus, coronavirus disease 2019 (COVID-19), has become a pandemic, implying the biggest health-related
80 battle in recent years. In Mexico, up to December 2020, have been produced more than 1 million
81 confirmed cases and ~130,000 deaths, according to WHO data⁴. The age group ranging between 30 and
82 79 years is the most highly affected, where 81% present mild symptoms, 14% severe and 5% critical,
83 requiring intensive care unit management.

84 SARS-CoV-2 is a single-stranded RNA virion, member of the *Betacoronavirus* genus⁵. SARS-CoV-2 has
85 an incubation period between 3 to 10 days, with different incubation periods related with different clinical
86 symptoms^{6 7}. It is transmitted through respiratory droplets from infected humans through contact with
87 contaminated fomites and aerosols; on the other hand, asymptomatic patients in close contact can transmit
88 the disease⁸. The mechanism through which the virus infects the respiratory cell is due to the angiotensin-
89 converting enzyme protein 2 (ACE-2). This receptor is found in multiple tissues such as the oral cavity,
90 brain, kidneys, intestine, and placenta⁹⁻¹¹.

91 Health personnel is not exempt from contracting the disease. In China, it was reported that 3.5-4.4% of
92 the infected population belonged to this group, and 14.8% presented characteristics of severity or critical
93 illness^{7 12 13}. In Italy, around 20% of healthcare professionals became infected¹⁴; mean age of health
94 workers who died was 55 years (range of 29-72 years) and mean period from hospital admission to death
95 was 19 days, (range 1-47 days)¹².

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3 96 Treatment of the SARS-Cov-2 infection has led different research groups to work on the development of
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5 97 vaccines. However, the use of vaccines can be a challenge. Early trials have shown minimal immune
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8 98 protection or long-term protection is low. On the other hand, because the virus is RNA and the mutation
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10 99 rate is high, we can expect new variants that reduce or nullify the effectiveness of vaccines. Therefore, it
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12 100 is not known if the vaccines that are now in the phase end of the clinical study and those that are
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15 101 administered will work with the same efficacy for the SARS-CoV-2 virus that gave rise to Covid-19.
16
17 102 Therefore, it is important to develop a pharmacological strategy that allows the use of prophylactic drugs
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19 103 for the prevention of SARS-CoV-2 infection.

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21
22 104 Chloroquine (CQ) and Hydroxychloroquine (HCQ) are known as an antimalarial agents; HCQ is a
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24 105 hydroxylated derivative from CQ. HCQ has been used in several viral infections, for example, as
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26 106 replication inhibitor for the dengue virus, decreasing *in vitro* virus infection and promoting activation of
27
28 107 different immunological signal pathways¹⁵. It has also been used to treat patients infected with hepatitis
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31 108 C virus decreasing viral load, with minimal adverse effects reported¹⁶. HCQ has been reported to block
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33 109 viral infection by increasing the endosomal pH required for virus fusion to the cell, as well as interfere
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35 110 with SARS-CoV-2 cell receptors, through inhibition of ACE2 glycosylation receptor^{17 18 19 20}. HCQ has
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38 111 immunomodulatory effects; it inhibits production and release of pro-inflammatory cytokines, that are
39
40 112 associated with severe disease development^{21 22}. Recently, it has been reported that HCQ works as a
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42 113 autophagy inhibitor, interfering with viral infection and replication²³. There is recent evidence that HCQ
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45 114 could be used to treat COVID-19; studies in high-risk patients show that the use of HCQ was associated
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47 115 with a lower risk of intubation or death²⁴. Finally, a recent study showed that pre-treatment with HCQ has
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49 116 shown a better effect on antiviral activity¹⁹.

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3 118 Another pharmacological option to treat SARS-CoV-2 infection is Bromhexine (BHH). BHH modifies
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6 119 the composition of mucus, increases ciliary clearance and decreases coughing, improving respiratory
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8 120 symptoms. It has also been reported to enhance the effects of some antibiotics²⁵. The mechanism by which
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10 121 SARS-CoV-2 enters human cells depends on the ACE-2 receptor and the human transmembrane serine
11
12 122 protease (TMPRSS2), on which BHH has a specific inhibitory effect^{26 27}. BHH has been used to treat
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15 123 pneumonic damage in both lungs during early infection²⁸. BHH turns out to be an ideal candidate for
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17 124 SARS-CoV-2 treatment, since it has few contraindications, and its side effects are minimal, demonstrating
18
19 125 an extensive margin of pharmacological safety. BHH is widely available over the counter, and its low cost
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22 126 makes it an ideal therapeutic option.

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24 127 According to a letter published in the New England Journal of Medicine, of 77,262 patients infected by
25
26 128 SARS-CoV-2, 3387 (4.4%) were health workers¹². Of these, 23 have died from this disease. The
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29 129 prevalence of infections in health personnel is alarming since health services in first world countries have
30
31 130 been overwhelmed by this disease. In Italy, around 20% of health professionals had a SARS-CoV-2
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33 131 infection¹⁴. Faced with a highly contagious disease, the care of health workers, who are first line of contact
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35 132 and on whom the health system of each country depends, is essential. This research regarding the use of
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38 133 HCQ and BHH in health personnel will allow us to determine and compare the effectiveness of both
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40 134 interventions, which is of vital importance to clarify whether these treatments may prevent the appearance
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42 135 of infection in this population. Describing for the first time that HCQ and/or BHH could function for
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45 136 disease prevention, would allow us to provide prophylaxis to health professionals worldwide. Therefore,
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47 137 the use of HCQ and BHH in healthy health personnel exposed in patients with confirmed or suspected
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49 138 SARS-CoV-2 will significantly reduce infection.

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53 54 140 **METHODS AND ANALYSIS**

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141 **Study design**

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

146 **Participants**

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

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

160 *Inclusion criteria*

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

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164 ▶ Exposition or caring for patients with suspected or confirmed SARS-CoV-2 infection.

165 ▶ Normal electrocardiogram.

Exclusion criteria

167 ▶ Positive quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) test for
168 SARS-CoV-2 at the time of inclusion.

169 ▶ Panel of IgG or IgM antibodies positive for SARS-CoV-2 at the time of inclusion.

170 ▶ Development of respiratory symptoms suspicious of SARS-CoV-2 infection during the first 7
171 days after treatment is initiated, confirmed by qRT-PCR.

172 ▶ Health personnel with comorbidities such as diabetes, hypertension, autoimmune diseases (i.e.,
173 porphyria, psoriasis, systemic lupus erythematosus), obesity (defined as body mass index ≥ 30),
174 cardiovascular diseases, respiratory diseases (such as asthma, chronic bronchitis, idiopathic
175 pulmonary fibrosis).

176 ▶ History of allergies to any hydroxychloroquine or bromhexine related compound or
177 medication.

178 ▶ Use of immunosuppressors for any reason.

179 ▶ History of bone marrow transplant.

180 ▶ Known glucose-6-phosphate dehydrogenase deficiency.

181 ▶ Chronic kidney disease or glomerular filtration < 20 ml/min.

182 ▶ Use of other drugs with reported pharmacological interactions (i.e., digitalis, flecainide,
183 amiodarone, procainamide, or propafenone).

184 ▶ History of long QT syndrome.

185 ▶ Electrocardiogram with QTc > 500 msec.

186 ▶ Pregnant or breastfeeding personnel.

187 ▶ Epilepsy.

188 ▶ Known liver disease.

189 *Elimination criteria*

190 ▶ Personnel who decide to leave the study for any reason not related to adverse events.

191 ▶ Personnel with incomplete information on the primary outcome (qRT-PCR for SARS-CoV-2).

192 ▶ Personnel who are relocated to work in another institution.

193 ▶ Personnel who do not wish to perform consecutive sample analysis for SARS-CoV-2 at 30 and
194 60 days after the start of pharmacological treatment.

195 **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, PaO ₂ / FiO ₂ ratio < 250, Infiltrates multilobars, confusion / disorientation, uremia [BUN > 20 mg / dL], leukopenia [$< 4,000$], thrombocytopenia [$< 100,000$ platelets / mm ³], hypothermia [core temperature < 36°C], 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 \leq 13, systolic pressure \leq 100 mm Hg, or respiratory rate \geq 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 \geq 65 mm Hg and lactate \geq 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

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3 204 of volunteers will allow us to find a difference of 16% between groups with a power of 80%. To ensure
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5 205 that desired sample size is reached, all health workers involved in managing patients suspected or infected
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8 206 by SARS-CoV-2 will be invited personally and by institutional email.
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10 207 11 12 208 **Interventions**

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15 209 Interventions will consist of low doses of HCQ 200 mg tablets every 24 hours for 60 days, and BHH 8
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17 210 mg tablets every 8 hours for 60 days. Study groups will be defined as follows: 1) HCQ 200 mg every 24
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19 211 hours plus BHH placebo; 2) BHH 8 mg every 8 hours plus HCQ placebo; 3) HCQ 200 mg every 24 hours
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22 212 plus BHH 8 mg every 8 hours; and 4) HCQ placebo plus BHH placebo. Fabrication of both drugs and
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24 213 placebos will be provided to our centre by a hired laboratory. Both drugs will be provided to participants
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26 214 directly at the hospital by a researcher blinded to group assignment process. To ensure that the intervention
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28 215 is carried out, each participant will be asked to keep a written record of the days and time the medication
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31 216 was administered. This document will be reviewed weekly to verify that more than 50% adherence to
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33 217 treatment is maintained. Participants will be asked to record any symptoms related to the use of the
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35 218 medication, which will be reviewed by a researcher blinded to group assignment, weekly, or at the
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38 219 participants' request.
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42 221 If any of the participants present symptoms of SARS-CoV-2 infection after the first 14 days from the
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44 222 beginning of the intervention or positive qRT-PCR is present, the drug will be discontinued. If the
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47 223 participant presents adverse events related to the drugs that are severe or intolerable, treatment will be
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49 224 suspended. If the participants report an adherence of less than 50% of the medication, the intervention will
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52 225 be discontinued. Use of drugs that interact with HCQ or BHH such as flecainide, digitalis, amiodarone,
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54 226 procainamide or propafenone will be prohibited. If a participant has to use these drugs during the study
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3 227 period, they will be eliminated from the study. A free diet and outdoor activity will be allowed since these
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6 228 do not intervene with the implementation of the treatment or have interaction with the drugs used. Finally,
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8 229 incidence of adverse events such as nausea, vomiting, abdominal pain, rash, itchy skin, hair loss,
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10 230 lengthening of the QT interval in the electrocardiogram, corneal opacity, cardiac arrhythmias, heart failure
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12 231 and death will be determined.
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16 17 233 **Randomization and treatment allocation**

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19 234 Group randomization will be in a centralized and straightforward way using the Web program
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22 235 www.randomization.com. It will be carried out independently by a researcher blinded to inclusion criteria,
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24 236 delivery of medication, participant follow-up, results, statistical analysis, and writing of the final
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26 237 manuscript. Allocation will be established, for 280 participants in blocks of 70 assigned. The selection of
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29 238 health workers will be made regardless of the hospital shift, work schedule, or assigned area. If the desired
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31 239 sample size is not reached, the inclusion of personnel involved in the first line of care of other referral
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33 240 hospitals for patients with SARS-CoV-2 will be considered.
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35 241 An independent researcher will allocate patients to the desired groups. Envelopes will be correctly sealed
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38 242 by the pharmacy department, and will contain HCQ, BHH or placebos as previously mentioned. In those
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40 243 who do not require HCQ or BHH, the drug will be replaced by tablets identical in colour and taste but
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42 244 lacking the active substance. In this way, drugs used in both groups will be indistinguishable.
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47 246 Researcher A will recruit the participants and assess the inclusion criteria according to the serological,
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49 247 electrocardiographic, and biochemical results. Once included, volunteers will go to another office with
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52 248 researcher B, who will be blinded to the first procedure and the rest of the study. Researcher B will assign
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54 249 the groups independently, centrally, and through the use of the web program. This same researcher will
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3 250 be the one who makes the packages indistinguishable to the person providing the drugs to the participant.
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5 251 Researcher C will provide treatment in a sealed envelope or box to the participant in the order of
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8 252 assignment, without knowing each participant's study group. This researcher will also be blinded to the
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10 253 rest of the results. Participants will be blinded to the treatment they will receive. The researchers
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12 254 performing follow-up, researchers for result assessment, and the researcher who performs the statistical
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15 255 analysis will be blinded.
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19 257 Informed consent will be obtained only by researcher A. If researcher A is not available, the study
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22 258 administrator may obtain informed consent for participation. The informed consent will contain the
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24 259 authorization to participate in the study and the authorization for taking biological samples,
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26 260 electrocardiogram, and authorization to handle personal information. All participants will complete a
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29 261 written informed consent included on the first page of the questionnaire that requires permission to
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31 262 participate in the study. No candidate is required to participate in the study, and their participation is based
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33 263 on the agreement that they may withdraw at any time. All participants have the right to withdraw from the
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35 264 study if they feel uncomfortable answering a question or with a test to be performed. Also, no one,
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38 265 including the research team, will require a reason why the participant decides to leave the study.
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40 266 In order to protect participant confidentiality, each one will be assigned a participation number, and all
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42 267 biological samples, as well as medical history information, will be identified by the participant's initials,
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45 268 date of birth and participant number. Part of the confidentiality protection process will include data capture
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47 269 only by the researcher in charge of data capture (researcher D), who will be the same for all participants
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49 270 and the entire study. Secondarily, the study administrator may also enter data into the database if
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52 271 researcher D is unavailable.
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3 273 The study administrator will be blinded to allocation and results of the participants. However, the
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6 274 administrator will be the only one who will be able to reveal the group and treatment assignment in any
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8 275 of the cases: major adverse events such as cardiac arrhythmias, heart failure, major neurological
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10 276 abnormalities, atrial or ventricular fibrillation, kidney failure, or any adverse event related to
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12 277 pharmacological treatment that endangers the life or any organ of the participant's body. The objective of
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15 278 revealing the assignment by the study administrator will be to provide the participant of a timely treatment
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17 279 according to the drugs ingested.
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21 **Participant timeline and intervention** 22 281

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24 282 The inclusion of participants will be evaluated according to the eligibility criteria and by invitation.
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26 283 Volunteers who wish to participate in the study will be summoned the next day at a specialized office to
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29 284 carry out all the relevant studies to ensure the inclusion criteria. These include a medical history,
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31 285 anthropometric measurements such as weight and body mass index, electrocardiogram, hematic biometry,
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33 286 complete blood chemistry, and serological test for antibodies and qRT-PCR for SARS-CoV-2. Volunteers
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35 287 will be asked for information to contact them once the serological results are obtained.
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38 288 Once the results are obtained (approximately 3 days), personnel eligible to participate in the study will be
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40 289 contacted. They will meet in a particular office to speak with a researcher who will be in charge of carrying
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42 290 out the eligibility criteria and medical history checklist. This researcher will be different from the one who
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45 291 makes the assignment, who delivers the medicine and the one who evaluates the results and performs the
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47 292 statistical analysis. The assignment of the group of each participant will be performed, and the participant
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49 293 will not know the group they have been assigned. This information will be known for the researcher in
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52 294 charge, unrelated to the delivery of the treatment, results, or inclusion of the participant in the study. After
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54 295 the assignment, the volunteers will receive the assigned treatment at the pharmacy using a code in a sealed
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3 296 envelope assigned by the Web. Participants who meet the inclusion criteria and there is no reason for
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5 297 exclusion will proceed to the second phase of group assignment with researcher B, the next business day
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8 298 at a different time or office than researcher A.
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12 300 The group of researchers in charge of monitoring the participants, who will be blinded to the group
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14 301 assignment at all times, will be in charge of assessing each participant's adverse event and treatment
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16 302 adherence record weekly. These follow-up researchers will be available 24 hours a day throughout the
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18 303 week if participants experience undesirable adverse events that require urgent attention or that do not
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20 304 allow them to continue with drug treatment. If this situation happens, the researcher in charge of the
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22 305 follow-up will contact the study administrator to reveal to the treating physicians the treatment received
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24 306 by the participant.
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28 307 At the end of the first 30 days, a new qRT-PCR will be requested from each participant. The same action
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31 308 will be carried out 60 days after the start of treatment for both groups. After 60 days, the treatment will be
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33 309 suspended and the results of the qRT-PCR samples for SARS-CoV-2 will be evaluated.
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37 310 38 311 **Outcome measures**

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40 312 This study compares the efficacy of the use of HCQ and/or BHH in prophylactic doses for 60 days in
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42 313 healthy health personnel exposed to the first line of care in confirmed patients with suspected infection by
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44 314 SARS-CoV-2.
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49 316 *Primary endpoint*

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51 317 The primary endpoint will be the proportion of health personnel infected by SARS-CoV-2 after seven
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53 318 days of inclusion and up 60 days after starting treatment, in all groups. The infection will be diagnosed
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3 319 using qRT-PCR for SARS-CoV-2 after day 7 of treatment. The study period will be 60 days. The
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5 320 proportion of infected personnel will be evaluated using relative risk (RR) and absolute risk increase (ARI)
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8 321 with their respective 95% confidence intervals, in the established time. The disease-free period in the 60
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10 322 days will also be evaluated by analysing the cumulative incidence of healthy patients, and the presence of
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12 323 confirmed infection by qRT-PCR of SARS-CoV-2 will be the outcome. The censoring variable will be
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15 324 the discontinuation of treatment either due to death, adverse events, or any elimination criteria. Since there
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17 325 is the possibility of false positives and negatives with qRT-PCR, we will perform qualitative
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19 326 measurements of IgM and IgG with the Elecsys® Anti-SARS-CoV-2 test from Roche laboratories.
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26 329 *Secondary endpoints*

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28 330 The secondary endpoint will be the proportion of health personnel infected by SARS-CoV-2 after seven
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31 331 days of inclusion and up to 30 days after starting treatment, in all groups. The infection will be diagnosed
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33 332 using qRT-PCR for SARS-CoV-2 after day 7 of the start of treatment. The study period will be 30 days.
34
35 333 The proportion of infected personnel will be evaluated using RR and ARI with their respective 95%
36
37
38 334 confidence intervals, in the established time. The disease-free period in the 30 days will also be evaluated
39
40 335 by analyzing the cumulative incidence of healthy patients, and the presence of confirmed infection by
41
42 336 qRT-PCR of SARS-CoV-2 will be the outcome. The censoring variable will be the discontinuation of
43
44
45 337 treatment either due to death, adverse events, or any elimination criteria.
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48
49 339 Another secondary endpoint will be adverse events, defined as the presence of any of the following during
50
51 340 the study period: death, nausea, vomiting, abdominal pain, diarrhoea, rash, itchy skin, hair loss,
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53
54 341 lengthening of the QT interval in the electrocardiogram (>500msec), corneal opacity, cardiac arrhythmias,
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3 342 heart failure or kidney failure (renal clearance <20ml/min). The proportion of the compound of adverse
4
5 343 events between the groups will be analysed using RR and ARI for 60 days with their respective 95%
6
7
8 344 confidence intervals.
9

10 345
11
12 346 The efficacy of the treatment will be established as the proportion of volunteers infected with SARS-CoV-
13
14
15 347 2. This difference should be sufficient to avoid overlapping of the 95% confidence intervals. It will be
16
17 348 considered effective if the intervals do not overlap and ineffective if when comparing groups, they have a
18
19 349 proportion of infected whose confidence intervals overlap. This type of evaluation will allow an adequate
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21
22 350 understanding of the efficacy of the treatment in both groups.
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26 352 **Handling and storage of data and documents**

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28
29 353 Before the start of the study, the researchers in charge of the recruitment, assignment, and delivery of
30
31 354 drugs will be trained to perform the task assigned to them at least 3 days before the start of the study.
32

33 355 Researcher A will assess the eligibility criteria of potential participants and perform a detailed clinical
34
35 356 examination to assess whether they can participate in the study. The data that will be collected initially
36
37
38 357 will be the following:
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40 358
41

