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

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ABSTRACT

Introduction SARS-CoV-2 infection in Mexico has caused ~2.7 million confirmed cases; around 20%–25% of health workers will be infected by the virus at their workplace, with approximately 4.4% of mortality. High infectivity of SARS-CoV-2 is related with cell entry mechanism, through the ACE receptor. SARS-CoV-2 requires transmembrane protease serine 2 to cleave its spike glycoprotein and ensure fusion of host cell and virus membrane. We propose studying prophylactic treatment with hydroxychloroquine (HCQ) and bromhexine (BHH), which have been shown to be effective in preventing SARS-CoV-2 infection progression when administered in early stages. The aim of this study is to assess the efficacy of HCQ and BHH as prophylactic treatments for SARS-CoV-2 infection in healthy health workers exposed to the virus.

Methods and analysis Double-blind randomised clinical trial, with parallel allocation at a 1:1 ratio with placebo, of low doses of HCQ plus BHH, for 60 days. Study groups will be defined as follows: (1) HCQ 200 mg/day+BHH 8 mg/8 hours versus (2) HCQ placebo plus BHH placebo. Primary endpoint will be efficacy of both interventions for the prevention of SARS-CoV-2 infection, determined by the risk ratio of infected personnel and the absolute risk. At least a 16% reduction in absolute risk is expected between the intervention and placebo groups; a minimum of 20% infection is expected in the placebo group. The sample size calculation estimated a total of 214 patients assigned: two groups of 107 participants each.

Ethics and dissemination This protocol has been approved by the local Medical Ethics Committee (National Institute of Rehabilitation 'Luis Guillermo Ibarra Ibarra', approval number INRLGII/25/20) and by the Federal Commission for Protection against Sanitary Risks (COFEPRIS, approval number 203 300 410A0058/2020). The results of the study will be submitted for publication in peer-reviewed journals and disseminated through conferences.

Trial registration number NCT04340349.

Strengths and limitations of this study

- This is a double-blind randomised single-centre clinical trial involving low doses of hydroxychloroquine and bromhexine (BHH), adequately powered to provide clinically relevant information regarding prophylactic treatment for SARS-CoV-2 infection in healthcare personnel.
- BHH has minimal side effects and is commercially available worldwide, so positive results could be applied in a timely fashion in different regions.
- Long-term use of hydroxychloroquine can cause heart rhythm problems.
- For the moment, people who are not candidates to receive the vaccine, due severe allergies, will not be included.
- Hydroxychloroquine has not been shown to be effective in monotherapy or with azithromycin, but adjunctive BHH could be an effective combination to inhibit SARS-CoV-2 infection.

INTRODUCTION

In Mexico, until July 2021, it has been reported more than 2.7 millions confirmed cases and ~238 000 deaths have arisen.[1] The age group ranging between 30 and 79 years is the most highly affected, where 81% present mild symptoms, 14% severe and 5% critical, requiring intensive care unit (ICU) management.

SARS-CoV-2 is a single-stranded RNA virion, member of the *Betacoronavirus* genus.[2] SARS-CoV-2 has an incubation period between 3 and 10 days, with different incubation periods related with different clinical symptoms.[3 4] It is transmitted through

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respiratory droplets from infected humans and through contact with contaminated fomites and aerosols; moreover, even asymptomatic persons in close contact can transmit the disease.[5] The mechanism through which the virus infects the respiratory cell is due to the ACE protein 2 (ACE-2) receptor. This receptor is found in multiple tissues such as the oral cavity, brain, kidneys, gut and placenta.[6–8]

