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Hydroxychloroquine alone or in combination with Cobicistat-boosted Darunavir for treatment of mild Covid-19: a cluster-randomized clinical trial

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Abstract:	Background No therapeutics have yet been proven effective for the treatment of mild-illness caused by SARS-CoV-2. We assessed the efficacy and safety of hydroxychloroquine (HCQ) alone or in combination with cobicistat-boosted darunavir (DRVc) for treating patients with mild Covid-19. Methods We conducted a randomized, prospective, controlled, open-label trial in three health regions of Catalonia. After confirmation of a case of Covid-19 disease, we enumerated on a list a ring of the case and all their contacts and randomly assigned the ring to either control or intervention arm on a 1:1 ratio. Here we present the methods concerning eligible index cases, which involved non-hospitalized adult patients with recently confirmed SARS-CoV-2 infection and less than seven days of symptoms. Patients were assigned to receive HCQ (800 mg on day 1, followed by 400 mg once daily for six days) in combination with DRVc (800 mg/150 mg tablets, once daily for seven days) or no antiviral treatment. The protocol was adapted during the course of the trial to use HCQ alone after findings of no benefit of the protease inhibitor lopinavir-ritonavir. Study outcomes were the reduction of viral RNA load in nasopharyngeal swabs and time to clinical improvement within 28 days of follow-up in the per-protocol population. Adverse events were assessed up to 28 days. The study is registered with ClinicalTrials.gov, NCT04304053 Findings Between Mar 17 and Apr 28, 2020, 353 Covid-19 patients met the criteria for the per-protocol analysis: 165 in the control arm and 142 in the intervention arm. The median time from symptom onset to treatment start was 3 days (IQR 2–4). The per-protocol analysis revealed no significant differences in the mean reduction of viral load in nasopharyngeal swabs at day-3 compared to baseline between the control group (-1·28 Log 10 copies/mL, SD 1·68) and the intervention group (-1·47, SD 1·50); difference -0·18 [95% CI -0.59 to 0·22]. The same pattern was observed at day-7 and -14 after treatment. Time to complete alleviation of symptoms was similar in both groups (22 vs. 20·5 days, p = 0·37). Adverse events included self-limited nausea and

	diarrhea. Twenty patients required hospitalization, all due to Covid-19 progression. No patients died during the study. Interpretation In patients with mild Covid-19, no benefit was observed with HCQ alone or in combination with DRVc beyond the usual care. Future testing of other agents in randomized trials may help to identify other drugs that provide a treatment benefit.

Hydroxychloroquine alone or in combination with Cobicistat-boosted Darunavir for treatment of mild Covid-19: a cluster-randomized clinical trial

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ABSTRACT

Background

No therapeutics have yet been proven effective for the treatment of mild-illness caused by SARS-CoV-2. We assessed the efficacy and safety of hydroxychloroquine (HCQ) alone or in combination with cobicistat-boosted darunavir (DRVc) for treating patients with mild Covid-19.

Methods

We conducted a randomized, prospective, controlled, open-label trial in three health regions of Catalonia. After confirmation of a case of Covid-19 disease, we enumerated on a list a ring of the case and all their contacts and randomly assigned the ring to either control or intervention arm on a 1:1 ratio. Here we present the methods concerning eligible index cases, which involved non-hospitalized adult patients with recently confirmed SARS-CoV-2 infection and less than seven days of symptoms. Patients were assigned to receive HCQ (800 mg on day 1, followed by 400 mg once daily for six days) in combination with DRVc (800 mg/150 mg tablets, once daily for seven days) or no antiviral treatment. The protocol was adapted during the course of the trial to use HCQ alone after findings of no benefit of the protease inhibitor lopinavir-ritonavir. Study outcomes were the reduction of viral RNA load in nasopharyngeal swabs and time to clinical improvement within 28 days of follow-up in the per-protocol population. Adverse events were assessed up to 28 days. The study is registered with ClinicalTrials.gov, NCT04304053