- 42 359 ► Medical history (includes personal data): study identifier number, history number, name, date of
43
44
45 360 birth, gender, occupation, marital status, nationality, current residence, degree of studies (primary,
46
47 361 secondary, upper secondary, bachelor`s degree, postgraduate), hospital service to which they
48
49 362 belong and the number of hours worked per week.
50
- 51
52 363 ► Personal history: alcohol intake (yes/no; how many glasses of beer or alcoholic beverages do you
53
54 364 consume per week), smoking habit (yes/ no; and number of cigarettes per day), drug use (yes/no),
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3 365 diet per week (dietary restrictions and number of meals per day) and number of hours of sleep per
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6 366 day.
- 7
8 367 ► Gynaecological history (in women): Number of pregnancies, number of live children, menarche,
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10 368 menopause.
- 11
12 369 ► History of respiratory disease, history of gastrointestinal disease, nephrological, neurological,
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14
15 370 haematological, cardiovascular, allergies.
- 16
17 371 ► Genetic family history, such as hypertension, diabetes, heart disease, kidney disease.
- 18
19 372 ► Physical examination: blood pressure, heart rate, respiratory rate, temperature, weight, height,
20
21
22 373 body mass index, skin lesions, head and neck inspection, respiratory inspection (chest symmetry,
23
24 374 lung expansion, palpation of the bases and preserved vertices, lung percussion, auscultation for
25
26 375 lung murmur, breath sounds). Cardiovascular inspection (palpation of the fifth intercostal space,
27
28
29 376 auscultation of heart sounds, pulses that are palpable and symmetrical), abdominal inspection
30
31 377 (palpation, percussion and auscultation of peristaltic sounds), neurological evaluation (Glasgow,
32
33 378 active motility, passive motility, reflex motility, cranial nerves, sensitivity).
- 34
35 379 ► Hematic biometry: haematocrit, leukocytes, segmented (%), lymphocytes (%), monocytes (%),
36
37
38 380 mean corpuscular volume, platelets.
- 39
40 381 ► Blood chemistry: glycaemia, urea, creatinine, sodium, potassium, chlorine, aspartate transaminase,
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42 382 alanine transaminase, alkaline phosphatase, total bilirubin.
- 43
44
45 383 ► Muscle enzymes.
- 46
47 384 ► Clotting times: thrombin time, prothrombin time, international normalized ratio.
- 48
49 385 ► Electrocardiogram: rhythm, heart rate, heart axis, evaluation of P wave, PR interval, duration of
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51
52 386 QRS, QT interval, time of T wave. The electrocardiogram will be performed using an instrument
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54 387 calibrated and validated for its use internationally, weekly.
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3 388 ► Molecular test results for IgG and IgM antibodies:
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6 389 ○ The FDA approved product called Cellex qSARS-CoV-2 IgG/IgM Rapid Test will be used
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8 390 for serological determination. The device cassette, sample, and buffer solution must be at
9
10 391 room temperature. The sample (10 µL) is transferred to the center of the sample well. After
11
12 392 the sample well is free of liquid, two drops of sample diluent are added. After fifteen to
13
14
15 393 twenty minutes, read the test results. Results should not be read after twenty minutes.
16
17 394 ○ A positive IgM result occurs when a coloured band appears on the M test line (M) and the
18
19 395 control line (C) and indicates that IgM against SARS-CoV-2 is present.
20
21
22 396 ○ A positive IgG result occurs when a coloured band appears on the G test line (G) and the
23
24 397 control line (C) and indicates that IgG against SARS-CoV-2 is present.
25
26 398 ○ A positive result for IgM and IgG occurs when coloured bands occur both M and G, as
27
28
29 399 well as C.
30
31 400 ○ A negative result occurs when a coloured band appears in C only and indicates that IgM
32
33 401 and IgG antibodies against SARS-CoV-2 were not detected.
34
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36 402 ○ An invalid result occurs when a colour band is not produced in C, and the test must be
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38 403 repeated.
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40 404 ► Official qRT-PCR results (carried out by INCMNSZ)
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45 406 All this information will be collected in a pre-established medical history questionnaire for each potential
46
47 407 participant. The information obtained from the weekly assessment of adverse events, and the results of
48
49 408 the qRT-PCR for SARS-CoV-2 at 30 and 60 days after starting treatment will be entered into an online
50
51
52 409 database. In order to ensure the quality of the data collection, the database will be built in CASTOR, a
53
54 410 database on the Web that allows entering all the pre-defined data for each participant, thus reducing human
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3 411 error. This information will be stored on a server in the United States of America and can only be accessed
4
5 412 by the study's administrator. The data may only be entered by a researcher in charge of collecting the data
6
7
8 413 sheets and emptying them.
9

10 414

11 12 415 **Monitoring and quality assurance** 13

14
15 416 Every possible adverse event will be noted daily by the participants in the agenda that will be delivered to
16
17 417 them. This agenda will be evaluated weekly by the researcher in charge of monitoring the participants
18
19 418 (who will be blinded to group assignment). In case of unbearable adverse events for the participants or
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21 419 that put their health at risk, an open line will be available 24 hours a day with direct communication to the
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24 420 researcher in charge of monitoring the study to report any event that requires hospitalization or immediate
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26 421 evaluation at the hospital. All participants with adverse events that put their life or health at risk may be
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28 422 urgently assessed by personnel from both INCMNSZ or INR-LGII, if possible, by the same staff within
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30
31 423 which are part of the study. Patient follow-up investigator will immediately contact the study administrator
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33 424 to disclose the participant's assignment to treating physicians at that institution, but the assignment will
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35 425 never be disclosed to other investigators related to the study. All the study expenses and/or attention of
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38 426 collateral effects will be covered by the current cost of the financing SECTEI/061/2020.
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40 427 Auditing will be carried out weekly, assessing adverse events, capturing data in the corresponding
41
42 428 datasheets by the study administrator. Likewise, the data entered in the CASTOR web base will be valued
43
44
45 429 to validate its quality. The paper data sheets must be kept in a special office dedicated to the study in
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47 430 folders separated by volunteers with the informed consent of each participant, the data of the medical
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49 431 history, laboratory results, eligibility criteria, adverse event sheet and results, molecular tests, as well as
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52 432 electrocardiogram. The letter of revocation of informed consent will also be protected if required. As part
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54 433 of the audit, an interim analysis will be carried out 30 days after the study starts to assess the possible
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3 434 adverse effects and whether these outweigh the potential benefits of the intervention. In the adverse event
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6 435 outweigh the potential benefits, termination of the study will be assessed. The approval of the research
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8 436 ethics committee of the INR-LGII of Mexico has been obtained.
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10 437 11 12 438 **Statistical analysis** 13

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15 439 Data analysis will be carried out by intention to treat, which means that each participant will be analysed
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17 440 according to the group assigned regardless of whether they modified their treatment. The study variables
18
19 441 will be divided according to the allocation group. The distribution of continuous variables will be assessed
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21
22 442 using the Shapiro Wilk test, Skewness and kurtosis. The variables of normal distribution will be compared
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24 443 using the Student's *t*-test, non-parametric distribution using the Mann-Whitney U test. Categorical
25
26 444 variables will be evaluated using the Chi-squared test.
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31 446 The primary objective will be expressed in number and proportion for each group. The RR will be obtained
32
33 447 as the division between the proportion of primary outcomes in the intervention group(s) by the proportion
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35 448 of primary outcomes in the double placebo group. Adjusted risk ratios (aRR) will be obtained using a log-
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37
38 449 binomial regression, adjusting for age and gender as pre-specified confounding variables. It will be
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40 450 expressed as RR with its respective 95% confidence interval for the initial time, which is 60 days.
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42 451 Likewise, the result will be expressed as absolute risk, which will be derived from the proportion of the
43
44
45 452 primary outcome in the intervention group minus the proportion of the primary outcome in the control
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47 453 group. Secondly, the primary objective will be analysed with the non-parametric estimate of the
48
49 454 survival and risk function using Kaplan-Meier curves for 60 days according to the allocation group. The
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51
52 455 primary endpoint will be SARS-CoV-2 infection within the 60-day period, and the silencing variable will
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54 456 be dropping out of the study for any reason. The comparison of the survival curves between both groups
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3 457 will be carried out using the log-rank test. To adjust the primary objective to possible confounders such
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6 458 as age, gender, service in which the participant works, body mass index, etc. Multiple regression will be
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8 459 performed using the Cox model to determine the adjusted primary endpoint hazard ratio.
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12 461 For secondary outcomes such as the analysis at 30 days, the same statistical analysis expressed in RR and
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15 462 absolute risk will be used. Survival analysis will be used for the primary endpoint only. An interim
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17 463 statistical analysis will be performed 30 days after the study starts to assess possible adverse effects and
18
19 464 the efficacy of the intervention. The study administrator will be the only one with access to the data. For
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21
22 465 the interim analysis and the final analysis, the administrator will export the data to Excel format to be
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24 466 analysed by the study statistician blinded to the assignment of groups, participants, or results.
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29 468 **Adverse events, serious adverse events and suspected unexpected serious adverse reactions**

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31 469 By requiring the use of drugs, the participant will be exposed to risks inherent to the drug used, ranging
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33 470 from mild to severe or death. Any unexpected risks that may occur during the study will be immediately
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35 471 explained to the participants and the ethics committee. Any adverse event will be compiled and will not
36
37
38 472 be disclosed under any condition to anyone other than the study administrator, treating physicians in case
39
40 473 of severe events, and the ethics committee. The results will be completely anonymous concerning the
41
42 474 names of the participants. The results will be compiled and reported as combined collective data.
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47 476 *Patient and public involvement*
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49 477 Patients were not involved in the development of this research. However, the results of the study will be
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52 478 communicated to the study participants by sending the end product (published article) to the provided
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54 479 email address.
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3 **480 ETHICS, DISSEMINATION AND SAFETY MONITORING**
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6 481 In case of adverse events or complications derived from the study, participants will be assured attention
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8 482 by the staff of the INCMNSZ in an enclosure that ensures the safety of the participant, not subjecting
9
10 483 volunteers to a higher risk of contamination. This care will be extended until adverse events are resolved.
11
12 484 In case of no adverse events during the study, medical attention will be extended at the aforementioned
13
14
15 485 institute until 15 days after the end of the study.
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17 486
18
19 487 This protocol has been approved by the local medical ethical review committee at the INR-LGII with the
20
21
22 488 internal number INRLGII/25/20, and by the Federal Commission for Protection against Sanitary Risks (in
23
24 489 Spanish, Comisión Federal para la Protección contra Riesgos Sanitarios, COFEPRIS), approval number
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26 490 203300410A0058/2020.
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31 492 The study results will be published in journals of worldwide impact affiliated with the Journal Citation
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33 493 Reports. Likewise, the results of the study will be disseminated in national and international media,
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35 494 exposed in international and national congresses, communicated to CONACYT, and recorded in
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37
38 495 Clinicaltrials.gov according to the study identifier number. The help of non-profit organizations will be
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40 496 sought to disseminate the results of the investigation to interest groups.
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45 498 The complete protocol will be published on Clinicaltrials.gov and the OSF - Center for Open Science
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47 499 platform <https://osf.io/>. Where a DOI will be assigned, and the amendments made to the original protocol
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49 500 will be assessed.
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3 502 Amendments to the protocol may be made before the start of the study and during the study. Any
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5 503 amendment to the protocol will be clarified and posted on Clinicaltrials.gov under the same identifier as
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7
8 504 this study. Likewise, any amendment will be sent to the ethics committee of the same hospital.
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11 12 13506 **AUTHOR CONTRIBUTIONS**

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15507 JGM is the lead study investigator, developed the study concepts and design, and wrote the manuscript by
16
17508 adapting the original study protocol for publication, subsequent reviews and amendments. EJHL, KMM
18
19
20509 and AAA contributed to the development and refining of the protocol, writing of manuscript and
21
22510 subsequent review. RJMP provided advanced methodological and statistical input, and contributed to the
23
24511 study design and subsequent amendments. RFC, TCH, PSSB, RAG and NML reviewed, commented and
25
26
27512 informed methodology, development and writing of the protocol.
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39 40 41518 **COMPETING INTERESTS STATEMENT**

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43519 None of the authors have conflict of interests, commercial agreements, or receive financial fees or
44
45
46520 compensation from any commercial or pharmaceutical company.
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49 50 51522 **ETHICS APPROVAL**

52
53523 This protocol has been approved by the local medical ethical review committee at the INR-LGII with the
54
55524 internal number INRLGII/25/20. Definitions of Research Risk Regulation of the General Health Law on
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3 525 Research for Health (in Spanish, Reglamento de la Ley General de Salud en Materia de Investigación para
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5 526 la Salud) <http://www.inr.gob.mx/Descargas/Investigacion/Reglamentos-LGS-Materia-Investigacion->
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8 527 Salud.pdf. ARTICLE 17; and by Federal Commission for Protection against Sanitary Risks (in Spanish,
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10 528 Comisión Federal para la Protección contra Riesgos Sanitarios, COFEPRIS), approval number
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16 17 531 **PROVENANCE AND PEER REVIEW**

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23 24 534 **ORCID ID**

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27 535 Julio Granados-Montiel <https://orcid.org/0000-0002-0611-64>
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For peer review only

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).

Journal:	<i>BMJ Open</i>
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Article Type:	Protocol
Date Submitted by the Author:	15-Feb-2021
Complete List of Authors:	<p>GRANADOS-MONTIEL, JULIO; Instituto Nacional de Rehabilitacion Luis Guillermo Ibarra Ibarra, Tissue Engineering and Regenerative Medicine Unit</p> <p>Hazan-Lasri, Eric; Instituto Nacional de Rehabilitacion Luis Guillermo Ibarra Ibarra, Division of Traumatology, Emergencies and Bone Infections</p> <p>Franco-Cendejas, Rafael; Instituto Nacional de Rehabilitacion Luis Guillermo Ibarra Ibarra, Infectology Laboratory</p> <p>Chavez-Heres, Tatiana; Instituto Nacional de Rehabilitacion Luis Guillermo Ibarra Ibarra, Service, Hospital Epidemiological Surveillance Unit</p> <p>Silva-Bermudez, Phaedra; Instituto Nacional de Rehabilitacion Luis Guillermo Ibarra Ibarra, Tissue Engineering and Regenerative Medicine Unit</p> <p>Aguilar-Gaytan, Rocio; Instituto Nacional de Rehabilitacion Luis Guillermo Ibarra Ibarra, Tissue Engineering and Regenerative Medicine Unit</p> <p>Manzano-Leon, Natalia; Instituto Nacional de Cancerologia, Basic Division Research</p> <p>Méndez-Maldonado, Karla; Instituto Nacional de Rehabilitacion Luis Guillermo Ibarra Ibarra, Tissue Engineering and Regenerative Medicine Unit</p> <p>Alvarez-Arce, Alejandro; Instituto Nacional de Rehabilitación Luis Guillermo Ibarra Ibarra, Tissue Engineering and Regenerative Medicine Unit</p> <p>Martinez-Portilla, Raigam ; National Institute of Perinatology, Clinical research division</p>
Primary Subject Heading:	Health services research
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Keywords:	INFECTIOUS DISEASES, PREVENTIVE MEDICINE, Clinical trials < THERAPEUTICS, COVID-19, CLINICAL PHARMACOLOGY

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3 1 **New prophylaxis regimen for SARS-CoV-2 infection in health**
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5 2 **professionals with low doses of Hydroxychloroquine and**
6
7 3 **Bromhexine: a randomized, double-blind placebo clinical trial**
8
9 4 **(ELEVATE Trial).**
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21 34 Keywords: prophylaxis, SARS-CoV-2, COVID-19, health workers,
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23 35 hydroxychloroquine, bromhexine.
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29 38 Word count: 5,851
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14 54 **ABSTRACT**

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17 55 **Introduction:** SARS-CoV-2 infection in Mexico has caused ~1
18
19 56 million confirmed cases; around 20-25% of health workers will
20
21 57 be infected by the virus at their workplace, with approximately
22
23 58 4.4% of mortality. High infectivity of SARS-CoV-2 is related
24
25 59 with cell entry mechanism, through the angiotensin-converting
26
27 60 enzyme (ACE) receptor. SARS-CoV-2 requires transmembrane
28
29 61 protease serine 2 (TMPRSS2) to cleave its spike glycoprotein
30
31 62 and ensure fusion of host cell and virus membrane. We propose
32
33 63 studying prophylactic treatment with hydroxychloroquine (HCQ)
34
35 64 and bromhexine (BHH), which have been shown to be effective in
36
37 65 preventing SARS-CoV-2 infection progression when administered
38
39 66 in early stages. The aim of this study is to assess the efficacy
40
41 67 of HCQ and BHH as prophylactic treatments for SARS-CoV-2
42
43 68 infection in healthy health workers exposed to the virus.

44
45
46 69 **Methods and analysis:** Double-blind randomized clinical trial,
47
48
49 70 with parallel allocation at a 1:1 ratio with placebo, of low
50
51 71 doses of HCQ plus BHH, for 60 days. Study groups will be defined
52
53 72 as follows: 1) HCQ 200mg/d + BHH 8mg/8h vs 2) HCQ placebo plus
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3 73 BHH placebo. Primary endpoint will be efficacy of both
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5 74 interventions for the prevention of SARS-CoV-2 infection,
6
7 75 determined by the risk ratio (RR) of infected personnel and
8
9 76 the absolute risk. At least a 16% reduction in absolute risk
10
11 77 is expected between the intervention and placebo groups; a
12
13 78 minimum of 20% infection is expected in the placebo group. The
14
15 79 sample size calculation estimated a total of 140 patients
16
17 80 assigned: two groups of 70 participants each.

21 **Ethics and dissemination:** This protocol has been approved by
22
23 82 the local Medical Ethics Committee (National Institute of
24
25 83 Rehabilitation 'Luis Guillermo Ibarra Ibarra', approval number
26
27 84 INRLGII/25/20) and by the Federal Commission for Protection
28
29 85 against Sanitary Risks (COFEPRIS, approval number
30
31 86 203300410A0058/2020). The results of the study will be
32
33 87 submitted for publication in peer-reviewed journals and
34
35 88 disseminated through conferences.

39 **Trial registration number 2a:** NCT04340349.

41 90 2b: NA

43 91 Protocol version: #3

45 92

48 93 **STRENGTHS AND LIMITATIONS OF THIS STUDY**

50 94 *Strengths*

52
53 95 ► This is a double-blind randomized single-centre clinical
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55 96 trial, involving low doses of hydroxychloroquine and
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1
2
3 97 bromhexine, adequately powered to provide clinically
4
5 98 relevant information regarding prophylactic treatment for
6
7 99 SARS-CoV-2 infection in health care personnel.
8
9

10 ▶ This study will include 140 participants who are health
11
12 101 workers exposed to SARS-CoV-2 patients with suspected or
13
14 102 confirmed infection, with short term follow-up (60 days).
15

16
17 103 ▶ A study of prophylactic treatment in this population is
18
19 104 of great value and could provide the basis for protecting
20
21 105 medical personnel around the world.
22

23
24 106 ▶ Bromhexine have minimal side effects and are commercially
25
26 107 available worldwide; findings could be applied in a timely
27
28 108 fashion in different regions.
29

30 109 *Limitations*

31
32
33 110 ▶ Long-term use of hydroxychloroquine can cause heart rhythm
34
35 111 problems

36
37 112 ▶ For the moment, people who are not candidates to receive
38
39 113 the vaccine, due to chronic diseases or severe allergies,
40
41 114 will not be included
42
43

44 115 **INTRODUCTION**

45
46 116 In Mexico, up to February 2021, have been produced more than
47
48 117 1.9 millions confirmed cases and ~170,000 deaths have
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50 118 arisen[1]. The age group ranging between 30 and 79 years is
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52 119 the most highly affected, where 81% present mild symptoms, 14%
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3 120 severe and 5% critical, requiring intensive care unit
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5 121 management.

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7 122 SARS-CoV-2 is a single-stranded RNA virion, member of the
8
9 123 *Betacoronavirus* genus [2]. SARS-CoV-2 has an incubation period
10
11 124 between 3 to 10 days, with different incubation periods related
12
13 125 with different clinical symptoms [3,4] . It is transmitted
14
15 126 through respiratory droplets from infected humans and through
16
17 127 contact with contaminated fomites and aerosols; moreover, even
18
19 128 asymptomatic persons in close contact can transmit the disease
20
21 129 [5]. The mechanism through which the virus infects the
22
23 130 respiratory cell is due to the angiotensin-converting enzyme
24
25 131 protein 2 (ACE-2). This receptor is found in multiple tissues
26
27 132 such as the oral cavity, brain, kidneys, intestine, and
28
29 133 placenta [6-8].

30
31 134 Health personnel is not exempt from contracting the disease.
32
33 135 In China, it was reported that 3.5-4.4% of the infected
34
35 136 population belonged to this group, and 14.8% presented
36
37 137 characteristics of severity or critical illness [4,9,10].
38
39 138 Italy, around 20% of healthcare professionals became infected
40
41 139 [11] ; mean age of health workers who died was 55 years (range
42
43 140 of 29-72 years) and mean period from hospital admission to
44
45 141 death was 19 days, (range 1-47 days) [9].

46
47 142 Treatment of the SARS-Cov-2 infection has led different
48
49 143 research groups to work on the development of vaccines.
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3 144 However, the use of vaccines can be a challenge. Early trials
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5 145 have shown minimal immune protection or long-term protection
6
7 146 is low. On the other hand, because the virus is RNA and the
8
9 147 mutation rate is high, we can expect new variants that reduce
10
11 148 or nullify the effectiveness of vaccines. Therefore, it is not
12
13 149 known if the vaccines that are now in the phase end of the
14
15 150 clinical study and those that are administered will work with
16
17 151 the same efficacy for the SARS-CoV-2 virus that gave rise to
18
19 152 Covid-19. On the other hand, around the world there are groups
20
21 153 of people who are against vaccination, or people that have
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23 154 severe allergies, as well as populations that will take much
24
25 155 longer to reach the moment when they can acquire the vaccine,
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27 156 so it is extremely necessary that people who do not vaccinate
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29 157 by choice, by disease or by the lack of the vaccine, have an
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31 158 alternative to avoid infection and avoid the spread of the
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33 159 virus.

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35 160 Therefore, it is important to develop a pharmacological
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37 161 strategy that allows the use of prophylactic drugs for the
38
39 162 prevention of SARS-CoV-2 infection.