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

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

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

Chloroquine (CQ) and hydroxychloroquine (HCQ) are known as an antimalarial agent; HCQ is a hydroxylated derivative from CQ. CQ and HCQ have gained attention as possible therapies in COVID-19 disease. In overdose, both drugs can cause severe, potentially life-threatening effects as visual disturbances, corneal opacities and irreversible retinopathy can occur with cumulative doses exceeding 100 g. When lower daily doses (250 mg are used), retinopathy may not occur after many years of treatment.[12] This indicates that the use of HCQ at low doses to avoid SARS-CoV-2 infection has a low possibility of being toxic and could be used as a prophylactic treatment. HCQ has been used in several viral infections, for example, as replication inhibitor for the dengue virus, decreasing in vitro virus infection and promoting activation of different immunological signal pathways.[13] It has also been used to treat hepatitis C virus infected patients to lower viral load, with minimal adverse effects reported.[14] HCQ has been reported to block viral infection by increasing the endosomal pH required for virus fusion to the cell, as well as interfering with cellular SARS-CoV-2 cell receptors, through inhibition of receptor glycosylation by ACE2.[15–18] HCQ has immunomodulatory effects; it inhibits production and release of proinflammatory cytokines that are associated with severe disease development.[19 20] Recently, it has been reported that HCQ works as a autophagy inhibitor, interfering with viral infection and replication.[21] There is recent evidence that HCQ could be used to treat COVID-19; studies in high-risk patients show that the use of HCQ was associated with a lower risk of intubation or death.[22] Recent study showed that pretreatment with HCQ has shown a better effect on antiviral activity,[17] and it has been reported that loading doses of 1600 mg HCQ followed by 600 mg daily doses are needed have a relevant effect to SARS-CoV-2 inhibition within 72 hours in 60% of patients with COVID-19.[23] Finally, a study where the antiviral mechanisms of CQ and adverse effects were evaluated showed that the use of CQ as a prophylactic treatment was more effective than when it is used as a therapeutic treatment.[24] However, CQ and HCQ have been reported to have various adverse effects, the CQ being the most toxic in overdose. However, it was recently published that in vivo trials are lacking to determine whether this drug is useful as a prophylactic treatment against SARS-CoV-2.[25] Therefore, further studies will be important to determine its effectiveness at low doses (<250 mg) as a prophylactic treatment.

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

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

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

METHODS AND ANALYSIS

Study design

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

Participants

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

Inclusion of participants will be assessed according to the eligibility criteria. Table 1 shows the classification and characteristics of study variables. Continuous variables will be assessed for normality. Variables with a normal distribution will be compared using Student's t-test, and non-parametric variables will be compared using the Mann-Whitney U test. Categorical variables will be evaluated using the χ^2 test.

Inclusion criteria

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

Exclusion criteria

- Positive quantitative reverse transcriptase-PCR (qRT-PCR) test for SARS-CoV-2 at the time of inclusion.
- Panel of IgG or IgM antibodies positive for SARS-CoV-2 at the time of inclusion.
- Development of respiratory symptoms suspicious of SARS-CoV-2 infection during the first 7 days after treatment is initiated, confirmed by qRT-PCR and IgG or IgM antibodies positive for SARS-CoV-2.
- History of allergies to any HCQ or BHH related compound or medication.
- Use of immunosuppressors for any reason.
- History of bone marrow transplant.
- Known glucose-6-phosphate dehydrogenase deficiency.
- Chronic kidney disease or glomerular filtration <20 mL/min.
- Use of other drugs with reported pharmacological interactions (ie, digitalis, flecainide, amiodarone, procainamide or propafenone).
- History of long QT syndrome.
- ECG with QTc >500 ms.
- Pregnant or breastfeeding personnel.
- Epilepsy.
- Known liver disease.
- Personnel who have received the COVID-19 vaccine.