40
41 163 Chloroquine (CQ) and Hydroxychloroquine (HCQ) are known as an
42
43 164 antimalarial agent; HCQ is a hydroxylated derivative from CQ.
44
45 165 CQ and HCQ have gained attention as possible therapies in
46
47 166 Covid-19 disease. In overdose, both drugs can cause severe,
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49 167 potentially life-threatening effects as visual disturbances,
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3 168 corneal opacities, irreversible retinopathy can occur with
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5 169 cumulative doses exceeding 100 grams. When lower daily doses
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7 170 (250 mg are used) retinopathy may not occur after many years
8
9 171 of treatment [12]. It has been reported in Cynomolgus Macaques
10
11 172 that the maximum concentration of HCQ was 293.33ng/mL in blood
12
13 173 and 36.90 ng/mL in plasma after single dose of 3 mg/kg [13].
14
15 174 HCQ has been used in several viral infections, for example, as
16
17 175 replication inhibitor for the dengue virus, decreasing in vitro
18
19 176 virus infection and promoting activation of different
20
21 177 immunological signal pathways [14]. It has also been used to
22
23 178 treat patients infected with hepatitis C virus decreasing viral
24
25 179 load, with minimal adverse effects reported [15]. HCQ has been
26
27 180 reported to block viral infection by increasing the endosomal
28
29 181 pH required for virus fusion to the cell, as well as interfere
30
31 182 with SARS-CoV-2 cell receptors, through inhibition of ACE2
32
33 183 glycosylation receptor [16-19]. HCQ has immunomodulatory
34
35 184 effects; it inhibits production and release of pro-inflammatory
36
37 185 cytokines, that are associated with severe disease development
38
39 186 [20,21] . Recently, it has been reported that HCQ works as a
40
41 187 autophagy inhibitor, interfering with viral infection and
42
43 188 replication [22]. There is recent evidence that HCQ could be
44
45 189 used to treat COVID-19; studies in high-risk patients show that
46
47 190 the use of HCQ was associated with a lower risk of intubation
48
49 191 or death [23]. Recent study showed that pre-treatment with HCQ
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3 192 has shown a better effect on antiviral activity [18] and it
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5 193 has been reported that loading doses of 1600 mg HCQ followed
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7 194 by 600 mg daily doses are needed have a relevant effect to
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9 195 SARS-CoV-2 inhibition within 72 hours in 60% of COVID-19
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11 196 patients [24]. Finally, a study where evaluated the antiviral
12
13 197 mechanisms of CQ and the adverse effects, repositioned the CQ
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15 198 to have more efficacy when used as a prophylactic treatment
16
17 199 rather than as a therapeutic [25]. On the other hand, CQ and
18
19 200 HCQ has been reported to have various adverse effects, the CQ
20
21 201 being the most toxic in overdose. However, it was recently
22
23 202 published that *in vivo* trials are lacking to determine whether
24
25 203 this drug is useful as a prophylactic treatment against SARS-
26
27 204 Cov-2 [26]. Based on the above, do more studies will be
28
29 205 important to determine its effectiveness at low doses (<250
30
31 206 mg) as a prophylactic treatment.
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37 207 Another pharmacological option to treat SARS-CoV-2 infection
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39 208 is Bromhexine (BHH). BHH modifies the composition of mucus,
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41 209 increases ciliary clearance and decreases coughing, improving
42
43 210 respiratory symptoms. It has also been reported to enhance the
44
45 211 effects of some antibiotics [27]. The mechanism by which SARS-
46
47 212 CoV-2 enters human cells depends on the ACE-2 receptor and the
48
49 213 human transmembrane serine protease (TMPRSS2), on which BHH
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51 214 has a specific inhibitory effect [28,29]. BHH has been used to
52
53 215 treat pneumonic damage in both lungs during early infection
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3 216 [30]. BHH turns out to be an ideal candidate for SARS-CoV-2
4
5 217 treatment, since it has few contraindications, and its side
6
7 218 effects are minimal, demonstrating an extensive margin of
8
9 219 pharmacological safety. BHH is widely available over the
10
11 220 counter, and its low cost makes it an ideal therapeutic option.
12
13 221 According to a letter published in the New England Journal of
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15 222 Medicine, of 77,262 patients infected by SARS-CoV-2, 3387
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17 223 (4.4%) were health workers [9]. Of these, 23 have died from
18
19 224 this disease. The prevalence of infections in health personnel
20
21 225 is alarming since health services in first world countries have
22
23 226 been overwhelmed by this disease. In Italy, around 20% of
24
25 227 health professionals had a SARS-CoV-2 infection [11]. Faced
26
27 228 with a highly contagious disease, the care of health workers,
28
29 229 who are first line of contact and on whom the health system of
30
31 230 each country depends, is essential. This research regarding
32
33 231 the use of HCQ and BHH in health personnel will allow us to
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35 232 determine and compare the effectiveness of both interventions,
36
37 233 which is of vital importance to clarify whether these
38
39 234 treatments may prevent the appearance of infection in this
40
41 235 population. Describing for the first time that HCQ plus BHH
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43 236 could function for disease prevention, would allow us to
44
45 237 provide prophylaxis to health professionals worldwide.
46
47 238 Therefore, the use of HCQ and BHH in healthy health personnel
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239 exposed to patients with confirmed or suspected SARS-CoV-2 will
240 significantly reduce infection.

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246 **METHODS AND ANALYSIS**

247 **Study design**

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

253

254

255 **Participants**

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

1
2
3 263 Phase 3). In the latter, it is assumed that every person who
4
5 264 enters a hospital is a potentially infected carrier; currently
6
7 265 our centre is in Phase 3. Likewise, health personnel who work
8
9 266 at the “Instituto Nacional de Ciencias Médicas y Nutrición,
10
11 267 Salvador Zubirán” (INCMNSZ), which is a COVID-19 designated
12
13 268 tertiary centre, and who meet inclusion criteria of the
14
15 269 protocol will be invited to participate in the study.

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17
18 270 Inclusion of participants will be assessed according to the
19
20 271 eligibility criteria. Table 1 shows the classification and
21
22 272 characteristics of study variables. Continuous variables will
23
24 273 be assessed for normality. Variables with a normal distribution
25
26 274 will be compared using Student's t-test, non-parametric
27
28 275 variables using the Mann-Whitney U-test. Categorical variables
29
30 276 will be evaluated using the Chi-squared test.

31
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33
34
35 277 *Inclusion criteria*

- 36
37 278 ▶ Health personnel working at INR LGII or INCMNSZ who wish
38
39 279 to participate in the study and sign the informed consent.
40
41 280 ▶ Over 18 and under 60 years of age, both genders.
42
43 281 ▶ Contacting with suspected or confirmed SARS-CoV-2
44
45 282 infection.
46
47 283 ▶ Normal electrocardiogram.

48
49
50 284 *Exclusion criteria*

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2
3 285 ▶ Positive quantitative reverse transcriptase-polymerase
4
5 286 chain reaction (qRT-PCR) test for SARS-CoV-2 at the time
6
7 287 of inclusion.
8
9
10 288 ▶ Panel of IgG or IgM antibodies positive for SARS-CoV-2 at
11
12 289 the time of inclusion.
13
14 290 ▶ Development of respiratory symptoms suspicious of SARS-
15
16 291 CoV-2 infection during the first 7 days after treatment
17
18 292 is initiated, confirmed by qRT-PCR and IgG or IgM
19
20 293 antibodies postiver for SARS-CoV-2.
21
22
23 294 ▶ Health personnel with comorbidities such as diabetes,
24
25 295 hypertension, autoimmune diseases (i.e., porphyria,
26
27 296 psoriasis, systemic lupus erythematosus), obesity
28
29 297 (defined as body mass index ≥ 30), cardiovascular diseases,
30
31 298 respiratory diseases (such as asthma, chronic bronchitis,
32
33 299 idiopathic pulmonary fibrosis).
34
35
36 300 ▶ History of allergies to any hydroxychloroquine or
37
38 301 bromhexine related compound or medication.
39
40
41 302 ▶ Use of immunosuppressors for any reason.
42
43
44 303 ▶ History of bone marrow transplant.
45
46
47 304 ▶ Known glucose-6-phosphate dehydrogenase deficiency.
48
49 305 ▶ Chronic kidney disease or glomerular filtration
50
51 306 < 20 ml/min.
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3 307 ▶ Use of other drugs with reported pharmacological
4
5 308 interactions (i.e., digitalis, flecainide, amiodarone,
6
7 309 procainamide, or propafenone).
8
9
10 310 ▶ History of long QT syndrome.
11
12 311 ▶ Electrocardiogram with QTc>500 msec.
13
14 312 ▶ Pregnant or breastfeeding personnel.
15
16 313 ▶ Epilepsy.
17
18 314 ▶ Known liver disease.
19
20 315 ▶ Personnel who have received the Covid-19 vaccine
21
22
23

24 316 *Elimination criteria*

- 25
26 317 ▶ Personnel who decide to leave the study for any reason
27
28 318 not related to adverse events.
29
30 319 ▶ Personnel with incomplete information on the primary
31
32 320 outcome (qRT-PCR for SARS-CoV-2).
33
34 321 ▶ Personnel who are relocated to work in another
35
36 322 institution.
37
38 323 ▶ Personnel who do not wish to participate in the study
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45 325 **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, PaO ₂ / FiO ₂ ratio <250, infiltrates multilobars, confusion / disorientation, uremia [BUN > 20 mg / dL], leukopenia [$<4,000$], thrombocytopenia [$<100,000$ platelets / mm ³], hypothermia [core temperature <36°C], 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

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327 **Sample size calculation**

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2
3 328 According to the study by Remuzzi A et al. [11], the proportion
4
5 329 of healthcare workers infected with SARS-CoV-2 and confirmed
6
7 330 by RT-PCR was 20%. Taking this 20% as our null hypothesis, we
8
9 331 estimate that the proportion of infections in the intervention
10
11 332 group will be 4%. Using a two-tailed test, with a type I error
12
13 333 of 0.05, a power of 90%, and taking into account a loss of 10%
14
15 334 of participants for each group, we estimate that a total of
16
17 335 214 participants will be required, distributed in parallel
18
19 336 groups (1:1) of 107 each. This number of volunteers will allow
20
21 337 us to find a difference of 16% between groups with a power of
22
23 338 90% and an attrition of 20%. To ensure that desired sample
24
25 339 size is reached, all health workers involved in managing
26
27 340 patients suspected or infected by SARS-CoV-2 will be invited
28
29 341 personally and by institutional email.
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37 343 **Interventions**

38
39 344 Interventions will consist of low doses of HCQ 200 mg tablets
40
41 345 every 24 hours for 60 days plus BHH 8 mg tablets every 8
42
43 346 hours for 60 days. Study groups will be defined as follows: 1)
44
45 347 HCQ plus BHH vs placebo for both drugs. Fabrication of both
46
47 348 drugs and placebos will be provided to our centre by a hired
48
49 349 laboratory. Both drugs will be provided to participants
50
51 350 directly at the hospital by a researcher blinded to group
52
53 351 assignment process. To ensure that the intervention is carried
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3 352 out, each participant will be asked to keep a written record
4
5 353 of the days and time the medication was administered. This
6
7 354 document will be reviewed weekly to verify that more than 50%
8
9 355 adherence to treatment is maintained. Participants will be
10
11 356 asked to record any symptoms related to the use of the
12
13 357 medication, which will be reviewed by a researcher blinded to
14
15 358 group assignment, weekly, or at the participants' request.
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21 360 If any of the participants present symptoms of SARS-CoV-2
22
23 361 infection after the first 14 days from the beginning of the
24
25 362 intervention or positive qRT-PCR is present, the drug will not
26
27 363 be discontinued. If the participant presents adverse events
28
29 364 related to the drugs that are severe or intolerable, treatment
30
31 365 will not be suspended. If the participants report an adherence
32
33 366 of less than 50% of the medication, the intervention will not
34
35 367 be discontinued to avoid imbalances between groups. Use of
36
37 368 drugs that interact with HCQ or BHH such as flecainide,
38
39 369 digitalis, amiodarone, procainamide or propafenone will be
40
41 370 prohibited. If a participant has to use these drugs during the
42
43 371 study period, they will be eliminated from the study. A free
44
45 372 diet and outdoor activity will be allowed since these do not
46
47 373 intervene with the implementation of the treatment or have
48
49 374 interaction with the drugs used. Finally, incidence of adverse
50
51 375 events such as nausea, vomiting, abdominal pain, rash, itchy
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3 376 skin, hair loss, lengthening of the QT interval in the
4
5 377 electrocardiogram, corneal opacity, cardiac arrhythmias, heart
6
7 378 failure and death will be determined.
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10 379

11
12 380 **Randomization and treatment allocation**
13

14 381 Group randomization will be in a centralized and
15
16 382 straightforward way using the Web program
17
18 383 www.randomization.com. It will be carried out independently by
19
20 384 a researcher blinded to inclusion criteria, delivery of
21
22 385 medication, participant follow-up, results, statistical
23
24 386 analysis, and writing of the final manuscript. Allocation will
25
26 387 be established, for 214 participants in blocks of 107 assigned.
27
28 388 The selection of health workers will be made regardless of the
29
30 389 hospital shift, work schedule, or assigned area. If the desired
31
32 390 sample size is not reached, the inclusion of personnel involved
33
34 391 in the first line of care of other referral hospitals for
35
36 392 patients with SARS-CoV-2 will be considered.

37
38 393 An independent researcher will allocate patients to the desired
39
40 394 groups. Envelopes will be correctly sealed by the pharmacy
41
42 395 department and will contain HCQ plus BHH or placebos as
43
44 396 previously mentioned. In those who do not require HCQ and BHH,
45
46 397 the drug will be replaced by tablets identical in colour and
47
48 398 taste but lacking the active substance. In this way, drugs used
49
50 399 in both groups will be indistinguishable.
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5 401 Researcher A will recruit the participants and assess the
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7 402 inclusion criteria according to the serological,
8
9 403 electrocardiographic, biochemical results and clinical
10
11 404 investigation. Once included, volunteers will go to another
12
13 405 office with researcher B, who will be blinded to the first
14
15 406 procedure and the rest of the study. Researcher B will assign
16
17 407 the groups independently, centrally, and through the use of
18
19 408 the web program. This same researcher will be the one who makes
20
21 409 the packages indistinguishable to the person providing the
22
23 410 drugs to the participant. Researcher C will provide treatment
24
25 411 in a sealed envelope or box to the participant in the order of
26
27 412 assignment, without knowing each participant's study group.
28
29 413 This researcher will also be blinded to the rest of the results.
30
31 414 Participants will be blinded to the treatment they will
32
33 415 receive. The researchers performing follow-up, researchers for
34
35 416 result assessment, and the researcher who performs the
36
37 417 statistical analysis will be blinded.
38
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45
46 419 Informed consent will be obtained only by researcher A. If
47
48 420 researcher A is not available, the study administrator may
49
50 421 obtain informed consent for participation. The informed consent
51
52 422 will contain the authorization to participate in the study and
53
54 423 the authorization for taking biological samples,
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3 424 electrocardiogram, and authorization to handle personal
4
5 425 information. All participants will complete a written informed
6
7 426 consent included on the first page of the questionnaire that
8
9 427 requires permission to participate in the study. No candidate
10
11 428 is required to participate in the study, and their
12
13 429 participation is based on the agreement that they may withdraw
14
15 430 at any time. All participants have the right to withdraw from
16
17 431 the study if they feel uncomfortable answering a question or
18
19 432 with a test to be performed. Also, no one, including the
20
21 433 research team, will require a reason why the participant
22
23 434 decides to leave the study.

24
25
26 435 In order to protect participant confidentiality, each one will
27
28 436 be assigned a participation number, and all biological samples,
29
30 437 as well as medical history information, will be identified by
31
32 438 the participant's initials and participant number. Part of the
33
34 439 confidentiality protection process will include data capture
35
36 440 only by the researcher in charge of data capture (researcher
37
38 441 D), who will be the same for all participants and the entire
39
40 442 study. Secondly, the study administrator may also enter data
41
42 443 into the database if researcher D is unavailable.

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48 445 The study administrator will be blinded to allocation and
49
50 446 results of the participants. However, the administrator will
51
52 447 be the only one who will be able to reveal the group and
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3 448 treatment assignment in any of the cases: major adverse events
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5 449 such as cardiac arrhythmias, heart failure, major neurological
6
7 450 abnormalities, atrial or ventricular fibrillation, kidney
8
9 451 failure, or any adverse event related to pharmacological
10
11 452 treatment that endangers the life or any organ of the
12
13 453 participant's body. The objective of revealing the assignment
14
15 454 by the study administrator will be to provide the participant
16
17 455 of a timely treatment according to the drugs ingested.
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23 457 **Participant timeline and intervention**

24
25 458 The inclusion of participants will be evaluated according to
26
27 459 the eligibility criteria and by invitation. Volunteers who wish
28
29 460 to participate in the study will be summoned the next day at a
30
31 461 specialized office to carry out all the relevant studies to
32
33 462 ensure the inclusion criteria. These include a medical history,
34
35 463 anthropometric measurements such as weight and body mass index,
36
37 464 electrocardiogram (at days 30, 60 and 90 or whe the participant
38
39 465 requests it if they have any discomfort), hematic biometry,
40
41
42 466 complete blood chemistry, and serological test for antibodies
43
44 467 and qRT-PCR for SARS-CoV-2. Volunteers will be asked for
45
46
47 468 information to contact them once the serological results are
48
49
50 469 obtained.

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52
53 470 Once the results are obtained (approximately 3 days), personnel
54
55 471 eligible to participate in the study will be contacted. They
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3 472 will meet in a particular office to speak with a researcher
4
5 473 who will be in charge of carrying out the eligibility criteria
6
7 474 and medical history checklist. This researcher will be
8
9 475 different from the one who makes the assignment, who delivers
10
11 476 the medicine and the one who evaluates the results and performs
12
13 477 the statistical analysis. The assignment of the group of each
14
15 478 participant will be performed, and the participant will not
16
17 479 know the group they have been assigned. This information will
18
19 480 be known for the researcher in charge, unrelated to the
20
21 481 delivery of the treatment, results, or inclusion of the
22
23 482 participant in the study. After the assignment, the volunteers
24
25 483 will receive the assigned treatment at the pharmacy using a
26
27 484 code in a sealed envelope assigned by the Web. Participants
28
29 485 who meet the inclusion criteria and there is no reason for
30
31 486 exclusion will proceed to the second phase of group assignment
32
33 487 with researcher B, the next business day at a different time
34
35 488 or office than researcher A.
36
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44 490 The group of researchers in charge of monitoring the
45
46 491 participants, who will be blinded to the group assignment at
47
48 492 all times, will be in charge of assessing each participant's
49
50 493 adverse event and treatment adherence record weekly. These
51
52 494 follow-up researchers will be available 24 hours a day
53
54 495 throughout the week if participants experience undesirable
55
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3 496 adverse events that require urgent attention or that do not
4
5 497 allow them to continue with drug treatment. If this situation
6
7 498 happens, the researcher in charge of the follow-up will contact
8
9 499 the study administrator to reveal to the treating physicians
10
11 500 the treatment received by the participant. Health evaluation
12
13 501 of all participants will be performed at day 30, day 60 and
14
15 502 day 90, this includes electrocardiogram analysis, blood
16
17 503 chemistry analysis, antibody test, qRT-PCR, or at request of
18
19 504 the participant due to adverse clinical symptoms.
20
21
22

23 505 At the end of the first 60 days, a new qRT-PCR will be requested
24
25 506 from each participant. All participants who present symptoms
26
27 507 after the first 7 days of initiation of the intervention, will
28
29 508 be considered as a positive individual for the analysis and
30
31 509 will not be excluded from the study. The same action will be
32
33 510 carried out 90 days after the start of treatment for both
34
35 511 groups. After 60 days, the treatment will be suspended and the
36
37 512 results of the qRT-PCR samples for SARS-CoV-2 will be
38
39 513 evaluated. After finishing the intervention (60 days), all
40
41 514 participants will be followed-up 30 more days with a new qRT-
42
43 515 PCR at day 90 after initiation of the intervention and 30 days
44
45 516 after the end of the intervention, to assess the efficacy or
46
47 517 the treatment during the follow-up period.
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55 519 **Outcome measures**
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3 520 This study compares the efficacy of the use of HCQ plus BHH
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5 521 (as a conjoined treatment) in prophylactic doses for 60 days
6
7 522 in healthy health personnel exposed to the first line of care
8
9 523 in confirmed patients with suspected infection by SARS-CoV-2.

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12 524

13
14 525 *Primary endpoint*

15
16 526 The primary endpoint will be the proportion of health personnel
17
18 527 infected by SARS-CoV-2 at 60 days after starting treatment, in
19
20 528 all groups. The infection will be diagnosed using qRT-PCR for
21
22 529 SARS-CoV-2 and IgM and IgG antibodies anti-SARS-CoV-2 after
23
24 530 day 7 of treatment. All participants presenting symptoms with
25
26 531 positive qRT-PCR after 7 days of initiation of the
27
28 532 intervention, will be considered positive and will be included
29
30 533 in the analysis. The study period will be 90 days (60 days for
31
32 534 the primary end point plus 30 days of follow-up). The
33
34 535 proportion of infected personnel will be evaluated using
35
36 536 relative risk (RR) and absolute risk increase (ARI) with their
37
38 537 respective 95% confidence intervals, in the established time.
39
40 538 The disease-free period in the 60 days will also be evaluated
41
42 539 by analysing the cumulative incidence of healthy personnel,
43
44 540 and the presence of confirmed infection by qRT-PCR of SARS-
45
46 541 CoV-2 and IgM and IgG antibodies anti-SARS-CoV-2 will be the
47
48 542 outcome. The censoring variable will be the discontinuation of
49
50 543 treatment either due to death, adverse events, or any
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3 544 elimination criteria. Since there is the possibility of false
4
5 545 positives and negatives with qRT-PCR, we will perform
6
7 546 qualitative measurements of IgM and IgG with the Elecsys® Anti-
8
9 547 SARS-CoV-2 test from Roche laboratories.
10
11

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13
14 549 *Secondary endpoints*
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16
17 550 The secondary endpoint will be the proportion of health
18
19 551 personnel infected 90 days after starting treatment in all
20
21 552 groups. The infection will be diagnosed using qRT-PCR for SARS-
22
23 553 CoV-2 and IgM and IgG antibodies anti-SARS-CoV-2 after day 7
24
25 554 of the start of treatment. The study period will be 90 days.
26
27 555 The proportion of infected personnel will be evaluated using
28
29 556 RR and ARI with their respective 95% confidence intervals, in
30
31 557 the established time. The disease-free period in the 90 days
32
33 558 will also be evaluated by analysing the cumulative incidence
34
35 559 of healthy personnel, and the presence of confirmed infection
36
37 560 by qRT-PCR of SARS-CoV-2 will be the outcome. The censoring
38
39 561 variable will be the discontinuation of treatment either due
40
41 562 to death, adverse events, or any elimination criteria.
42
43

44
45 563 Also, secondary outcomes will be, in case of a positive SARS-
46
47 564 CoV-2 result, the need for oxygen use, admission to the
48
49 565 intensive care unit (ICU), presence of pneumonia by computer
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51 566 tomography scan (CT), death, severe pneumonia defined by the
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3 567 American Thoracic Association, time from hospitalization to
4
5 568 recovery in days.
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7 569 Another secondary endpoint will be adverse events, defined as
8
9 570 the presence of any of the following during the study period:
10
11 571 death, nausea, vomiting, abdominal pain, diarrhea, rash, itchy
12
13 572 skin, hair loss, lengthening of the QT interval in the
14
15 573 electrocardiogram (>500msec), corneal opacity, cardiac
16
17 574 arrhythmias, heart failure or kidney failure (renal clearance
18
19 575 <20ml/min). The proportion of the compound of adverse events
20
21 576 between the groups will be analyzed using RR and ARI for 60 days
22
23 577 with their respective 95% confidence intervals.
24
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28 578

29
30 579 The efficacy of the treatment will be established as the
31
32 580 proportion of volunteers infected with SARS-CoV-2. This
33
34 581 difference should be sufficient to avoid overlapping of the
35
36 582 95% confidence intervals. It will be considered effective if
37
38 583 the intervals do not overlap and ineffective if when comparing
39
40 584 groups, they have a proportion of infected whose confidence
41
42 585 intervals overlap. This type of evaluation will allow an
43
44 586 adequate understanding of the efficacy of the treatment in both
45
46 587 groups.
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5 592 **Handling and storage of data and documents**
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7 593 Before the start of the study, the researchers in charge of
8
9 594 the recruitment, assignment, and delivery of drugs will be
10
11 595 trained to perform the task assigned to them at least 3 days
12
13 596 before the start of the study.
14

15
16 597 Researcher A will assess the eligibility criteria of potential
17
18 598 participants and perform a detailed clinical examination to
19
20 599 assess whether they can participate in the study. The data that
21
22 600 will be collected initially will be the following:
23

24
25 601
26

27
28 602 ► Medical history (includes personal data): study
29
30 603 identifier number, history number, name, date of birth,
31
32 604 gender, occupation, marital status, nationality, current
33
34 605 residence, degree of studies (primary, secondary, upper
35
36 606 secondary, bachelor`s degree, postgraduate), hospital
37
38 607 service to which they belong and the number of hours
39
40 608 worked per week.
41

42
43
44 609 ► Personal history: alcohol intake (yes/no; how many glasses
45
46 610 of beer or alcoholic beverages do you consume per week),
47
48 611 smoking habit (yes/ no; and number of cigarettes per day),
49
50 612 drug use (yes/no), diet per week (dietary restrictions
51
52 613 and number of meals per day) and number of hours of sleep
53
54 614 per day.
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3 615 ▶ Gynaecological history (in women): Number of pregnancies,
4
5 616 number of live children, menarche, menopause.
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7
8 617 ▶ History of respiratory disease, history of
9
10 618 gastrointestinal disease, nephrological, neurological,
11
12 619 haematological, cardiovascular, allergies.
13
14
15 620 ▶ Genetic family history, such as hypertension, diabetes,
16
17 621 heart disease, kidney disease.
18
19 622 ▶ Physical examination: blood pressure, heart rate,
20
21 623 respiratory rate, temperature, weight, height, body mass
22
23 624 index, skin lesions, head and neck inspection, respiratory
24
25 625 inspection (chest symmetry, lung expansion, palpation of
26
27 626 the bases and preserved vertices, lung percussion,
28
29 627 auscultation for lung murmur, breath sounds).
30
31
32 628 Cardiovascular inspection (palpation of the fifth
33
34 629 intercostal space, auscultation of heart sounds, pulses
35
36 630 that are palpable and symmetrical), abdominal inspection
37
38 631 (palpation, percussion and auscultation of peristaltic
39
40 632 sounds), neurological evaluation (Glasgow, active
41
42 633 motility, passive motility, reflex motility, cranial
43
44 634 nerves, sensitivity).
45
46
47
48 635 ▶ Hematic biometry: haematocrit, leukocytes, segmented (%),
49
50 636 lymphocytes (%), monocytes (%), mean corpuscular volume,
51
52 637 platelets.
53
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3 638 ▶ Blood chemistry: glycaemia, urea, creatinine, sodium,
4
5 639 potassium, chlorine, aspartate transaminase, alanine
6
7 640 transaminase, alkaline phosphatase, total bilirubin.
8
9
10 641 ▶ Muscle enzymes.
11
12 642 ▶ Clotting times: thrombin time, prothrombin time,
13
14 643 international normalized ratio.
15
16
17 644 ▶ Electrocardiogram: rhythm, heart rate, heart axis,
18
19 645 evaluation of P wave, PR interval, duration of QRS, QT
20
21 646 interval, time of T wave. The electrocardiogram will be
22
23 647 performed using an instrument calibrated and validated
24
25 648 for its use internationally, weekly.
26
27
28 649
29
30
31 650 ▶ Molecular test results for IgG and IgM antibodies:
32
33 651
34 652 ○ The FDA approved product called Cellex qSARS-CoV-2
35
36 653 IgG/IgM Rapid Test will be used for serological
37
38 654 determination. The device cassette, sample, and
39
40 655 buffer solution must be at room temperature. The
41
42 656 sample (10 µL) is transferred to the center of the
43
44 657 sample well. After the sample well is free of liquid,
45
46 658 two drops of sample diluent are added. After fifteen
47
48 659 to twenty minutes, read the test results. Results
49
50 660 should not be read after twenty minutes.
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2
3 661 o A positive IgM result occurs when a coloured band
4
5 662 appears on the M test line (M) and the control line
6
7 663 (C) and indicates that IgM against SARS-CoV-2 is
8
9
10 664 present.
- 11
12 665 o A positive IgG result occurs when a coloured band
13
14 666 appears on the G test line (G) and the control line
15
16 667 (C) and indicates that IgG against SARS-CoV-2 is
17
18 668 present.
- 19
20
21 669 o A positive result for IgM and IgG occurs when
22
23 670 coloured bands occur both M and G, as well as C.
- 24
25 671 o A negative result occurs when a coloured band appears
26
27 672 in C only and indicates that IgM and IgG antibodies
28
29 673 against SARS-CoV-2 were not detected.
- 30
31
32 674 o An invalid result occurs when a colour band is not
33
34 675 produced in C, and the test must be repeated.

36
37 676 ▶ Official qRT-PCR results (carried out by INCMNSZ)

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39 677

40
41
42 678 All this information will be collected in a pre-established
43
44 679 medical history questionnaire for each potential participant.

45
46 680 The information obtained from the weekly assessment of adverse

47
48 681 events, and the results of the qRT-PCR for SARS-CoV-2 at 60

49
50 682 and 90 days (60 for the primary end point plus 30 more days of

51
52 683 follow-up) after starting treatment will be entered into an

53
54 684 online database. In order to ensure the quality of the data

1
2
3 685 collection, the database will be built in CASTOR, a database
4
5 686 on the Web that allows entering all the pre-defined data for
6
7 687 each participant, thus reducing human error. This information
8
9 688 will be stored on a server in the United States of America and
10
11 689 can only be accessed by the study's administrator. The data
12
13 690 may only be entered by a researcher in charge of collecting
14
15 691 the data sheets and emptying them.
16
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21 **Monitoring and quality assurance**

22
23 694 Every possible adverse event will be noted daily by the
24
25 695 participants in the agenda that will be delivered to them. This
26
27 696 agenda will be evaluated weekly by the researcher in charge of
28
29 697 monitoring the participants (who will be blinded to group
30
31 698 assignment). In case of unbearable adverse events for the
32
33 699 participants or that put their health at risk, an open line
34
35 700 will be available 24 hours a day with direct communication to
36
37 701 the researcher in charge of monitoring the study to report any
38
39 702 event that requires hospitalization or immediate evaluation at
40
41 703 the hospital. All participants with adverse events that put
42
43 704 their life or health at risk may be urgently assessed by
44
45 705 personnel from both INCMNSZ and INR-LGII, if possible, by the
46
47 706 staff involved into the study. Patient follow-up investigator
48
49 707 will immediately contact the study administrator to disclose
50
51 708 the participant's assignment to treating physicians at that
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3 709 institution, but the assignment will never be disclosed to
4
5 710 other investigators related to the study. All the study
6
7 711 expenses and/or attention of collateral effects will be covered
8
9 712 by the current cost of the financing SECTEI/061/2020.
10
11
12 713 Auditing will be carried out weekly, assessing adverse events,
13
14 714 capturing data in the corresponding datasheets by the study
15
16 715 administrator. Likewise, the data entered in the CASTOR web
17
18 716 base will be valued to validate its quality. The paper data
19
20 717 sheets must be kept in a special office dedicated to the study
21
22 718 in folders separated by volunteers with the informed consent
23
24 719 of each participant, the data of the medical history,
25
26 720 laboratory results, eligibility criteria, adverse event sheet
27
28 721 and results, molecular tests, as well as electrocardiogram.
29
30
31 722 The letter of revocation of informed consent will also be
32
33 723 protected if required. As part of the audit, an interim
34
35 724 analysis will be carried out 30 days after the study starts to
36
37 725 assess the possible adverse effects and whether these outweigh
38
39 726 the potential benefits of the intervention. In the adverse
40
41 727 event outweigh the potential benefits, termination of the study
42
43 728 will be assessed. The approval of the research ethics committee
44
45 729 of the INR-LGII of Mexico has been obtained.
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53 **731 Statistical analysis**
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3 732 Data analysis will be carried out by intention to treat, which
4
5 733 means that each participant will be analysed according to the
6
7 734 group assigned regardless of whether they modified their
8
9 735 treatment. The study variables will be divided according to
10
11 736 the allocation group. The statistical analysis will be carried
12
13 737 out by evaluation the difference between the different groups
14
15 738 of HCQ plus BHH versus placebos. Missing data will be handled
16
17 739 by multiple imputation analysis when missing at random. Deaths
18
19 740 will be censored.
20
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24
25 742 The primary objective will be expressed in number and
26
27 743 proportion for each group. The RR will be obtained as the
28
29 744 division between the proportion of primary outcomes in the
30
31 745 intervention group(s) by the proportion of primary outcomes in
32
33 746 the double placebo group. Adjusted risk ratios (aRR) will be
34
35 747 obtained using a log-binomial regression, adjusting for age
36
37 748 and gender as pre-specified confounding variables. It will be
38
39 749 expressed as RR with its respective 95% confidence interval
40
41 750 for the initial time, which is 60 days. Likewise, the result
42
43 751 will be expressed as absolute risk, which will be derived from
44
45 752 the proportion of the primary outcome in the intervention group
46
47 753 minus the proportion of the primary outcome in the control
48
49 754 group. Secondly, the primary objective will be analysed with
50
51 755 the non-parametric estimate of the survival and risk function
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1
2
3 756 using Kaplan-Meier curves for 60 days according to the
4
5 757 allocation group. The primary endpoint will be SARS-CoV-2
6
7 758 infection within the 60-day period, and the silencing variable
8
9 759 will be dropping out of the study for any reason. The comparison
10
11 760 of the survival curves between both groups will be carried out
12
13 761 using the log-rank test. To adjust the primary objective to
14
15 762 possible confounders such as age, gender, service in which the
16
17 763 participant works, body mass index, etc. Multiple regression
18
19 764 will be performed using the Cox model to determine the adjusted
20
21 765 primary endpoint hazard ratio.
22
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28 767 For secondary outcomes such as the analysis at 90 days, the
29
30 768 same statistical analysis expressed in RR and absolute risk
31
32 769 will be used. Survival analysis will be used for the primary
33
34 770 endpoint only. An interim statistical analysis will be
35
36 771 performed 30 days after the study starts to assess possible
37
38 772 adverse effects and the efficacy of the intervention. The study
39
40 773 administrator will be the only one with access to the data.
41
42 774 For the interim analysis and the final analysis, the
43
44 775 administrator will export the data to Excel format to be
45
46 776 analysed by the study statistician blinded to the assignment
47
48 777 of groups, participants, or results.
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3 779 **Adverse events, serious adverse events and suspected unexpected**
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5 780 **serious adverse reactions**
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7 781 By requiring the use of drugs, the participant will be exposed
8
9 782 to risks inherent to the drug used, ranging from mild to severe
10
11 783 or death. Any unexpected risks that may occur during the study
12
13 784 will be immediately explained to the participants and the
14
15 785 ethics committee. Any adverse event will be compiled and will
16
17 786 not be disclosed under any condition to anyone other than the
18
19 787 study administrator, treating physicians in case of severe
20
21 788 events, and the ethics committee. The results will be
22
23 789 completely anonymous concerning the names of the participants.
24
25 790 The results will be compiled and reported as combined
26
27 791 collective data.
28
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33
34 793 *Patient and public involvement*
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36 794 Patients were not involved in the development of this research.
37
38 795 However, the results of the study will be communicated to the
39
40 796 study participants by sending the end product (published
41
42 797 article) to the provided email address.
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48 799 **ETHICS, DISSEMINATION AND SAFETY MONITORING**
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50 800 In case of adverse events or complications derived from the
51
52 801 study, participants will be assured attention by the staff of
53
54 802 the INCMNSZ in an enclosure that ensures the safety of the
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3 803 participant, not subjecting volunteers to a higher risk of
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5 804 contamination. This care will be extended until adverse events
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7 805 are resolved. In case of no adverse events during the study,
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9 806 medical attention will be extended at the aforementioned
10
11 807 institute until 15 days after the end of the study.
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13

14 808

15
16 809 This protocol has been approved by the local medical ethical
17
18 810 review committee at the INR-LGII with the internal number
19
20 811 INRLGII/25/20, and by the Federal Commission for Protection
21
22 812 against Sanitary Risks (in Spanish, Comisión Federal para la
23
24 813 Protección contra Riesgos Sanitarios, COFEPRIS), approval
25
26 814 number 203300410A0058/2020.
27

28
29 815 The study results will be published in journals of worldwide
30
31 816 impact affiliated with the Journal Citation Reports. Likewise,
32
33 817 the results of the study will be disseminated in national and
34
35 818 international media, exposed in international and national
36
37 819 congresses, communicated to CONACYT, and recorded in
38
39 820 Clinicaltrials.gov according to the study identifier number.
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41

42
43 821 The help of non-profit organizations will be sought to
44
45 822 disseminate the results of the investigation to interest
46
47 823 groups.
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51 825 The complete protocol will be published on Clinicaltrials.gov
52
53 826 and the OSF - Center for Open Science platform <https://osf.io/>.
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3 827 Where a DOI will be assigned, and the amendments made to the
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5 828 original protocol will be assessed.
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10 830 Amendments to the protocol may be made before the start of the
11
12 831 study and during the study. Any amendment to the protocol will
13
14 832 be clarified and posted on Clinicaltrials.gov under the same
15
16 833 identifier as this study. Likewise, any amendment will be sent
17
18 834 to the ethics committee of the same hospital.
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22 23 836 **AUTHOR CONTRIBUTIONS**

24
25 837 JGM is the lead study investigator, developed the study
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27 838 concepts and design, and wrote the manuscript by adapting the
28
29 839 original study protocol for publication, subsequent reviews
30
31 840 and amendments. EJHL, KMM and AAA contributed to the
32
33 841 development and refining of the protocol, writing of manuscript
34
35 842 and subsequent review. RJMP provided advanced methodological
36
37 843 and statistical input, and contributed to the study design and
38
39 844 subsequent amendments. RFC, TCH, PSSB, RAG and NML reviewed,
40
41 845 commented and informed methodology, development and writing of
42
43 846 the protocol.
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50
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52
53 850 Technology and Innovation Department (in Spanish, Secretaría
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3 851 de Educación, Ciencia, Tecnología e Innovación), grant number
4
5 852 SECTEI/061/20.
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10 854 **COMPETING INTERESTS STATEMENT**

11
12 855 None of the authors have conflict of interests, commercial
13
14 856 agreements, or receive financial fees or compensation from any
15
16 857 commercial or pharmaceutical company.
17

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21 859 **ETHICS APPROVAL**

22
23 860 This protocol has been approved by the local medical ethical
24
25 861 review committee at the INR-LGII with the internal number
26
27 862 INRLGII/25/20. Definitions of Research Risk Regulation of the
28
29 863 General Health Law on Research for Health (in Spanish,
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31 864 Reglamento de la Ley General de Salud en Materia de
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33 865 Investigación para la Salud)
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35 866 <http://www.inr.gob.mx/Descargas/Investigacion/Reglamentos->
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37 867 [LGS-Materia-Investigacion-Salud.pdf](http://www.inr.gob.mx/Descargas/Investigacion/Reglamentos-LGS-Materia-Investigacion-Salud.pdf). ARTICLE 17; and by
38
39 868 Federal Commission for Protection against Sanitary Risks (in
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41 869 Spanish, Comisión Federal para la Protección contra Riesgos
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43 870 Sanitarios, COFEPRIS), approval number 203300410A0058/2020.
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53 873 **PROVENANCE AND PEER REVIEW**

54
55 874 Not commissioned; externally peer reviewed.
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For peer review only

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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).

Journal:	<i>BMJ Open</i>
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3 1 **New prophylaxis regimen for SARS-CoV-2 infection in health**
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5 2 **professionals with low doses of Hydroxychloroquine and**
6
7 3 **Bromhexine: a randomized, double-blind placebo clinical trial**
8
9 4 **(ELEVATE Trial).**
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19 33
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21 34 Keywords: prophylaxis, SARS-CoV-2, COVID-19, health workers,
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23 35 hydroxychloroquine, bromhexine.
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29 38 Word count: 5,666
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14 54 **ABSTRACT**

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16
17 55 **Introduction:** SARS-CoV-2 infection in Mexico has caused ~1
18
19 56 million confirmed cases; around 20-25% of health workers will
20
21 57 be infected by the virus at their workplace, with approximately
22
23 58 4.4% of mortality. High infectivity of SARS-CoV-2 is related
24
25 59 with cell entry mechanism, through the angiotensin-converting
26
27 60 enzyme (ACE) receptor. SARS-CoV-2 requires transmembrane
28
29 61 protease serine 2 (TMPRSS2) to cleave its spike glycoprotein
30
31 62 and ensure fusion of host cell and virus membrane. We propose
32
33 63 studying prophylactic treatment with hydroxychloroquine (HCQ)
34
35 64 and bromhexine (BHH), which have been shown to be effective in
36
37 65 preventing SARS-CoV-2 infection progression when administered
38
39 66 in early stages. The aim of this study is to assess the efficacy
40
41 67 of HCQ and BHH as prophylactic treatments for SARS-CoV-2
42
43 68 infection in healthy health workers exposed to the virus.

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45
46 69 **Methods and analysis:** Double-blind randomized clinical trial,
47
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49 70 with parallel allocation at a 1:1 ratio with placebo, of low
50
51 71 doses of HCQ plus BHH, for 60 days. Study groups will be defined
52
53 72 as follows: 1) HCQ 200mg/d + BHH 8mg/8h vs 2) HCQ placebo plus
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3 73 BHH placebo. Primary endpoint will be efficacy of both
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5 74 interventions for the prevention of SARS-CoV-2 infection,
6
7 75 determined by the risk ratio (RR) of infected personnel and
8
9 76 the absolute risk. At least a 16% reduction in absolute risk
10
11 77 is expected between the intervention and placebo groups; a
12
13 78 minimum of 20% infection is expected in the placebo group. The
14
15 79 sample size calculation estimated a total of 214 patients
16
17 80 assigned: two groups of 107 participants each.

21 **Ethics and dissemination:** This protocol has been approved by
22
23 82 the local Medical Ethics Committee (National Institute of
24
25 83 Rehabilitation 'Luis Guillermo Ibarra Ibarra', approval number
26
27 84 INRLGII/25/20) and by the Federal Commission for Protection
28
29 85 against Sanitary Risks (COFEPRIS, approval number
30
31 86 203300410A0058/2020). The results of the study will be
32
33 87 submitted for publication in peer-reviewed journals and
34
35 88 disseminated through conferences.

39 **Trial registration number 2a:** NCT04340349.

41 90 2b: NA

43 91 Protocol version: #4

44 92

48 93 **STRENGTHS AND LIMITATIONS OF THIS STUDY**

50 94 *Strengths*

52
53 95 ► This is a double-blind randomized single-centre clinical
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55 96 trial, involving low doses of hydroxychloroquine and
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2
3 97 bromhexine, adequately powered to provide clinically
4
5 98 relevant information regarding prophylactic treatment for
6
7 99 SARS-CoV-2 infection in health care personnel.

10 100 ► Bromhexine have minimal side effects, has been used in
11
12 101 the early infection with SARS-CoV-2 showing satisfactory
13
14 102 results and are commercially available worldwide.
15
16 103 Findings could be applied in a timely fashion in different
17
18 104 regions.

21 105

23 106 *Limitations*

26 107 ► Long-term use of hydroxychloroquine can cause heart rhythm
27
28 108 problems

30 109 ► For the moment, people who are not candidates to receive
31
32 110 the vaccine, due to chronic diseases or severe allergies,
33
34 111 will not be included.

37 112 ► Hydroxychloroquine has not been shown to have a
38
39 113 prophylactic effect; however, we believe that the use of
40
41 114 HCQ in conjunction with BHH may inhibit SARS-Cov-2
42
43 115 infection.

46 116 **INTRODUCTION**

48
49 117 In Mexico, up to February 2021, have been produced more than
50
51 118 1.9 millions confirmed cases and ~170,000 deaths have
52
53 119 arisen[1]. The age group ranging between 30 and 79 years is
54
55 120 the most highly affected, where 81% present mild symptoms, 14%

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3 121 severe and 5% critical, requiring intensive care unit
4
5 122 management.