Elimination criteria

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

Sample size calculation

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

Interventions

Interventions will consist of low doses of HCQ 200 mg tablets every 24 hours for 60 days plus BHH 8 mg tablets every 8 hours for 60 days. Study groups will be defined as follows: (1) HCQ plus BHH versus placebo for both

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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, microentrepreneur or saleswoman, administrative employee, professional and entrepreneur	Qualitative nominal
Civil status	Civil status of the individual	Married, single, widowed, divorced and 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 and 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 and glass	Consumption of drugs	Qualitative nominal
Hypertension	Elevation of blood pressure >130/80	Positive/negative	Qualitative nominal
Asthma	Chronic inflammatory disease characterised 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 characterised 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

Continued

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Table 1 Continued

Variable	Conceptual definition	Operational definition	Type
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, PaO2/FiO_2 ratio <250, infiltrates multilobars, confusion/disorientation, uremia (>20 mg/dL), leucopenia (<4000), thrombocytopaenia (<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°C	Positive/negative	Qualitative nominal
Thrombocytopaenia	Total platelets less than 100 000 per mm^3	Positive/negative	Qualitative nominal
Arterial hypotension	Systolic blood pressure less than 90 mm Hg or mean arterial pressure less than 60 mm Hg	Positive/negative	Qualitative nominal
Sepsis	Rapid SOFA score (qSOFA) with two 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 (mean arterial pressure) ≥65 mm Hg and lactate ≥2 mmol/L (18 mg/dL) in the absence of hypovolaemia	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 ECG, 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 and diarrhoea	Positive/negative	Qualitative nominal

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drugs. Fabrication of both drugs and placebos will be provided to our centre by a hired laboratory. Both drugs will be provided to participants directly at the hospital by a researcher blinded to group assignment process. To ensure that the intervention is carried out, each participant will be asked to keep a written record of the days and time the medication was administrated. This document will be reviewed weekly to verify that more than 50% adherence to treatment is maintained. Participants will be asked to record any symptoms related to the use of the medication, which will be reviewed by a researcher blinded to group assignment, weekly, or at the participants' request.

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

Randomisation and treatment allocation

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

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

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

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

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

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

Participant timeline and intervention

The inclusion of participants will be evaluated according to the eligibility criteria and by invitation. Volunteers who wish to participate in the study will be scheduled the next

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day at a specialised office to carry out all the relevant studies to ensure the inclusion criteria. These include a medical history, anthropometric measurements such as weight and body mass index, ECG (every week until the end of the study or when the participant requests it if they have any discomfort), complete blood count, complete blood chemistry and serological test for antibodies and qRT-PCR for SARS-CoV-2. Volunteers will be asked for information to contact them once the serological results are obtained.

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

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

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

Outcome measures

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

Primary endpoint

The primary endpoint will be the proportion of health personnel infected by SARS-CoV-2 at 60 days after starting treatment in both groups. The infection will be diagnosed using qRT-PCR for relative expression of the mRNA SARS-CoV-2 and the measure of IgM and IgG antibodies anti-SARS-CoV-2 after day 7 of treatment using rapid test Cellex qSARS-CoV-2 IgG/IgM. All participants presenting symptoms with positive qRT-PCR after 7 days of initiation of the intervention will be considered positive and will be included in the analysis. The study period will be 90 days (60 days for the primary end point plus 30 days of follow-up). The proportion of infected personnel will be evaluated using relative risk (RR) and absolute risk increase (ARI) with their respective 95% CIs in the established time. The disease-free period in the 60 days will also be evaluated, analysing the acumulated incidence of healthy personnel. The presence of infection will be confirmed by qRT-PCR for SARS-CoV-2 and by presence of IgM and IgG antibodies for SARS-CoV-2. The censoring variable will be interruption of treatment either due to death, adverse events or any elimination criteria. Since there is the possibility of false positives and negatives with qRT-PCR, we will perform qualitative measurements of IgM and IgG with the Cellex qSARS-CoV-2 IgG/IgM Rapid test, which is authorised by Food Drug Administration (FDA). The test can be used on serum, plasma or whole blood samples. The clinical sensitivity of the assay was 93.8%, and the clinical specificity was 96%.[30]