6
7 123 SARS-CoV-2 is a single-stranded RNA virion, member of the
8
9 124 *Betacoronavirus* genus [2]. SARS-CoV-2 has an incubation period
10
11 125 between 3 to 10 days, with different incubation periods related
12
13 126 with different clinical symptoms [3,4] . It is transmitted
14
15 127 through respiratory droplets from infected humans and through
16
17 128 contact with contaminated fomites and aerosols; moreover, even
18
19 129 asymptomatic persons in close contact can transmit the disease
20
21 130 [5]. The mechanism through which the virus infects the
22
23 131 respiratory cell is due to the angiotensin-converting enzyme
24
25 132 protein 2 (ACE-2). This receptor is found in multiple tissues
26
27 133 such as the oral cavity, brain, kidneys, intestine, and
28
29 134 placenta [6-8].

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34 135 Health personnel is not exempt from contracting the disease.
35
36 136 In China, it was reported that 3.5-4.4% of the infected
37
38 137 population belonged to this group, and 14.8% presented
39
40 138 characteristics of severity or critical illness [4,9,10].
41
42 139 Italy, around 20% of healthcare professionals became infected
43
44 140 [11]; mean age of health workers who died was 55 years (range
45
46 141 of 29-72 years) and mean period from hospital admission to
47
48 142 death was 19 days, (range 1-47 days) [9].

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51 143 Treatment of the SARS-Cov-2 infection has led different
52
53 144 research groups to work on the development of vaccines.
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3 145 However, the use of vaccines can be a challenge. The first
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5 146 trials have shown that the immune protection is not 100% and
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7 147 the protection is only temporary, so it will be necessary to
8
9 148 carry out the vaccination periodically. On the other hand,
10
11 149 because the virus is RNA and the mutation rate is high, we can
12
13 150 expect new variants that reduce or nullify the effectiveness
14
15 151 of the vaccines, this depends on the origin of vaccine, if it
16
17 152 is made with viral vectors (such as from CanSino or
18
19 153 AstraZeneca), if it is mRNA (such as from Moderna or Pfizer-
20
21 154 BioNTech) or if it is inactivated virus (Sinovac). Mainly
22
23 155 because the development of vaccine that can be efficient for
24
25 156 the new variants could be delayed and this could once again
26
27 157 increase the number of people who acquire the SARS-CoV-2 virus.
28
29 158 On the other hand, around the world there are groups of people
30
31 159 who are against vaccination, or people that have severe
32
33 160 allergies, as well as populations that will take much longer
34
35 161 to reach the moment when they can acquire the vaccine, so it
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37 162 is extremely necessary that people who do not vaccinate by
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39 163 choice, by disease or by the lack of the vaccine, have an
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41 164 alternative to avoid infection and avoid the spread of the
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43 165 virus.
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45 166 Therefore, it is important to develop a pharmacological
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47 167 strategy that allows the use of prophylactic drugs for the
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49 168 prevention of SARS-CoV-2 infection.
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3 169 Chloroquine (CQ) and Hydroxychloroquine (HCQ) are known as an
4
5 170 antimalarial agent; HCQ is a hydroxylated derivative from CQ.
6
7 171 CQ and HCQ have gained attention as possible therapies in
8
9 172 Covid-19 disease. In overdose, both drugs can cause severe,
10
11 173 potentially life-threatening effects as visual disturbances,
12
13 174 corneal opacities, irreversible retinopathy can occur with
14
15 175 cumulative doses exceeding 100 grams. When lower daily doses
16
17 176 (250 mg are used) retinopathy may not occur after many years
18
19 177 of treatment [12]. The above indicates that the use of HCQ at
20
21 178 low doses, to avoid SARS-CoV-2 infection, does not produce
22
23 179 toxicity and could be used as a prophylactic treatment. HCQ
24
25 180 has been used in several viral infections, for example, as
26
27 181 replication inhibitor for the dengue virus, decreasing in vitro
28
29 182 virus infection and promoting activation of different
30
31 183 immunological signal pathways [13]. It has also been used to
32
33 184 treat patients infected with hepatitis C virus decreasing viral
34
35 185 load, with minimal adverse effects reported [14]. HCQ has been
36
37 186 reported to block viral infection by increasing the endosomal
38
39 187 pH required for virus fusion to the cell, as well as interfere
40
41 188 with SARS-CoV-2 cell receptors, through inhibition of ACE2
42
43 189 glycosylation receptor [15-18]. HCQ has immunomodulatory
44
45 190 effects; it inhibits production and release of pro-inflammatory
46
47 191 cytokines, that are associated with severe disease development
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51 192 [19,20] . Recently, it has been reported that HCQ works as a
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3 193 autophagy inhibitor, interfering with viral infection and
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5 194 replication [21]. There is recent evidence that HCQ could be
6
7 195 used to treat COVID-19; studies in high-risk patients show that
8
9 196 the use of HCQ was associated with a lower risk of intubation
10
11 197 or death [22]. Recent study showed that pre-treatment with HCQ
12
13 198 has shown a better effect on antiviral activity [17] and it
14
15 199 has been reported that loading doses of 1600 mg HCQ followed
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17 200 by 600 mg daily doses are needed have a relevant effect to
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19 201 SARS-CoV-2 inhibition within 72 hours in 60% of COVID-19
20
21 202 patients [23]. Finally, a study where evaluated the antiviral
22
23 203 mechanisms of CQ and the adverse effects, repositioned the CQ
24
25 204 to have more efficacy when used as a prophylactic treatment
26
27 205 rather than as a therapeutic [24]. On the other hand, CQ and
28
29 206 HCQ has been reported to have various adverse effects, the CQ
30
31 207 being the most toxic in overdose. However, it was recently
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33 208 published that *in vivo* trials are lacking to determine whether
34
35 209 this drug is useful as a prophylactic treatment against SARS-
36
37 210 Cov-2 [25]. Based on the above, do more studies will be
38
39 211 important to determine its effectiveness at low doses (<250
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41 212 mg) as a prophylactic treatment.
42
43 213 Another pharmacological option to treat SARS-CoV-2 infection
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45 214 is Bromhexine (BHH). BHH modifies the composition of mucus,
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47 215 increases ciliary clearance and decreases coughing, improving
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49 216 respiratory symptoms. It has also been reported to enhance the
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3 217 effects of some antibiotics [26]. The mechanism by which SARS-
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5 218 CoV-2 enters human cells depends on the ACE-2 receptor and the
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7 219 human transmembrane serine protease (TMPRSS2), on which BHH
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9 220 has a specific inhibitory effect [27,28]. BHH has been used to
10
11 221 treat pneumonic damage in both lungs during early infection
12
13 222 [29]. BHH turns out to be an ideal candidate for SARS-CoV-2
14
15 223 treatment, since it has few contraindications, and its side
16
17 224 effects are minimal, demonstrating an extensive margin of
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19 225 pharmacological safety. BHH is widely available over the
20
21 226 counter, and its low cost makes it an ideal therapeutic option.
22
23 227 According to a letter published in the New England Journal of
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25 228 Medicine, of 77,262 patients infected by SARS-CoV-2, 3387
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27 229 (4.4%) were health workers [9]. Of these, 23 have died from
28
29 230 this disease. The prevalence of infections in health personnel
30
31 231 is alarming since health services in first world countries have
32
33 232 been overwhelmed by this disease. In Italy, around 20% of
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35 233 health professionals had a SARS-CoV-2 infection [11]. Faced
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37 234 with a highly contagious disease, the care of health workers,
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39 235 who are first line of contact and on whom the health system of
40
41 236 each country depends, is essential. This research regarding
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43 237 the use of HCQ and BHH in health personnel will allow us to
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45 238 determine and compare the effectiveness of both interventions,
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47 239 which is of vital importance to clarify whether these
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49 240 treatments may prevent the appearance of infection in this
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241 population. Describing for the first time that HCQ plus BHH
242 could function for disease prevention, would allow us to
243 provide prophylaxis to health professionals worldwide.
244 Therefore, the use of HCQ and BHH in healthy health personnel
245 exposed to patients with confirmed or suspected SARS-CoV-2 will
246 significantly reduce infection.

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252 **METHODS AND ANALYSIS**

253 **Study design**

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

259

260

261 **Participants**

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

1
2
3 265 been designated as a COVID-19 centre. The Mexican government
4
5 266 defined 3 phases to determine risk for SARS-CoV-2 infection:
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7 267 imported cases from outside Mexico; community infection and
8
9 268 spread of the disease throughout the country (also known as
10
11 269 Phase 3). In the latter, it is assumed that every person who
12
13
14 270 enters a hospital is a potentially infected carrier; currently
15
16 271 our centre is in Phase 3. Likewise, health personnel who work
17
18 272 at the "Instituto Nacional de Ciencias Médicas y Nutrición,
19
20 273 Salvador Zubirán" (INCMNSZ), which is a COVID-19 designated
21
22 274 tertiary centre, and who meet inclusion criteria of the
23
24 275 protocol will be invited to participate in the study.

26
27
28 276 Inclusion of participants will be assessed according to the
29
30 277 eligibility criteria. Table 1 shows the classification and
31
32 278 characteristics of study variables. Continuous variables will
33
34 279 be assessed for normality. Variables with a normal distribution
35
36 280 will be compared using Student's t-test, non-parametric
37
38 281 variables using the Mann-Whitney U-test. Categorical variables
39
40 282 will be evaluated using the Chi-squared test.

41 42 43 44 283 *Inclusion criteria*

- 45
46 284 ► Health personnel working at INR LGII or INCMNSZ who wish
47
48 285 to participate in the study and sign the informed consent.
49
50 286 ► Over 18 and under 60 years of age, both genders.
51
52 287 ► Contacting with suspected or confirmed SARS-CoV-2
53
54 288 infection.
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3 289 ▶ Normal electrocardiogram.
4

5 290 *Exclusion criteria*
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7
8 291 ▶ Positive quantitative reverse transcriptase-polymerase
9
10 292 chain reaction (qRT-PCR) test for SARS-CoV-2 at the time
11
12 293 of inclusion.
13

14 294 ▶ Panel of IgG or IgM antibodies positive for SARS-CoV-2 at
15
16
17 295 the time of inclusion.
18

19 296 ▶ Development of respiratory symptoms suspicious of SARS-
20
21 297 CoV-2 infection during the first 7 days after treatment
22
23 298 is initiated, confirmed by qRT-PCR and IgG or IgM
24
25 299 antibodies postiver for SARS-CoV-2.
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27

28 300 ▶ Health personnel with comorbidities such as diabetes,
29
30 301 hypertension, autoimmune diseases (i.e., porphyria,
31
32 302 psoriasis, systemic lupus erythematosus), obesity
33
34 303 (defined as body mass index ≥ 30), cardiovascular diseases,
35
36 304 respiratory diseases (such as asthma, chronic bronchitis,
37
38 305 idiopathic pulmonary fibrosis).
39
40

41
42 306 ▶ History of allergies to any hydroxychloroquine or
43
44 307 bromhexine related compound or medication.
45

46 308 ▶ Use of immunosuppressors for any reason.
47

48
49 309 ▶ History of bone marrow transplant.
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51 310 ▶ Known glucose-6-phosphate dehydrogenase deficiency.
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3 311 ▶ Chronic kidney disease or glomerular filtration
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5 312 <20ml/min.
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7 313 ▶ Use of other drugs with reported pharmacological
8
9 314 interactions (i.e., digitalis, flecainide, amiodarone,
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11 315 procainamide, or propafenone).
12
13 316 ▶ History of long QT syndrome.
14
15 317 ▶ Electrocardiogram with QTc>500 msec.
16
17 318 ▶ Pregnant or breastfeeding personnel.
18
19 319 ▶ Epilepsy.
20
21 320 ▶ Known liver disease.
22
23 321 ▶ Personnel who have received the Covid-19 vaccine
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29 *Elimination criteria*

- 30
31 323 ▶ Personnel who decide to leave the study for any reason
32
33 324 not related to adverse events.
34
35 325 ▶ Personnel with incomplete information on the primary
36
37 326 outcome (qRT-PCR for SARS-CoV-2).
38
39 327 ▶ Personnel who are relocated to work in another
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41 328 institution.
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43 329 ▶ Personnel who do not wish to participate in the study
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50 331 **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, PaO ₂ / FiO ₂ ratio <250, Infiltrates multilobars, confusion / disorientation, uremia [BUN > 20 mg / dL], leukopenia [$<4,000$], thrombocytopenia [$<100,000$ platelets / mm ³], hypothermia [core temperature <36°C], 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

332

333 **Sample size calculation**

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2
3 334 According to the study by Remuzzi A et al. [11], the proportion
4
5 335 of healthcare workers infected with SARS-CoV-2 and confirmed
6
7 336 by RT-PCR was 20%. Taking this 20% as our null hypothesis, we
8
9
10 337 estimate that the proportion of infections in the intervention
11
12 338 group will be 4%. Using a two-tailed test, with a type I error
13
14 339 of 0.05, a power of 90%, and taking into account a loss of 10%
15
16 340 of participants for each group, we estimate that a total of
17
18 341 214 participants will be required, distributed in parallel
19
20
21 342 groups (1:1) of 107 each. This number of volunteers will allow
22
23 343 us to find a difference of 16% between groups with a power of
24
25 344 90% and an attrition of 20%. To ensure that desired sample
26
27 345 size is reached, all health workers involved in managing
28
29 346 patients suspected or infected by SARS-CoV-2 will be invited
30
31 347 personally and by institutional email.
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35 348

37 349 **Interventions**

39 350 Interventions will consist of low doses of HCQ 200 mg tablets
40
41 351 every 24 hours for 60 days plus BHH 8 mg tablets every 8
42
43 352 hours for 60 days. Study groups will be defined as follows: 1)
44
45 353 HCQ plus BHH vs placebo for both drugs. Fabrication of both
46
47 354 drugs and placebos will be provided to our centre by a hired
48
49 355 laboratory. Both drugs will be provided to participants
50
51 356 directly at the hospital by a researcher blinded to group
52
53 357 assignment process. To ensure that the intervention is carried
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3 358 out, each participant will be asked to keep a written record
4
5 359 of the days and time the medication was administered. This
6
7 360 document will be reviewed weekly to verify that more than 50%
8
9 361 adherence to treatment is maintained. Participants will be
10
11 362 asked to record any symptoms related to the use of the
12
13 363 medication, which will be reviewed by a researcher blinded to
14
15 364 group assignment, weekly, or at the participants' request.
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21 366 If any of the participants present symptoms of SARS-CoV-2
22
23 367 infection after the first 14 days from the beginning of the
24
25 368 intervention or positive qRT-PCR is present, the drug will not
26
27 369 be discontinued. If the participant presents adverse events
28
29 370 related to the drugs that are severe or intolerable, treatment
30
31 371 will be suspended. If the participants report an adherence of
32
33 372 less than 50% of the medication, the intervention will not be
34
35 373 discontinued to avoid imbalances between groups. Use of drugs
36
37 374 that interact with HCQ or BHH such as flecainide, digitalis,
38
39 375 amiodarone, procainamide or propafenone will be prohibited. If
40
41 376 a participant has to use these drugs during the study period,
42
43 377 they will be eliminated from the study. A free diet and outdoor
44
45 378 activity will be allowed since these do not intervene with the
46
47 379 implementation of the treatment or have interaction with the
48
49 380 drugs used. Finally, incidence of adverse events such as
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51 381 nausea, vomiting, abdominal pain, rash, itchy skin, hair loss,
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3 382 lengthening of the QT interval in the electrocardiogram,
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5 383 corneal opacity, cardiac arrhythmias, heart failure and death
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7 384 will be determined.
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12 386 **Randomization and treatment allocation**
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14 387 Group randomization will be in a centralized and
15
16 388 straightforward way using the Web program
17
18 389 www.randomization.com. It will be carried out independently by
19
20
21 390 a researcher blinded to inclusion criteria, delivery of
22
23 391 medication, participant follow-up, results, statistical
24
25 392 analysis, and writing of the final manuscript. Allocation will
26
27 393 be established, for 214 participants in blocks of 107 assigned.
28
29 394 The selection of health workers will be made regardless of the
30
31 395 hospital shift, work schedule, or assigned area. If the desired
32
33 396 sample size is not reached, the inclusion of personnel involved
34
35 397 in the first line of care of other referral hospitals for
36
37 398 patients with SARS-CoV-2 will be considered.

38
39
40 399 An independent researcher will allocate patients to the desired
41
42 400 groups. Envelopes will be correctly sealed by the pharmacy
43
44 401 department and will contain HCQ plus BHH or placebos as
45
46 402 previously mentioned. In those who do not require HCQ and BHH,
47
48 403 the drug will be replaced by tablets identical in colour and
49
50 404 taste but lacking the active substance. In this way, drugs used
51
52 405 in both groups will be indistinguishable.
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5 407 Researcher A will recruit the participants and assess the
6
7 408 inclusion criteria according to the serological,
8
9 409 electrocardiographic, biochemical results and clinical
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11 410 investigation. Once included, volunteers will go to another
12
13 411 office with researcher B, who will be blinded to the first
14
15 412 procedure and the rest of the study. Researcher B will assign
16
17 413 the groups independently, centrally, and through the use of
18
19 414 the web program. This same researcher will be the one who makes
20
21 415 the packages indistinguishable to the person providing the
22
23 416 drugs to the participant. Researcher C will provide treatment
24
25 417 in a sealed envelope or box to the participant in the order of
26
27 418 assignment, without knowing each participant's study group.
28
29 419 This researcher will also be blinded to the rest of the results.
30
31 420 Participants will be blinded to the treatment they will
32
33 421 receive. The researchers performing follow-up, researchers for
34
35 422 result assessment, and the researcher who performs the
36
37 423 statistical analysis will be blinded.
38
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44 424

45
46 425 Informed consent will be obtained only by researcher A. If
47
48 426 researcher A is not available, the study administrator may
49
50 427 obtain informed consent for participation. The informed consent
51
52 428 will contain the authorization to participate in the study and
53
54 429 the authorization for taking biological samples,
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3 430 electrocardiogram, and authorization to handle personal
4
5 431 information. All participants will complete a written informed
6
7 432 consent included on the first page of the questionnaire that
8
9 433 requires permission to participate in the study. No candidate
10
11 434 is required to participate in the study, and their
12
13 435 participation is based on the agreement that they may withdraw
14
15 436 at any time. All participants have the right to withdraw from
16
17 437 the study if they feel uncomfortable answering a question or
18
19 438 with a test to be performed. Also, no one, including the
20
21 439 research team, will require a reason why the participant
22
23 440 decides to leave the study.

24
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27
28 441 In order to protect participant confidentiality, each one will
29
30 442 be assigned a participation number, and all biological samples,
31
32 443 as well as medical history information, will be identified by
33
34 444 the participant's initials and participant number. Part of the
35
36 445 confidentiality protection process will include data capture
37
38 446 only by the researcher in charge of data capture (researcher
39
40 447 D), who will be the same for all participants and the entire
41
42 448 study. Secondly, the study administrator may also enter data
43
44 449 into the database if researcher D is unavailable.

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50 451 The study administrator will be blinded to allocation and
51
52 452 results of the participants. However, the administrator will
53
54 453 be the only one who will be able to reveal the group and
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3 454 treatment assignment in any of the cases: major adverse events
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5 455 such as cardiac arrhythmias, heart failure, major neurological
6
7 456 abnormalities, atrial or ventricular fibrillation, kidney
8
9
10 457 failure, or any adverse event related to pharmacological
11
12 458 treatment that endangers the life or any organ of the
13
14 459 participant's body. The objective of revealing the assignment
15
16 460 by the study administrator will be to provide the participant
17
18 461 of a timely treatment according to the drugs ingested.
19
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21 462

22 23 463 **Participant timeline and intervention**

24
25 464 The inclusion of participants will be evaluated according to
26
27 465 the eligibility criteria and by invitation. Volunteers who wish
28
29 466 to participate in the study will be summoned the next day at a
30
31 467 specialized office to carry out all the relevant studies to
32
33 468 ensure the inclusion criteria. These include a medical history,
34
35 469 anthropometric measurements such as weight and body mass index,
36
37 470 electrocardiogram (every week until the end of the study or
38
39 471 when the participant requests it if they have any discomfort),
40
41 472 hematic biometry, complete blood chemistry, and serological
42
43 473 test for antibodies and qRT-PCR for SARS-CoV-2. Volunteers will
44
45 474 be asked for information to contact them once the serological
46
47 475 results are obtained.
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51
52 476 Once the results are obtained (approximately 3 days), personnel
53
54 477 eligible to participate in the study will be contacted. They
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3 478 will meet in a particular office to speak with a researcher
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5 479 who will be in charge of carrying out the eligibility criteria
6
7 480 and medical history checklist. This researcher will be
8
9 481 different from the one who makes the assignment, who delivers
10
11 482 the medicine and the one who evaluates the results and performs
12
13 483 the statistical analysis. The assignment of the group of each
14
15 484 participant will be performed, and the participant will not
16
17 485 know the group they have been assigned. This information will
18
19 486 be known for the researcher in charge, unrelated to the
20
21 487 delivery of the treatment, results, or inclusion of the
22
23 488 participant in the study. After the assignment, the volunteers
24
25 489 will receive the assigned treatment at the pharmacy using a
26
27 490 code in a sealed envelope assigned by the Web. Participants
28
29 491 who meet the inclusion criteria and there is no reason for
30
31 492 exclusion will proceed to the second phase of group assignment
32
33 493 with researcher B, the next business day at a different time
34
35 494 or office than researcher A.
36
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44 496 The group of researchers in charge of monitoring the
45
46 497 participants, who will be blinded to the group assignment at
47
48 498 all times, will be in charge of assessing each participant's
49
50 499 adverse event and treatment adherence record weekly. These
51
52 500 follow-up researchers will be available 24 hours a day
53
54 501 throughout the week if participants experience undesirable
55
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3 502 adverse events that require urgent attention or that do not
4
5 503 allow them to continue with drug treatment. If this situation
6
7 504 happens, the researcher in charge of the follow-up will contact
8
9 505 the study administrator to reveal to the treating physicians
10
11 506 the treatment received by the participant. Health evaluation
12
13 507 of all participants will be performed at day 30, day 60 and
14
15 508 day 90, this includes electrocardiogram analysis, blood
16
17 509 chemistry analysis, antibody test, qRT-PCR, or at request of
18
19 510 the participant due to adverse clinical symptoms.
20
21
22

23 511 At the end of the first 60 days, a new qRT-PCR will be requested
24
25 512 from each participant. All participants who present symptoms
26
27 513 after the first 7 days of initiation of the intervention, will
28
29 514 be considered as a positive individual for the analysis and
30
31 515 will not be excluded from the study. The same action will be
32
33 516 carried out 90 days after the start of treatment for both
34
35 517 groups. After 60 days, the treatment will be suspended and the
36
37 518 results of the qRT-PCR samples for SARS-CoV-2 will be
38
39 519 evaluated. After finishing the intervention (60 days), all
40
41 520 participants will be followed-up 30 more days with a new qRT-
42
43 521 PCR at day 90 after initiation of the intervention and 30 days
44
45 522 after the end of the intervention, to assess the efficacy or
46
47 523 the treatment during the follow-up period.
48
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55 525 **Outcome measures**
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3 526 This study compares the efficacy of the use of HCQ plus BHH
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5 527 (as a conjoined treatment) in prophylactic doses for 60 days
6
7 528 in healthy health personnel exposed to the first line of care
8
9 529 in confirmed patients with suspected infection by SARS-CoV-2.

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13
14 531 *Primary endpoint*

15
16 532 The primary endpoint will be the proportion of health personnel
17
18 533 infected by SARS-CoV-2 at 60 days after starting treatment, in
19
20 534 both groups. The infection will be diagnosed using qRT-PCR for
21
22 535 relative expression of the mRNA SARS-CoV-2 and the measure of
23
24 536 IgM and IgG antibodies anti-SARS-CoV-2 after day 7 of treatment
25
26 537 using rapid test Cellex qSARS-CoV-2 IgG/IgM. All participants
27
28 538 presenting symptoms with positive qRT-PCR after 7 days of
29
30 539 initiation of the intervention, will be considered positive
31
32 540 and will be included in the analysis. The study period will be
33
34 541 90 days (60 days for the primary end point plus 30 days of
35
36 542 follow-up). The proportion of infected personnel will be
37
38 543 evaluated using relative risk (RR) and absolute risk increase
39
40 544 (ARI) with their respective 95% confidence intervals, in the
41
42 545 established time. The disease-free period in the 60 days will
43
44 546 also be evaluated by analysing the cumulative incidence of
45
46 547 healthy personnel, and the presence of confirmed infection by
47
48 548 qRT-PCR of SARS-CoV-2 and IgM and IgG antibodies anti-SARS-
49
50 549 CoV-2 will be the outcome. The censoring variable will be the
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3 550 discontinuation of treatment either due to death, adverse
4
5 551 events, or any elimination criteria. Since there is the
6
7 552 possibility of false positives and negatives with qRT-PCR, we
8
9 553 will perform qualitative measurements of IgM and IgG with the
10
11 554 Cellex qSARS-CoV-2 IgG/IgM Rapid test from test from which is
12
13 555 authorized by FDA. The test can be used on serum, plasma, or
14
15 556 whole blood samples. The clinical sensitivity of the assay was
16
17 557 93.8% and the clinical specificity was 96%[30].
18
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23 559 *Secondary endpoints*

24
25 560 The secondary endpoint will be the proportion of health
26
27 561 personnel infected 90 days after starting treatment in both
28
29 562 groups. The infection will be diagnosed using qRT-PCR for
30
31 563 relative expression of the mRNA of SARS-CoV-2 and the measure
32
33 564 of IgM and IgG antibodies anti-SARS-CoV-2 after day 7 of the
34
35 565 start of treatment using rapid test Cellex qSARS-CoV-2 IgG/IgM.
36
37 566 The study period will be 90 days. The proportion of infected
38
39 567 personnel will be evaluated using RR and ARI with their
40
41 568 respective 95% confidence intervals, in the established time.
42
43 569 The disease-free period in the 90 days will also be evaluated
44
45 570 by analysing the cumulative incidence of healthy personnel,
46
47 571 and the presence of confirmed infection by qRT-PCR of SARS-
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49 572 CoV-2 will be the outcome. The censoring variable will be the
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3 573 discontinuation of treatment either due to death, adverse
4
5 574 events, or any elimination criteria.

6
7 575 Also, secondary outcomes will be, in case of a positive SARS-
8
9 576 CoV-2 result, the need for oxygen use, admission to the
10
11 577 intensive care unit (ICU), presence of pneumonia by computer
12
13 578 tomography scan (CT), death, severe pneumonia defined by the
14
15 579 American Thoracic Association, time from hospitalization to
16
17 580 recovery in days.

18
19 581 Another secondary endpoint will be adverse events, defined as
20
21 582 the presence of any of the following during the study period:
22
23 583 death, nausea, vomiting, abdominal pain, diarrhea, rash, itchy
24
25 584 skin, hair loss, lengthening of the QT interval in the
26
27 585 electrocardiogram (>500msec), corneal opacity, cardiac
28
29 586 arrhythmias, heart failure or kidney failure (renal clearance
30
31 587 <20ml/min). The proportion of the compound of adverse events
32
33 588 between the groups will be analysed using RR and ARI for 60
34
35 589 days with their respective 95% confidence intervals.

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42
43 591 The efficacy of the treatment will be established as the
44
45 592 proportion of volunteers infected with SARS-CoV-2. This
46
47 593 difference should be sufficient to avoid overlapping of the
48
49 594 95% confidence intervals. It will be considered effective if
50
51 595 the intervals do not overlap and ineffective if when comparing
52
53 596 groups, they have a proportion of infected whose confidence
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3 597 intervals overlap. This type of evaluation will allow an
4
5 598 adequate understanding of the efficacy of the treatment in both
6
7 599 groups.
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19 604 **Handling and storage of data and documents**

20
21 605 Before the start of the study, the researchers in charge of
22
23 606 the recruitment, assignment, and delivery of drugs will be
24
25 607 trained to perform the task assigned to them at least 3 days
26
27 608 before the start of the study.

28
29
30 609 Researcher A will assess the eligibility criteria of potential
31
32 610 participants and perform a detailed clinical examination to
33
34 611 assess whether they can participate in the study. The data that
35
36 612 will be collected initially will be the following:

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41 614 ► Medical history (includes personal data): study
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43 615 identifier number, history number, name, date of birth,
44
45 616 gender, occupation, marital status, nationality, current
46
47 617 residence, degree of studies (primary, secondary, upper
48
49 618 secondary, bachelor`s degree, postgraduate), hospital
50
51 619 service to which they belong and the number of hours
52
53 620 worked per week.
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3 621 ▶ Personal history: alcohol intake (yes/no; how many glasses
4
5 622 of beer or alcoholic beverages do you consume per week),
6
7 623 smoking habit (yes/ no; and number of cigarettes per day),
8
9 624 drug use (yes/no), diet per week (dietary restrictions
10
11 625 and number of meals per day) and number of hours of sleep
12
13 626 per day.
14
15
16 627 ▶ Gynaecological history (in women): Number of pregnancies,
17
18 628 number of live children, menarche, menopause.
19
20
21 629 ▶ History of respiratory disease, history of
22
23 630 gastrointestinal disease, nephrological, neurological,
24
25 631 haematological, cardiovascular, allergies.
26
27
28 632 ▶ Genetic family history, such as hypertension, diabetes,
29
30 633 heart disease, kidney disease.
31
32
33 634 ▶ Physical examination: blood pressure, heart rate,
34
35 635 respiratory rate, temperature, weight, height, body mass
36
37 636 index, skin lesions, head and neck inspection, respiratory
38
39 637 inspection (chest symmetry, lung expansion, palpation of
40
41 638 the bases and preserved vertices, lung percussion,
42
43 639 auscultation for lung murmur, breath sounds).
44
45 640 Cardiovascular inspection (palpation of the fifth
46
47 641 intercostal space, auscultation of heart sounds, pulses
48
49 642 that are palpable and symmetrical), abdominal inspection
50
51 643 (palpation, percussion and auscultation of peristaltic
52
53 644 sounds), neurological evaluation (Glasgow, active
54
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3 645 motility, passive motility, reflex motility, cranial
4
5 646 nerves, sensitivity).

6
7 647 ► Hematic biometry: haematocrit, leukocytes, segmented (%),
8
9 lymphocytes (%), monocytes (%), mean corpuscular volume,
10 648
11 platelets.
12 649

13
14 650 ► Blood chemistry: glycaemia, urea, creatinine, sodium,
15
16 potassium, chlorine, aspartate transaminase, alanine
17 651
18 transaminase, alkaline phosphatase, total bilirubin.
19 652

20
21 653 ► Muscle enzymes.

22
23 654 ► Clotting times: thrombin time, prothrombin time,
24
25 international normalized ratio.
26 655

27
28 656 ► Electrocardiogram: rhythm, heart rate, heart axis,
29
30 evaluation of P wave, PR interval, duration of QRS, QT
31 657
32 interval, time of T wave. The electrocardiogram will be
33 658
34 performed using an instrument calibrated and validated
35 659
36 for its use internationally, weekly.
37 660

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41
42 662 ► Molecular test results for IgG and IgM antibodies:

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44 663
45 664 o The FDA approved product called Cellex qSARS-CoV-2
46
47 IgG/IgM Rapid Test will be used for serological
48 665
49 determination. The device cassette, sample, and
50 666
51 buffer solution must be at room temperature. The
52 667
53 sample (10 µL) is transferred to the center of the
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2
3 669 sample well. After the sample well is free of liquid,
4
5 670 two drops of sample diluent are added. After fifteen
6
7 671 to twenty minutes, read the test results. Results
8
9 672 should not be read after twenty minutes.

11
12 673 o A positive IgM result occurs when a coloured band
13
14 674 appears on the M test line (M) and the control line
15
16 675 (C) and indicates that IgM against SARS-CoV-2 is
17
18 676 present.

19
20
21 677 o A positive IgG result occurs when a coloured band
22
23 678 appears on the G test line (G) and the control line
24
25 679 (C) and indicates that IgG against SARS-CoV-2 is
26
27 680 present.

28
29
30 681 o A positive result for IgM and IgG occurs when
31
32 682 coloured bands occur both M and G, as well as C.

33
34 683 o A negative result occurs when a coloured band appears
35
36 684 in C only and indicates that IgM and IgG antibodies
37
38 685 against SARS-CoV-2 were not detected.

39
40
41 686 o An invalid result occurs when a colour band is not
42
43 687 produced in C, and the test must be repeated.

44
45
46 688 ► Official qRT-PCR results (carried out by INCMNSZ)

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48 689

49
50
51 690 All this information will be collected in a pre-established
52
53 691 medical history questionnaire for each potential participant.

54
55 692 The information obtained from the weekly assessment of adverse
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57
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1
2
3 693 events, and the results of the qRT-PCR for SARS-CoV-2 at 60
4
5 694 and 90 days (60 for the primary end point plus 30 more days of
6
7 695 follow-up) after starting treatment will be entered into an
8
9 696 online database. In order to ensure the quality of the data
10
11 697 collection, the database will be built in CASTOR, a database
12
13 698 on the Web that allows entering all the pre-defined data for
14
15 699 each participant, thus reducing human error. This information
16
17 700 will be stored on a server in the United States of America and
18
19 701 can only be accessed by the study's administrator. The data
20
21 702 may only be entered by a researcher in charge of collecting
22
23 703 the data sheets and emptying them.
24
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30 **Monitoring and quality assurance**

31
32 706 Every possible adverse event will be noted daily by the
33
34 707 participants in the agenda that will be delivered to them. This
35
36 708 agenda will be evaluated weekly by the researcher in charge of
37
38 709 monitoring the participants (who will be blinded to group
39
40 710 assignment). In case of unbearable adverse events for the
41
42 711 participants or that put their health at risk, an open line
43
44 712 will be available 24 hours a day with direct communication to
45
46 713 the researcher in charge of monitoring the study to report any
47
48 714 event that requires hospitalization or immediate evaluation at
49
50 715 the hospital. All participants with adverse events that put
51
52 716 their life or health at risk may be urgently assessed by
53
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3 717 personnel from both INCMNSZ and INR-LGII, if possible, by the
4
5 718 staff involved into the study. Patient follow-up investigator
6
7 719 will immediately contact the study administrator to disclose
8
9 720 the participant's assignment to treating physicians at that
10
11 721 institution, but the assignment will never be disclosed to
12
13 722 other investigators related to the study. All the study
14
15 723 expenses and/or attention of collateral effects will be covered
16
17 724 by the current cost of the financing SECTEI/061/2020.
18
19 725 Auditing will be carried out weekly, assessing adverse events,
20
21 726 capturing data in the corresponding datasheets by the study
22
23 727 administrator. Likewise, the data entered in the CASTOR web
24
25 728 base will be valued to validate its quality. The paper data
26
27 729 sheets must be kept in a special office dedicated to the study
28
29 730 in folders separated by volunteers with the informed consent
30
31 731 of each participant, the data of the medical history,
32
33 732 laboratory results, eligibility criteria, adverse event sheet
34
35 733 and results, molecular tests, as well as electrocardiogram.
36
37 734 The letter of revocation of informed consent will also be
38
39 735 protected if required. As part of the audit, an interim
40
41 736 analysis will be carried out 30 days after the study starts to
42
43 737 assess the possible adverse effects and whether these outweigh
44
45 738 the potential benefits of the intervention. In the adverse
46
47 739 event outweigh the potential benefits, termination of the study
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3 740 will be assessed. The approval of the research ethics committee
4
5 741 of the INR-LGII of Mexico has been obtained.
6

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9
10 743 **Statistical analysis**

11
12 744 Data analysis will be carried out by intention to treat, which
13
14 745 means that each participant will be analysed according to the
15
16 746 group assigned regardless of whether they modified their
17
18 747 treatment. The study variables will be divided according to
19
20 748 the allocation group. The statistical analysis will be carried
21
22 749 out by evaluation the difference between the different groups
23
24 750 of HCQ plus BHH versus placebos. Missing data will be handled
25
26 751 by multiple imputation analysis when missing at random. Deaths
27
28 752 will be censored.
29

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32 753

33
34 754 The primary objective will be expressed in number and
35
36 755 proportion for each group. The RR will be obtained as the
37
38 756 division between the proportion of primary outcomes in the
39
40 757 intervention group(s) by the proportion of primary outcomes in
41
42 758 the double placebo group. Adjusted risk ratios (aRR) will be
43
44 759 obtained using a log-binomial regression, adjusting for age
45
46 760 and gender as pre-specified confounding variables. It will be
47
48 761 expressed as RR with its respective 95% confidence interval
49
50 762 for the initial time, which is 60 days. Likewise, the result
51
52 763 will be expressed as absolute risk, which will be derived from
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1
2
3 764 the proportion of the primary outcome in the intervention group
4
5 765 minus the proportion of the primary outcome in the control
6
7 766 group. Secondly, the primary objective will be analysed with
8
9 767 the non-parametric estimate of the survival and risk function
10
11 768 using Kaplan-Meier curves for 60 days according to the
12
13 769 allocation group. The primary endpoint will be SARS-CoV-2
14
15 770 infection within the 60-day period, and the silencing variable
16
17 771 will be dropping out of the study for any reason. The comparison
18
19 772 of the survival curves between both groups will be carried out
20
21 773 using the log-rank test. Risk ratio will be used for treatment
22
23 774 effect. A log-binomial regression adjusted by age, gender,
24
25 775 service in which the participant works, body mass index, will
26
27 776 be used.

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33
34
35 778 For secondary outcomes such as the analysis at 90 days, the
36
37 779 same statistical analysis expressed in RR and absolute risk
38
39 780 will be used. Survival analysis will be used for the primary
40
41 781 endpoint only. An interim statistical analysis will be
42
43 782 performed 30 days after the study starts to assess possible
44
45 783 adverse effects and the efficacy of the intervention. The study
46
47 784 administrator will be the only one with access to the data.
48
49 785 For the interim analysis and the final analysis, the
50
51 786 administrator will export the data to Excel format to be
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3 787 analysed by the study statistician blinded to the assignment
4
5 788 of groups, participants, or results.
6

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9
10 790 **Adverse events, serious adverse events and suspected unexpected**
11
12 791 **serious adverse reactions**
13

14 792 By requiring the use of drugs, the participant will be exposed
15
16 793 to risks inherent to the drug used, ranging from mild to severe
17
18 794 or death. Any unexpected risks that may occur during the study
19
20 795 will be immediately explained to the participants and the
21
22 796 ethics committee. Any adverse event will be compiled and will
23
24 797 not be disclosed under any condition to anyone other than the
25
26 798 study administrator, treating physicians in case of severe
27
28 799 events, and the ethics committee. The results will be
29
30 800 completely anonymous concerning the names of the participants.
31
32 801 The results will be compiled and reported as combined
33
34 802 collective data.
35
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40
41 804 *Patient and public involvement*
42

43
44 805 Patients were not involved in the development of this research.
45
46 806 However, the results of the study will be communicated to the
47
48 807 study participants by sending the end product (published
49
50 808 article) to the provided email address.
51

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55 810 **ETHICS, DISSEMINATION AND SAFETY MONITORING**
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3 811 In case of adverse events or complications derived from the
4
5 812 study, participants will be assured attention by the staff of
6
7 813 the INCMNSZ in an enclosure that ensures the safety of the
8
9 814 participant, not subjecting volunteers to a higher risk of
10
11 815 contamination. This care will be extended until adverse events
12
13 816 are resolved. In case of no adverse events during the study,
14
15 817 medical attention will be extended at the aforementioned
16
17 818 institute until 15 days after the end of the study.
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23 820 This protocol has been approved by the local medical ethical
24
25 821 review committee at the INR-LGII with the internal number
26
27 822 INRLGII/25/20, and by the Federal Commission for Protection
28
29 823 against Sanitary Risks (in Spanish, Comisión Federal para la
30
31 824 Protección contra Riesgos Sanitarios, COFEPRIS), approval
32
33 825 number 203300410A0058/2020.
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36

37 826 The study results will be published in journals of worldwide
38
39 827 impact affiliated with the Journal Citation Reports. Likewise,
40
41 828 the results of the study will be disseminated in national and
42
43 829 international media, exposed in international and national
44
45 830 congresses, communicated to CONACYT, and recorded in
46
47 831 Clinicaltrials.gov according to the study identifier number.
48
49 832 The help of non-profit organizations will be sought to
50
51 833 disseminate the results of the investigation to interest
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53 834 groups.
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5 836 The complete protocol will be published on Clinicaltrials.gov
6
7 837 and the OSF - Center for Open Science platform <https://osf.io/>.
8
9 838 Where a DOI will be assigned, and the amendments made to the
10
11
12 839 original protocol will be assessed.
13

14 840
15

16 841 Amendments to the protocol may be made before the start of the
17
18 842 study and during the study. Any amendment to the protocol will
19
20
21 843 be clarified and posted on Clinicaltrials.gov under the same
22
23 844 identifier as this study. Likewise, any amendment will be sent
24
25 845 to the ethics committee of the same hospital.
26
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30 847 **AUTHOR CONTRIBUTIONS**
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32 848 JGM is the lead study investigator, developed the study
33
34 849 concepts and design, and wrote the manuscript by adapting the
35
36
37 850 original study protocol for publication, subsequent reviews
38
39 851 and amendments. EJHL, KMM and AAA contributed to the
40
41 852 development and refining of the protocol, writing of manuscript
42
43 853 and subsequent review. RJMP provided advanced methodological
44
45 854 and statistical input, and contributed to the study design and
46
47 855 subsequent amendments. RFC, TCH, PSSB, RAG and NML reviewed,
48
49 856 commented and informed methodology, development and writing of
50
51 857 the protocol.
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2
3 **859 FUNDING STATEMENT**
4

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6
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8
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10
11
12 863 SECTEI/061/20.
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15

16 **865 COMPETING INTERESTS STATEMENT**
17

18 866 None of the authors have conflict of interests, commercial
19
20
21 867 agreements, or receive financial fees or compensation from any
22
23 868 commercial or pharmaceutical company.
24

25 869
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27
28 **870 ETHICS APPROVAL**
29

30 871 This protocol has been approved by the local medical ethical
31
32 872 review committee at the INR-LGII with the internal number
33
34 873 INRLGII/25/20. Definitions of Research Risk Regulation of the
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36 874 General Health Law on Research for Health (in Spanish,
37
38 875 Reglamento de la Ley General de Salud en Materia de
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40 876 Investigación para la Salud)
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42 877 <http://www.inr.gob.mx/Descargas/Investigacion/Reglamentos->
43
44 878 [LGS-Materia-Investigacion-Salud.pdf](http://www.inr.gob.mx/Descargas/Investigacion/Reglamentos-LGS-Materia-Investigacion-Salud.pdf). ARTICLE 17; and by
45
46 879 Federal Commission for Protection against Sanitary Risks (in
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48 880 Spanish, Comisión Federal para la Protección contra Riesgos
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50 881 Sanitarios, COFEPRIS), approval number 203300410A0058/2020.
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5 884 **PROVENANCE AND PEER REVIEW**

6
7 885 Not commissioned; externally peer reviewed.

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12 887 **ORCID ID**

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14 888 Julio Granados-Montiel <https://orcid.org/0000-0002-0611-64>

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32 896 **REFERENCES**

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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

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).