Secondary endpoints

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

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

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

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

Handling and storage of data and documents

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

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

- Medical history (includes personal data): study identifier number, history number, name, date of birth, gender, occupation, marital status, nationality, current residence, degree of studies (primary, secondary, upper secondary, bachelor degree and postgraduate), hospital service to which they belong and the number of hours worked per week.
- Personal history: alcohol intake (yes/no; how many glasses of beer or alcoholic beverages do you consume per week), smoking habit (yes/no; and number of cigarettes per day), drug use (yes/no), diet per week (dietary restrictions and number of meals per day) and number of hours of sleep per day.
- Gynaecological history (in women): number of pregnancies, number of live children, menarche and menopause.
- History of respiratory disease, history of gastrointestinal disease, nephrological, neurological, haematological, cardiovascular and allergies.
- Genetic family history, such as hypertension, diabetes, heart disease and 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 and breath sounds). Cardiovascular inspection (palpation of the fifth intercostal space, auscultation of heart sounds, pulses that are palpable and symmetrical), abdominal inspection (palpation, percussion and auscultation of peristaltic sounds), neurological evaluation (Glasgow, active motility, passive motility, reflex motility, cranial nerves and sensitivity).
- Complete blood count: haematocrit, leucocytes, segmented (%), lymphocytes (%), monocytes (%), mean corpuscular volume and platelets.
- Blood chemistry: glycaemia, urea, creatinine, sodium, potassium, chlorine, aspartate transaminase, alanine transaminase, alkaline phosphatase and total bilirubin.
- Muscle enzymes (creatine kinase).
- Clotting times: thrombin time, prothrombin time and international normalised ratio.
- ECG: rhythm, heart rate, heart axis, evaluation of P wave, PR interval, duration of QRS, QT interval and time of T wave. The ECG will be performed using an instrument calibrated and validated for its use internationally, weekly.
- Molecular test results for IgG and IgM antibodies:
  The FDA-approved product called Cellex qSARS-CoV-2 IgG/IgM Rapid Test will be used for serological determination. The device cassette, sample and buffer solution must be at room temperature. The sample (10 µL) is transferred to the centre of the sample well. After the sample well is free of liquid, two drops of sample diluent are added. After 15–20 min, read the test results. Results should not be read after 20 min.
  A positive IgM result occurs when a coloured band appears on the M test line (M) and the control line (C) and indicates that IgM against SARS-CoV-2 is present.
  A positive IgG result occurs when a coloured band appears on the G test line (G) and the control line (C) and indicates that IgG against SARS-CoV-2 is present.
  A positive result for IgM and IgG occurs when coloured bands occur both M and G, as well as C.
  A negative result occurs when a coloured band appears in C only and indicates that IgM and IgG antibodies against SARS-CoV-2 were not detected.
  An invalid result occurs when a colour band is not produced in C, and the test must be repeated.
- Official qRT-PCR results.

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

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treatment will be entered into an online database. In order to ensure the quality of the data collection, the database will be built in CASTOR, a database on the web that allows entering all the predefined data for each participant, thus reducing human error. This information will be stored on a server in the USA and can only be accessed by the study's administrator. The data may only be entered by a researcher in charge of collecting the data sheets and emptying them.

Monitoring and quality assurance

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

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

Statistical analysis

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

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

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

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

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

Patient and public involvement

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

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Ethics, dissemination and safety monitoring

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

This protocol has been approved by the local medical ethical review committee at the INR-LGII with the internal number INRLGII/25/20 and by the Federal Commission for Protection against Sanitary Risks (in Spanish, Comisión Federal para la Protección contra Riesgos Sanitarios, COFEPRIS), approval number 203 300 410A0058/2020.

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

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

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

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Contributors JG-M is the lead study investigator, developed the study concepts and design and wrote the manuscript by adapting the original study protocol for publication, subsequent reviews and amendments. EH-L, KM-M and AA-A contributed to the development and refining of the protocol, writing of manuscript and subsequent review. RJM-P provided advanced methodological and statistical input and contributed to the study design and subsequent amendments. RF-C, TC-H, PS-B, RA-G and NM-L reviewed, commented and informed methodology, development and writing of the protocol.

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Competing interests None declared.

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

Patient consent for publication Not required.

Ethics approval The approval of the research ethics committee of the INR-LGII of Mexico has been obtained.

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