Journal:	<i>BMJ Open</i>
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4 1 **New prophylaxis regimen for SARS-CoV-2 infection in health professionals with low doses**
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7 2 **of Hydroxychloroquine and Bromhexine: a randomized, double-blind placebo clinical trial**
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10 3 **(ELEVATE Trial).**
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37 27 Keywords: prophylaxis, SARS-CoV-2, COVID-19, health workers, hydroxychloroquine,
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40 28 bromhexine.
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ABSTRACT

For peer review only

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4 48 **Introduction:** SARS-CoV-2 infection in Mexico has caused ~2.5 million confirmed cases;
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7 49 around 20-25% of health workers will be infected by the virus at their workplace, with
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10 50 approximately 4.4% of mortality. High infectivity of SARS-CoV-2 is related with cell entry
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13 51 mechanism, through the angiotensin-converting enzyme (ACE) receptor. SARS-CoV-2
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17 52 requires transmembrane protease serine 2 (TMPRSS2) to cleave its spike glycoprotein and
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20 53 ensure fusion of host cell and virus membrane. We propose studying prophylactic treatment
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24 54 with hydroxychloroquine (HCQ) and bromhexine (BHH), which have been shown to be
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27 55 effective in preventing SARS-CoV-2 infection progression when administered in early
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30 56 stages. The aim of this study is to assess the efficacy of HCQ and BHH as prophylactic
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34 57 treatments for SARS-CoV-2 infection in healthy health workers exposed to the virus.

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37 58 **Methods and analysis:** Double-blind randomized clinical trial, with parallel allocation at a
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40 59 1:1 ratio with placebo, of low doses of HCQ plus BHH, for 60 days. Study groups will be
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44 60 defined as follows: 1) HCQ 200mg/d + BHH 8mg/8h vs 2) HCQ placebo plus BHH placebo.
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47 61 Primary endpoint will be efficacy of both interventions for the prevention of SARS-CoV-2
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50 62 infection, determined by the risk ratio (RR) of infected personnel and the absolute risk. At
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54 63 least a 16% reduction in absolute risk is expected between the intervention and placebo
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3 64 groups; a minimum of 20% infection is expected in the placebo group. The sample size

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7 65 calculation estimated a total of 214 patients assigned: two groups of 107 participants each.

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10 66 **Ethics and dissemination:** This protocol has been approved by the local Medical Ethics

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13 67 Committee (National Institute of Rehabilitation ‘Luis Guillermo Ibarra Ibarra’, approval

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16 68 number INRLGII/25/20) and by the Federal Commission for Protection against Sanitary

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19 69 Risks (COFEPRIS, approval number 203300410A0058/2020). The results of the study will

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22 70 be submitted for publication in peer-reviewed journals and disseminated through

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25 71 conferences.

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28 72 **Trial registration number 2a:** NCT04340349.

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31 73 2b: NA

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34 74 Protocol version: #4

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40 76 **STRENGTHS AND LIMITATIONS OF THIS STUDY**

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43 77 *Strengths*

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46 78 ► This is a double-blind randomized single-centre clinical trial, involving low doses of

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49 79 hydroxychloroquine and bromhexine, adequately powered to provide clinically

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3 80 relevant information regarding prophylactic treatment for SARS-CoV-2 infection in

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7 81 health care personnel.

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10 82 ► Bromhexine has minimal side effects and is commercially available worldwide so,

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13 83 positive results could be applied in a timely fashion in different regions.

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20 85 *Limitations*

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23 86 ► Long-term use of hydroxychloroquine can cause heart rhythm problems

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27 87 ► For the moment, people who are not candidates to receive the vaccine, due severe

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30 88 allergies, will not be included.

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33 89 ► Hydroxychloroquine has not been shown to be effective in monotherapy or with

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37 90 azithromycin, but adjunctive BHH could be an effective combination to inhibit

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40 91 SARS-Cov-2 infection.

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45 92 **INTRODUCTION**

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48 93 In Mexico, up to June 2021, have been produced more than 2.5 millions confirmed cases and

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51 94 ~232,000 deaths have arisen[1]. The age group ranging between 30 and 79 years is the most

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4 95 highly affected, where 81% present mild symptoms, 14% severe and 5% critical, requiring
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7 96 intensive care unit management.
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10 97 SARS-CoV-2 is a single-stranded RNA virion, member of the *Betacoronavirus* genus [2].
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13 98 SARS-CoV-2 has an incubation period between 3 to 10 days, with different incubation
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17 99 periods related with different clinical symptoms [3,4] . It is transmitted through respiratory
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20 100 droplets from infected humans and through contact with contaminated fomites and aerosols;
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23 101 moreover, even asymptomatic persons in close contact can transmit the disease [5]. The
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27 102 mechanism through which the virus infects the respiratory cell is due to the angiotensin-
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30 103 converting enzyme protein 2 (ACE-2) receptor. This receptor is found in multiple tissues
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33 104 such as the oral cavity, brain, kidneys, gut, and placenta [6–8].
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37 105 Health personnel is not exempt from contracting the disease. In China, it was reported that
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40 106 3.5-4.4% of the infected population belonged to this group, and 14.8% presented
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43 107 characteristics of severity or critical illness [4,9,10]. Italy, around 20% of healthcare
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47 108 professionals became infected [11]; mean age of health workers who died was 55 years
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50 109 (range of 29-72 years) and mean period from hospital admission to death was 19 days, (range
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54 110 1-47 days) [9].
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4 111 Treatment of the SARS-Cov-2 infection has led different research groups to work on the
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7 112 development of vaccines. However, the use of vaccines can be a challenge. The first trials
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10 113 have shown that the immune protection is not 100% and protection may wane over time so
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13 114 periodic vaccination or booster shots for new variants may be needed . On the other hand,
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16 115 because the virus is RNA and the mutation rate is high, we can expect new variants that
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20 116 reduce or nullify the effectiveness of the vaccines, this depends on the origin of vaccine, if it
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23 117 is made with viral vectors (such as from CanSino or AstraZeneca), if it is mRNA (such as
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26 118 from Moderna or Pfizer-BioNTech) or if it is inactivated virus (Sinovac). Mainly because the
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30 119 development of vaccine that can be efficient for the new variants could be delayed and this
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33 120 could once again increase the number of people who acquire the SARS-CoV-2 virus. On the
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37 121 other hand, around the world there are groups of people who are against vaccination, or
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40 122 people that have severe allergies, as well as populations that will take much longer to reach
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43 123 the moment when they can acquire the vaccine, so it is extremely necessary that people who
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46 124 do not vaccinate by choice, by disease or by the lack of the vaccine, have an alternative to
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50 125 avoid infection and avoid the spread of the virus.
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3 126 Therefore, it is important to develop a pharmacological strategy that allows the use of
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7 127 prophylactic drugs for the prevention of SARS-CoV-2 infection.
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10 128 Chloroquine (CQ) and Hydroxychloroquine (HCQ) are known as an antimalarial agent; HCQ
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13 129 is a hydroxylated derivative from CQ. CQ and HCQ have gained attention as possible
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17 130 therapies in Covid-19 disease. In overdose, both drugs can cause severe, potentially life-
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20 131 threatening effects as visual disturbances, corneal opacities, irreversible retinopathy can
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24 132 occur with cumulative doses exceeding 100 grams. When lower daily doses (250 mg are
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27 133 used) retinopathy may not occur after many years of treatment [12]. The above indicates that
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30 134 the use of HCQ at low doses to avoid SARS-CoV-2 infection, has a low possibility of being
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34 135 toxic and could be used as a prophylactic treatment. HCQ has been used in several viral
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37 136 infections, for example, as replication inhibitor for the dengue virus, decreasing in vitro virus
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40 137 infection and promoting activation of different immunological signal pathways [13]. It has
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44 138 also been used to treat patients infected with hepatitis C virus decreasing viral load, with
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47 139 minimal adverse effects reported [14]. HCQ has been reported to block viral infection by
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50 140 increasing the endosomal pH required for virus fusion to the cell, as well as interfering with
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54 141 cellular SARS-CoV-2 cell receptors, through inhibition of receptor glycosylation by ACE2
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4 142 [15–18]. HCQ has immunomodulatory effects; it inhibits production and release of pro-
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7 143 inflammatory cytokines, that are associated with severe disease development [19,20] .
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10 144 Recently, it has been reported that HCQ works as a autophagy inhibitor, interfering with viral
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13 145 infection and replication [21]. There is recent evidence that HCQ could be used to treat
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16 146 COVID-19; studies in high-risk patients show that the use of HCQ was associated with a
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20 147 lower risk of intubation or death [22]. Recent study showed that pre-treatment with HCQ has
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23 148 shown a better effect on antiviral activity [17] and it has been reported that loading doses of
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26 149 1600 mg HCQ followed by 600 mg daily doses are needed have a relevant effect to SARS-
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30 150 CoV-2 inhibition within 72 hours in 60% of COVID-19 patients [23]. Finally, a study where
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33 151 evaluated the antiviral mechanisms of CQ and the adverse effects, repositioned the CQ to
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36 152 have more efficacy when used as a prophylactic treatment rather than as a therapeutic [24].
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40 153 On the other hand, CQ and HCQ has been reported to have various adverse effects, the CQ
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43 154 being the most toxic in overdose. However, it was recently published that *in vivo* trials are
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46 155 lacking to determine whether this drug is useful as a prophylactic treatment against SARS-
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50 156 Cov-2 [25]. Based on the above, do more studies will be important to determine its
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53 157 effectiveness at low doses (<250 mg) as a prophylactic treatment.
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4 158 Another pharmacological option to treat SARS-CoV-2 infection is Bromhexine (BHH). BHH
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7 159 modifies the composition of mucus, increases ciliary clearance and decreases coughing,
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10 160 improving respiratory symptoms. It has also been reported to enhance the effects of some
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13 161 antibiotics [26]. The mechanism by which SARS-CoV-2 enters human cells depends on the
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16 162 ACE-2 receptor and the human transmembrane serine protease (TMPRSS2), on which BHH
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20 163 has a specific inhibitory effect [27,28]. BHH has been used to treat pneumonic damage in
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23 164 both lungs during early infection [29]. BHH turns out to be an ideal candidate for SARS-
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26 165 CoV-2 treatment, since it has few contraindications, and its side effects are minimal,
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30 166 demonstrating an extensive margin of pharmacological safety. BHH is widely available over
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33 167 the counter, and its low cost makes it an ideal therapeutic option.
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37 168 According to a letter published in the New England Journal of Medicine, of 77,262 patients
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40 169 infected by SARS-CoV-2, 3387 (4.4%) were health workers [9]. Of these, 23 have died from
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43 170 this disease. The prevalence of infections in health personnel is alarming since health services
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47 171 in first world countries have been overwhelmed by this disease. In Italy, around 20% of health
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50 172 professionals had a SARS-CoV-2 infection [11]. Faced with a highly contagious disease, the
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53 173 care of health workers, who are first line of contact and on whom the health system of each
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4 174 country depends, is essential. This research regarding the use of HCQ and BHH in health
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7 175 personnel will allow us to determine and compare the effectiveness of both interventions,
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10 176 which is of vital importance to clarify whether these treatments may prevent the appearance
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13 177 of infection in this population. Describing for the first time that HCQ plus BHH could
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16 178 function for disease prevention, would allow us to provide prophylaxis to health
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20 179 professionals worldwide. Therefore, the use of HCQ and BHH in healthy health personnel
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23 180 exposed to patients with confirmed or suspected SARS-CoV-2 will significantly reduce
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27 181 infection.

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4 182 **METHODS AND ANALYSIS**
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7 183 **Study design**
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10 184 Double-blind randomized clinical trial, with parallel allocation at a 1:1 ratio with HCQ +
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13 185 BHH vs placebo for both drugs for 60 days, to determine the efficacy of the combined drugs
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17 186 for the prevention of SARS-CoV-2 infection in healthcare workers.
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23 188 **Participants**
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27 189 The study will be carried out at the “Instituto Nacional de Rehabilitación, Luis Guillermo
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30 190 Ibarra Ibarra” (INR-LGII). This institution is a tertiary hospital that at this time has not been
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33 191 designated as a COVID-19 centre. The Mexican government defined 3 phases to determine
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37 192 risk for SARS-CoV-2 infection: imported cases from outside Mexico; community infection
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40 193 and spread of the disease throughout the country (also known as Phase 3). In the latter, it is
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44 194 assumed that every person who enters a hospital is a potentially infected carrier; currently
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47 195 our centre is in Phase 3. Likewise, health personnel who work at the “Instituto Nacional de
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50 196 Ciencias Médicas y Nutrición, Salvador Zubirán” (INCMNSZ), which is a COVID-19
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4 197 designated tertiary centre, and who meet inclusion criteria of the protocol will be invited to
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7 198 participate in the study.
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10 199 Inclusion of participants will be assessed according to the eligibility criteria. Table 1 shows
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13 200 the classification and characteristics of study variables. Continuous variables will be assessed
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17 201 for normality. Variables with a normal distribution will be compared using Student's t-test,
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20 202 non-parametric variables using the Mann-Whitney U-test. Categorical variables will be
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23 203 evaluated using the Chi-squared test.
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30 205 *Inclusion criteria*
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33 206 ▶ Health personnel working at INR LGII or INCMNSZ who wish to participate in the
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37 207 study and sign the informed consent.
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40 208 ▶ Over 18 and under 60 years of age, both genders.
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43 209 ▶ Contacting with suspected or confirmed SARS-CoV-2 infection.
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47 210 ▶ Normal electrocardiogram.
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50 211 *Exclusion criteria*
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4 212 ▶ Positive quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) test
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7 213 for SARS-CoV-2 at the time of inclusion.
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10 214 ▶ Panel of IgG or IgM antibodies positive for SARS-CoV-2 at the time of inclusion.
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13 215 ▶ Development of respiratory symptoms suspicious of SARS-CoV-2 infection during
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15
16 216 the first 7 days after treatment is initiated, confirmed by qRT-PCR and IgG or IgM
17
18
19 217 antibodies positive for SARS-CoV-2.
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21
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23 218 ▶ History of allergies to any hydroxychloroquine or bromhexine related compound or
24
25
26 219 medication.
27
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29
30 220 ▶ Use of immunosuppressors for any reason.
31
32
33 221 ▶ History of bone marrow transplant.
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36 222 ▶ Known glucose-6-phosphate dehydrogenase deficiency.
37
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39 223 ▶ Chronic kidney disease or glomerular filtration <20ml/min.
40
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43 224 ▶ Use of other drugs with reported pharmacological interactions (i.e., digitalis,
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45
46 225 flecainide, amiodarone, procainamide, or propafenone).
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50 226 ▶ History of long QT syndrome.
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53 227 ▶ Electrocardiogram with QTc>500 msec.
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228 ▶ Pregnant or breastfeeding personnel.

229 ▶ Epilepsy.

230 ▶ Known liver disease.

231 ▶ Personnel who have received the Covid-19 vaccine

232 *Elimination criteria*

233 ▶ Personnel who decide to leave the study for any reason not related to adverse events.

234 ▶ Personnel with incomplete information on the primary outcome (qRT-PCR for
235 SARS-CoV-2).

236 ▶ Personnel who are relocated to work in another institution.

237 ▶ Personnel who do not wish to participate in the study

238

239 **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, PaO ₂ / FiO ₂ ratio <250, Infiltrates multilobars, confusion / disorientation, uremia [BUN > 20 mg / dL], leukopenia [<4,000], thrombocytopenia [<100,000 platelets / mm ³], hypothermia [core temperature <36°C], 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

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241 **Sample size calculation**

242 According to the study by Remuzzi A et al. [11], the proportion of healthcare workers

243 infected with SARS-CoV-2 and confirmed by RT-PCR was 20%. Taking this 20% as our

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3 244 null hypothesis, we estimate that the proportion of infections in the intervention group will
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7 245 be 4%. Using a two-tailed test, with a type I error of 0.05, a power of 90%, and taking into
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10 246 account a loss of 10% of participants for each group, we estimate that a total of 214
11
12
13 247 participants will be required, distributed in parallel groups (1:1) of 107 each. This number of
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16
17 248 volunteers will allow us to find a difference of 16% between groups with a power of 90%
18
19
20 249 and an attrition of 20%. To ensure that desired simple size is reached, all health workers
21
22
23 250 involved in managing patients suspected or infected by SARS-CoV-2 will be invited
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27 251 personally and by institutional email.
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30 252

33 253 **Interventions**

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37 254 Interventions will consist of low doses of HCQ 200 mg tablets every 24 hours for 60 days
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40 255 plus BHH 8 mg tablets every 8 hours for 60 days. Study groups will be defined as follows:
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43
44 256 1) HCQ plus BHH vs placebo for both drugs. Fabrication of both drugs and placebos will be
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46
47 257 provided to our centre by a hired laboratory. Both drugs will be provided to participants
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49
50 258 directly at the hospital by a researcher blinded to group assignment process. To ensure that
51
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54 259 the intervention is carried out, each participant will be asked to keep a written record of the
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3 260 days and time the medication was administrated. This document will be reviewed weekly to
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7 261 verify that more than 50% adherence to treatment is maintained. Participants will be asked
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10 262 to record any symptoms related to the use of the medication, which will be reviewed by a
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13 263 researcher blinded to group assignment, weekly, or at the participants' request.
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20 265 If any of the participants present symptoms of SARS-CoV-2 infection after the first 14 days
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23 266 from the beginning of the intervention or positive qRT-PCR is present, the drug will not be
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27 267 discontinued. If the participant presents adverse events related to the drugs that are severe or
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30 268 intolerable, treatment will be suspended. If the participants report an adherence of less than
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33 269 50% of the medication, the intervention will not be discontinued to avoid imbalances between
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36
37 270 groups. Use of drugs that interact with HCQ or BHH such as flecainide, digitalis,
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40 271 amiodarone, procainamide or propafenone will be prohibited. If a participant has to use these
41
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43 272 drugs during the study period, they will be eliminated from the study. A free diet and outdoor
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47 273 activity will be allowed since these do not intervene with the implementation of the treatment
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49
50 274 or have interaction with the drugs used. Finally, incidence of adverse events such as nausea,
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53 275 vomiting, abdominal pain, rash, itchy skin, hair loss, lengthening of the QT interval in the
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3 276 electrocardiogram, corneal opacity, cardiac arrhythmias, heart failure and death will be
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7 277 determined.

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13 279 **Randomization and treatment allocation**

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17 280 Group randomization will be in a centralized and straightforward way using the Web program
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19
20 281 www.randomization.com. It will be carried out independently by a researcher blinded to
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23 282 inclusion criteria, delivery of medication, participant follow-up, results, statistical analysis,
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25
26
27 283 and writing of the final manuscript. Allocation will be established, for 214 participants in
28
29
30 284 blocks of 107 assigned. The selection of health workers will be made regardless of the
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34 285 hospital shift, work schedule, or assigned area. If the desired sample size is not reached, the
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37 286 inclusion of personnel involved in the first line of care of other referral hospitals for patients
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39
40 287 with SARS-CoV-2 will be considered.

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42
43
44 288 An independent researcher will allocate patients to the desired groups. Envelopes will be
45
46
47 289 correctly sealed by the pharmacy department and will contain HCQ plus BHH or placebos
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49
50 290 as previously mentioned. In those who do not require HCQ and BHH, the drug will be
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3 291 replaced by tablets identical in colour and taste but lacking the active substance. In this way,
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7 292 drugs used in both groups will be indistinguishable.
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13 294 Researcher A will recruit the participants and assess the inclusion criteria according to the
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16 295 serological, electrocardiographic, biochemical results and clinical investigation. Once
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19 296 included, volunteers will go to another office with researcher B, who will be blinded to the
20
21

22 297 first procedure and the rest of the study. Researcher B will assign the groups independently,
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25 298 centrally, and through the use of the web program. This same researcher will be the one who
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28 299 makes the packages indistinguishable to the person providing the drugs to the participant.
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31 300 Researcher C will provide treatment in a sealed envelope or box to the participant in the order
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33

34 301 of assignment, without knowing each participant's study group. This researcher will also be
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36

37 302 blinded to the rest of the results. Participants will be blinded to the treatment they will receive.
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40 303 The researchers performing follow-up, researchers for result assessment, and the researcher
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43 304 who performs the statistical analysis will be blinded.
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3 306 Informed consent will be obtained only by researcher A. If researcher A is not available, the
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7 307 study administrator may obtain informed consent for participation. The informed consent
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10 308 will contain the authorization to participate in the study and the authorization for taking
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12
13 309 biological samples, electrocardiogram, and authorization to handle personal information. All
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15
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17 310 participants will complete a written informed consent included on the first page of the
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19
20 311 questionnaire that requires permission to participate in the study. No candidate is required to
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22
23 312 participate in the study, and their participation is based on the agreement that they may
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27 313 withdraw at any time. All participants have the right to withdraw from the study if they feel
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30 314 uncomfortable answering a question or with a test to be performed. Also, no one, including
31
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33 315 the research team, will require a reason why the participant decides to leave the study.
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37 316 In order to protect participant confidentiality, each one will be assigned a participation
38
39
40 317 number, and all biological samples, as well as medical history information, will be identified
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42
43 318 by the participant's initials and participant number. Part of the confidentiality protection
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45
46
47 319 process will include data capture only by the researcher in charge of data capture (researcher
48
49
50 320 D), who will be the same for all participants and the entire study. Secondly, the study
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53 321 administrator may also enter data into the database if researcher D is unavailable.
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7 323 The study administrator will be blinded to allocation and results of the participants. However,
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10 324 the administrator will be the only one who will be able to reveal the group and treatment
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13 325 assignment in any of the cases: major adverse events such as cardiac arrhythmias, heart
14
15
16 326 failure, major neurological abnormalities, atrial or ventricular fibrillation, kidney failure, or
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18
19
20 327 any adverse event related to pharmacological treatment that endangers the life or any organ
21
22
23 328 of the participant's body. The objective of revealing the assignment by the study
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25
26 329 administrator will be to provide the participant of a timely treatment according to the drugs
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29
30 330 ingested.
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37 332 **Participant timeline and intervention**
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40 333 The inclusion of participants will be evaluated according to the eligibility criteria and by
41
42
43 334 invitation. Volunteers who wish to participate in the study will be scheduled the next day at
44
45
46
47 335 a specialized office to carry out all the relevant studies to ensure the inclusion criteria. These
48
49
50 336 include a medical history, anthropometric measurements such as weight and body mass
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52
53 337 index, electrocardiogram (every week until the end of the study or when the participant
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3 338 requests it if they have any discomfort), complete blood count (cbc), complete blood
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7 339 chemistry, and serological test for antibodies and qRT-PCR for SARS-CoV-2. Volunteers
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10 340 will be asked for information to contact them once the serological results are obtained.
11
12
13 341 Once the results are obtained (approximately 3 days), personnel eligible to participate in the
14
15
16 342 study will be contacted. They will meet in a particular office to speak with a researcher who
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18
19 343 will be in charge of carrying out the eligibility criteria and medical history checklist. This
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21
22 344 researcher will be different from the one who makes the assignment, who delivers the
23
24
25 345 medicine and the one who evaluates the results and performs the statistical analysis. The
26
27
28 346 assignment of the group of each participant will be performed, and the participant will not
29
30
31 347 know the group they have been assigned. This information will be known for the researcher
32
33
34 348 in charge, unrelated to the delivery of the treatment, results, or inclusion of the participant in
35
36
37 349 the study. After the assignment, the volunteers will receive the assigned treatment at the
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39
40 350 pharmacy using a code in a sealed envelope assigned by the Web. Participants who meet the
41
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43 351 inclusion criteria and there is no reason for exclusion will proceed to the second phase of
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46 352 group assignment with researcher B, the next business day at a different time or office than
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49 353 researcher A.
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7 355 The group of researchers in charge of monitoring the participants, who will be blinded to the

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9
10 356 group assignment at all times, will be in charge of assessing each participant's adverse event

11
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13 357 and treatment adherence record weekly. These follow-up researchers will be available 24

14
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16 358 hours a day throughout the week if participants experience undesirable adverse events that

17
18
19 359 require urgent attention or that do not allow them to continue with drug treatment. If this

20
21
22 360 situation happens, the researcher in charge of the follow-up will contact the study

23
24
25 361 administrator to reveal to the treating physicians the treatment received by the participant.

26
27
28 362 Health evaluation of all participants will be performed at day 30, day 60 and day 90, this

29
30
31 363 includes electrocardiogram analysis, blood chemistry analysis, antibody test, qRT-PCR, or

32
33
34 364 at request of the participant due to adverse clinical symptoms.

35
36
37 365 At the end of the first 60 days, a new qRT-PCR will be requested from each participant. All

38
39
40 366 participants who present symptoms after the first 7 days of initiation of the intervention, will

41
42
43 367 be considered as a positive individual for the analysis and will not be excluded from the

44
45
46 368 study. The same action will be carried out 90 days after the start of treatment for both groups.

47
48
49 369 After 60 days, the treatment will be suspended and the results of the qRT-PCR samples for

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3 370 SARS-CoV-2 will be evaluated. After finishing the intervention (60 days), all participants
4
5
6
7 371 will be followed-up 30 more days with a new qRT-PCR at day 90 after initiation of the
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10 372 intervention and 30 days after the end of the intervention, to assess the efficacy or the
11
12
13 373 treatment during the follow-up period.
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20 375 **Outcome measures**
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22
23 376 This study compares the efficacy of the use of HCQ plus BHH (as a conjoined treatment) in
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27 377 prophylactic doses for 60 days in healthy health personnel exposed to the first line of care in
28
29
30 378 confirmed patients with suspected infection by SARS-CoV-2.
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37 380 *Primary endpoint*
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40 381 The primary endpoint will be the proportion of health personnel infected by SARS-CoV-2 at
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42
43 382 60 days after starting treatment, in both groups. The infection will be diagnosed using qRT-
44
45
46
47 383 PCR for relative expression of the mRNA SARS-CoV-2 and the measure of IgM and IgG
48
49
50 384 antibodies anti-SARS-CoV-2 after day 7 of treatment using rapid test Cellex qSARS-CoV-2
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52
53 385 IgG/IgM. All participants presenting symptoms with positive qRT-PCR after 7 days of
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4 386 initiation of the intervention, will be considered positive and will be included in the analysis.
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7 387 The study period will be 90 days (60 days for the primary end point plus 30 days of follow-
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9

10 388 up). The proportion of infected personnel will be evaluated using relative risk (RR) and
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12

13 389 absolute risk increase (ARI) with their respective 95% confidence intervals, in the established
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16 390 time. The disease-free period in the 60 days will also be evaluated, analysing the cumulative
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18

19 391 incidence of healthy personnel, presence of infection will be confirmed by qRT-PCR for
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22 392 SARS-CoV-2 and by presence of IgM and IgG antibodies for SARS-CoV-2. The censoring
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24

25 393 variable will be interruption of treatment either due to death, adverse events, or any
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27

28 394 elimination criteria. Since there is the possibility of false positives and negatives with qRT-
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31 395 PCR, we will perform qualitative measurements of IgM and IgG with the Cellex qSARS-
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33

34 396 CoV-2 IgG/IgM Rapid test which is authorized by FDA. The test can be used on serum,
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37 397 plasma, or whole blood samples. The clinical sensitivity of the assay was 93.8% and the
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40 398 clinical specificity was 96%[30].
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50 400 *Secondary endpoints*
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4 401 The secondary endpoint will be the proportion of health personnel infected 90 days after
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7 402 starting treatment in both groups. The infection will be diagnosed using qRT-PCR for relative
8
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10 403 expression of the mRNA of SARS-CoV-2 and the measure of IgM and IgG antibodies anti-
11
12
13 404 SARS-CoV-2 after day 7 of the start of treatment using rapid test Cellex qSARS-CoV-2
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17 405 IgG/IgM. The study period will be 90 days. The proportion of infected personnel will be
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19
20 406 evaluated using RR and ARI with their respective 95% confidence intervals, in the
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22
23 407 established time. The disease-free period in the 90 days will also be evaluated by analysing
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26
27 408 the cumulative incidence of healthy personnel, and the presence of confirmed infection by
28
29
30 409 qRT-PCR of SARS-CoV-2 will be the outcome. The censoring variable will be the
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34 410 discontinuation of treatment either due to death, adverse events, or any elimination criteria.
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37 411 Also, secondary outcomes will be, in case of a positive SARS-CoV-2 result, the need for
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40 412 oxygen use, admission to the intensive care unit (ICU), presence of pneumonia by computer
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44 413 tomography scan (CT), death, severe pneumonia defined by the American Thoracic
45
46
47 414 Association, time from hospitalization to recovery in days.
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50 415 Another secondary endpoint will be adverse events, defined as the presence of any of the
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54 416 following during the study period: death, nausea, vomiting, abdominal pain, diarrhoea, rash,
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4 417 itchy skin, hair loss, lengthening of the QT interval in the electrocardiogram (>500msec),
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7 418 corneal opacity, cardiac arrhythmias, heart failure or kidney failure (renal clearance
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10 419 <20ml/min). The proportion of the compound of adverse events between the groups will be
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12
13 420 analysed using RR and ARI for 60 days with their respective 95% confidence intervals.
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20 422 The efficacy of the treatment will be established as the proportion of volunteers infected with
21
22
23 423 SARS-CoV-2. This difference should be sufficient to avoid overlapping of the 95%
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26
27 424 confidence intervals. It will be considered effective if the intervals do not overlap and
28
29
30 425 ineffective if when comparing groups, they have a proportion of infected whose confidence
31
32
33 426 intervals overlap. This type of evaluation will allow an adequate understanding of the
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35
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37 427 efficacy of the treatment in both groups.
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40 428 **Handling and storage of data and documents**

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42
43 429 Before the start of the study, the researchers in charge of the recruitment, assignment, and
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47 430 delivery of drugs will be trained to perform the task assigned to them at least 3 days before
48
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50 431 the start of the study.
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4 432 Researcher A will assess the eligibility criteria of potential participants and perform a detailed
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7 433 clinical examination to assess whether they can participate in the study. The data that will be
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10 434 collected initially will be the following:

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16
17 436 ► Medical history (includes personal data): study identifier number, history number,
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20 437 name, date of birth, gender, occupation, marital status, nationality, current residence,
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23 438 degree of studies (primary, secondary, upper secondary, bachelor degree,
24
25
26
27 439 postgraduate), hospital service to which they belong and the number of hours worked
28
29
30 440 per week.

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34 441 ► Personal history: alcohol intake (yes/no; how many glasses of beer or alcoholic
35
36
37 442 beverages do you consume per week), smoking habit (yes/ no; and number of
38
39
40 443 cigarettes per day), drug use (yes/no), diet per week (dietary restrictions and number
41
42
43
44 444 of meals per day) and number of hours of sleep per day.

45
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47 445 ► Gynaecological history (in women): Number of pregnancies, number of live children,
48
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50 446 menarche, menopause.

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4 447 ▶ History of respiratory disease, history of gastrointestinal disease, nephrological,
5
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7 448 neurological, haematological, cardiovascular, allergies.
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10 449 ▶ Genetic family history, such as hypertension, diabetes, heart disease, kidney disease.
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13 450 ▶ Physical examination: blood pressure, heart rate, respiratory rate, temperature,
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15
16
17 451 weight, height, body mass index, skin lesions, head and neck inspection, respiratory
18
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20 452 inspection (chest symmetry, lung expansion, palpation of the bases and preserved
21
22
23 453 vertices, lung percussion, auscultation for lung murmur, breath sounds).
24
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26
27 454 Cardiovascular inspection (palpation of the fifth intercostal space, auscultation of
28
29
30 455 heart sounds, pulses that are palpable and symmetrical), abdominal inspection
31
32
33 456 (palpation, percussion and auscultation of peristaltic sounds), neurological evaluation
34
35
36
37 457 (Glasgow, active motility, passive motility, reflex motility, cranial nerves,
38
39
40 458 sensitivity).
41
42
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44 459 ▶ Complete blood count: haematocrit, leukocytes, segmented (%), lymphocytes (%),
45
46
47 460 monocytes (%), mean corpuscular volume, platelets.
48
49
50 461 ▶ Blood chemistry: glycaemia, urea, creatinine, sodium, potassium, chlorine, aspartate
51
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54 462 transaminase, alanine transaminase, alkaline phosphatase, total bilirubin.
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4 463 ▶ Muscle enzymes (creatine kinase)
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7 464 ▶ Clotting times: thrombin time, prothrombin time, international normalized ratio.
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10 465 ▶ Electrocardiogram: rhythm, heart rate, heart axis, evaluation of P wave, PR interval,
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13 466 duration of QRS, QT interval, time of T wave. The electrocardiogram will be
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17 467 performed using an instrument calibrated and validated for its use internationally,
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19
20 468 weekly.
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23 469 ▶ Molecular test results for IgG and IgM antibodies:
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26
27 470 ○ The FDA approved product called Cellex qSARS-CoV-2 IgG/IgM Rapid Test
28
29
30 471 will be used for serological determination. The device cassette, sample, and
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33 472 buffer solution must be at room temperature. The sample (10 µL) is
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37 473 transferred to the centre of the sample well. After the sample well is free of
38
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40 474 liquid, two drops of sample diluent are added. After fifteen to twenty minutes,
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42
43
44 475 read the test results. Results should not be read after twenty minutes.
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46
47 476 ○ A positive IgM result occurs when a coloured band appears on the M test line
48
49
50 477 (M) and the control line (C) and indicates that IgM against SARS-CoV-2 is
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54 478 present.
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4 479 ○ A positive IgG result occurs when a coloured band appears on the G test line
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7 480 (G) and the control line (C) and indicates that IgG against SARS-CoV-2 is
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10 481 present.
- 11
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13 482 ○ A positive result for IgM and IgG occurs when coloured bands occur both M
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15
16 483 and G, as well as C.
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19
20 484 ○ A negative result occurs when a coloured band appears in C only and indicates
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23 485 that IgM and IgG antibodies against SARS-CoV-2 were not detected.
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27 486 ○ An invalid result occurs when a colour band is not produced in C, and the test
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30 487 must be repeated.

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33 488 ▶ Official qRT-PCR results

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37 489 All this information will be collected in a pre-established medical history questionnaire for
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40 490 each potential participant. The information obtained from the weekly assessment of adverse
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43 491 events, and the results of the qRT-PCR for SARS-CoV-2 at 60 and 90 days (60 for the
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47 492 primary end point plus 30 more days of follow-up) after starting treatment will be entered
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49
50 493 into an online database. In order to ensure the quality of the data collection, the database will
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54 494 be built in CASTOR, a database on the Web that allows entering all the pre-defined data for

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4 495 each participant, thus reducing human error. This information will be stored on a server in
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7 496 the United States of America and can only be accessed by the study's administrator. The data
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9
10 497 may only be entered by a researcher in charge of collecting the data sheets and emptying
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13 498 them.

17 499 **Monitoring and quality assurance**

20 500 Every possible adverse event will be noted daily by the participants in the agenda that will
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22
23 501 be delivered to them. This agenda will be evaluated weekly by the researcher in charge of
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26 502 monitoring the participants (who will be blinded to group assignment). In case of unbearable
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30 503 adverse events for the participants or that put their health at risk, an open line will be available
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33 504 24 hours a day with direct communication to the researcher in charge of monitoring the study
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37 505 to report any event that requires hospitalization or immediate evaluation at the hospital. All
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40 506 participants with adverse events that put their life or health at risk may be urgently assessed
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44 507 by personnel from both INCMNSZ and INR-LGII, if possible, by the staff involved into the
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47 508 study. Patient follow-up investigator will immediately contact the study administrator to
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50 509 disclose the participant's assignment to treating physicians at that institution, but the
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54 510 assignment will never be disclosed to other investigators related to the study. All the study
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3 511 expenses and/or attention of collateral effects will be covered by the current cost of the
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7 512 financing SECTEI/061/2020.
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10 513 Auditing will be carried out weekly, assessing adverse events, capturing data in the
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13 514 corresponding datasheets by the study administrator. Likewise, the data entered in the
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16 515 CASTOR web base will be valued to validate its quality. The paper data sheets must be kept
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19
20 516 in a special office dedicated to the study in folders separated by volunteers with the informed
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23 517 consent of each participant, the data of the medical history, laboratory results, eligibility
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26 518 criteria, adverse event sheet and results, molecular tests, as well as electrocardiogram. The
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30 519 letter of revocation of informed consent will also be protected if required. As part of the audit,
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33 520 an interim analysis will be carried out 30 days after the study starts to assess the possible
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36 521 adverse effects and whether these outweigh the potential benefits of the intervention. In the
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40 522 adverse event outweigh the potential benefits, termination of the study will be assessed. The
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43 523 approval of the research ethics committee of the INR-LGII of Mexico has been obtained.
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50 525 **Statistical analysis**
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3 526 Data analysis will be carried out by intention to treat, which means that each participant will
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7 527 be analysed according to the group assigned regardless of whether they modified their
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10 528 treatment. The study variables will be divided according to the allocation group. The
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13 529 statistical analysis will be carried out by evaluation the difference between the different
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16 530 groups of HCQ plus BHH versus placebos. Missing data will be handled by multiple
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20 531 imputation analysis when missing at random. Deaths will be censored.
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27 533 The primary objective will be expressed in number and proportion for each group. The RR
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30 534 will be obtained as the division between the proportion of primary outcomes in the
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33 535 intervention group(s) by the proportion of primary outcomes in the double placebo group.
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37 536 Adjusted risk ratios (aRR) will be obtained using a log-binomial regression, adjusting for age
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40 537 and gender as pre-specified confounding variables. It will be expressed as RR with its
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43 538 respective 95% confidence interval for the initial time, which is 60 days. Likewise, the result
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47 539 will be expressed as absolute risk, which will be derived from the proportion of the primary
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50 540 outcome in the intervention group minus the proportion of the primary outcome in the control
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54 541 group. Secondly, the primary objective will be analysed with the non-parametric estimate
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4 542 of the survival and risk function using Kaplan-Meier curves for 60 days according to the
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7 543 allocation group. The primary endpoint will be SARS-CoV-2 infection within the 60-day
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10 544 period, and the silencing variable will be dropping out of the study for any reason. The
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13 545 comparison of the survival curves between both groups will be carried out using the log-rank
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17 546 test. Risk ratio will be used for treatment effect. A log-binomial regression adjusted by age,
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20 547 gender, service in which the participant works, body mass index, will be used.
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27 549 For secondary outcomes such as the analysis at 90 days, the same statistical analysis
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30 550 expressed in RR and absolute risk will be used. Survival analysis will be used for the primary
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34 551 endpoint only. An interim statistical analysis will be performed 30 days after the study starts
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36
37 552 to assess possible adverse effects and the efficacy of the intervention. The study administrator
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40 553 will be the only one with access to the data. For the interim analysis and the final analysis,
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44 554 the administrator will export the data to Excel format to be analysed by the study statistician
45
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47 555 blinded to the assignment of groups, participants, or results.
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54 557 **Adverse events, serious adverse events and suspected unexpected serious adverse reactions**
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3 558 By requiring the use of drugs, the participant will be exposed to risks inherent to the drug
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7 559 used, ranging from mild to severe or death. Any unexpected risks that may occur during the
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10 560 study will be immediately explained to the participants and the ethics committee. Any
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13 561 adverse event will be compiled and will not be disclosed under any condition to anyone other
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17 562 than the study administrator, treating physicians in case of severe events, and the ethics
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20 563 committee. The results will be completely anonymous concerning the names of the
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23 564 participants. The results will be compiled and reported as combined collective data.
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33 567 *Patient and public involvement*

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37 568 Patients were not involved in the development of this research. However, the results of the
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40 569 study will be communicated to the study participants by sending the end product (published
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43 570 article) to the provided email address.
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50 572 **ETHICS, DISSEMINATION AND SAFETY MONITORING**
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3 573 In case of adverse events or complications derived from the study, participants will be assured
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7 574 attention by the staff of the INCMNSZ in an enclosure that ensures the safety of the
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10 575 participant, not subjecting volunteers to a higher risk of contamination. This care will be
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13 576 extended until adverse events are resolved. In case of no adverse events during the study,
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17 577 medical attention will be extended at the aforementioned institute until 15 days after the end
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20 578 of the study.
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27 580 This protocol has been approved by the local medical ethical review committee at the INR-
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30 581 LGII with the internal number INRLGII/25/20, and by the Federal Commission for
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33 582 Protection against Sanitary Risks (in Spanish, Comisión Federal para la Protección contra
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37 583 Riesgos Sanitarios, COFEPRIS), approval number 203300410A0058/2020.
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40 584 The study results will be published in journals of worldwide impact affiliated with the Journal
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43 585 Citation Reports. Likewise, the results of the study will be disseminated in national and
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47 586 international media, exposed in international and national congresses, communicated to
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50 587 CONACYT, and recorded in Clinicaltrials.gov according to the study identifier number. The
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3 588 help of non-profit organizations will be sought to disseminate the results of the investigation

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7 589 to interest groups.

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10 590 The complete protocol will be published on Clinicaltrials.gov and the OSF - Center for Open

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13 591 Science platform <https://osf.io/>. Where a DOI will be assigned, and the amendments made to

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17 592 the original protocol will be assessed.

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23 594 Amendments to the protocol may be made before the start of the study and during the study.

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26 595 Any amendment to the protocol will be clarified and posted on Clinicaltrials.gov under the

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30 596 same identifier as this study. Likewise, any amendment will be sent to the ethics committee

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33 597 of the same hospital.

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40 599 **AUTHOR CONTRIBUTIONS**

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43 600 JGM is the lead study investigator, developed the study concepts and design, and wrote the

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46 601 manuscript by adapting the original study protocol for publication, subsequent reviews and

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48
49
50 602 amendments. EJHL, KMM and AAA contributed to the development and refining of the

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53 603 protocol, writing of manuscript and subsequent review. RJMP provided advanced

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3 604 methodological and statistical input, and contributed to the study design and subsequent
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7 605 amendments. RFC, TCH, PSSB, RAG and NML reviewed, commented and informed
8
9
10 606 methodology, development and writing of the protocol.
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17 608 **FUNDING STATEMENT**
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19
20 609 This work was supported by the Mexican Education, Science, Technology and Innovation
21
22
23 610 Department (in Spanish, Secretaría de Educación, Ciencia, Tecnología e Innovación), grant
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25
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27 611 number SECTEI/061/20.
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33 613 **COMPETING INTERESTS STATEMENT**
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37 614 None of the authors have conflict of interests, commercial agreements, or receive financial
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40 615 fees or compensation from any commercial or pharmaceutical company.
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47 617 **ETHICS APPROVAL**
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50 618 This protocol has been approved by the local medical ethical review committee at the INR-
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53 619 LGII with the internal number INRLGII/25/20. Definitions of Research Risk Regulation of
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3 620 the General Health Law on Research for Health (in Spanish, Reglamento de la Ley General
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7 621 de Salud en Materia de Investigación para la Salud)
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10 622 <http://www.inr.gob.mx/Descargas/Investigacion/Reglamentos-LGS-Materia-Investigacion->
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13 623 Salud.pdf. ARTICLE 17; and by Federal Commission for Protection against Sanitary Risks
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17 624 (in Spanish, Comisión Federal para la Protección contra Riesgos Sanitarios, COFEPRIS),
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20 625 approval number 203300410A0058/2020.
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27 627 **PROVENANCE AND PEER REVIEW**
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30 628 Not commissioned; externally peer reviewed.
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37 630 **ORCID ID**
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40 631 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