Findings

Between Mar 17 and Apr 28, 2020, 353 Covid-19 patients met the criteria for the per-protocol analysis: 165 in the control arm and 142 in the intervention arm. The median time from symptom onset to treatment start was 3 days (IQR 2–4). The per-protocol analysis revealed no significant differences in the mean reduction of viral load in nasopharyngeal swabs at day-3 compared to baseline between the control group (-1·28 Log_{10} copies/mL, SD 1·68) and the intervention group (-1·47, SD 1·50); difference -0·18 [95% CI -0.59 to 0·22]. The same pattern was observed at day-7 and -14 after treatment. Time to complete alleviation of symptoms was similar in both groups (22 vs. 20·5 days, $p = 0·37$). Adverse events

included self-limited nausea and diarrhea. Twenty patients required hospitalization, all due to Covid-19 progression. No patients died during the study.

Interpretation

In patients with mild Covid-19, no benefit was observed with HCQ alone or in combination with DRVc beyond the usual care. Future testing of other agents in randomized trials may help to identify other drugs that provide a treatment benefit.

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PANEL RESEARCH IN CONTEXT

Research in context:

We searched PubMed on March 6, 2020, using the terms "Covid-19", "hydroxychloroquine (HCQ)", "lopinavir/ritonavir (LPVr)", "darunavir/cobicistat (DRVc)", and "trial" for articles in English published up to the date of the search. Our search yielded no randomized controlled trials assessing HCQ alone or in combination with DRVc in the treatment of patients with coronavirus disease 2019 (Covid-19). Empirical data for the efficacy of HCQ in hospitalized Covid-19 patients became available after the start of this study; HCQ administration did not result in a significantly higher PCR negative conversion in a RCT including 150 patients and there was no reduction in the risk of death/intubation in two large observational studies. The quality of the evidence was low, comprising primarily observational studies in hospitalized patients. On the other hand, when high-quality empirical data for the inefficacy of LPVr to reduce viral shedding in hospitalized Covid-19 patients became available, we stopped the use of the closely related drug DRVr,

Added value of this study:

This is the first randomized controlled trial on HCQ—alone or combined with DRVc to treat patients with mild Covid-19. The experimental treatment did not show any benefit in the reduction of viral load of nasopharyngeal swab specimens at 3, 7, and 14 days after treatment start. This treatment regimen also did not result in a shortened time to alleviation of symptoms or a lower rate of deterioration of clinical status requiring hospitalization. HCQ alone was shown to be safe, with minor and self-limiting gastrointestinal adverse events of diarrhea. No cardiovascular events related to the study drug were reported.

Implications of all the available evidence:

This study showed that early treatment with HCQ—alone or in combination with DRVc—was not effective in shortening the duration of virus shedding, alleviating symptoms, and reducing complication rate of patients with mild Covid-19. Future testing of other agents in randomized studies to assess their relative effectiveness against Covid-19 is needed to identify which drugs slow viral replication and disease progression.

INTRODUCTION

Since the emergence of the novel SARS-CoV-2 coronavirus in December 2019, various drugs have been proposed as antiviral agents for treating the coronavirus disease-2019 (Covid-19), including the aminoquinolines chloroquine and hydroxychloroquine (HCQ), and the inhibitors of HIV protease lopinavir and ritonavir (LPVr) and cobicistat-boosted darunavir (DRVc).[1]

Chloroquine and HCQ have been extensively used for treating malaria and various autoimmune diseases, although other therapeutic effects, including antiviral activity, have been increasingly recognized.[2,3] In-vitro studies showed that both drugs can block the viral spread of SARS-CoV-2 in cell cultures,[4–6] and that HCQ appears to have more potent antiviral activity.[6] Nevertheless, as of 22 May 2020, publicly available clinical data on the effectiveness of chloroquine and HCQ for treating Covid-19 are limited to two small randomized clinical trials in hospitalized patients and six observational studies.[7–14] Furthermore, these studies showed conflicting results, and those concluding on the potential efficacy of chloroquine and/or HCQ lack internal validity high enough to broadly recommend these drugs for routine treatment of Covid-19. An open-label study of 36 adult patients with Covid-19 found that treatment with HCQ (200 mg three times per day for 10 days) was associated with a significantly lower rate of detectable SARS-CoV-2 RNA on nasopharyngeal swabs at day 6 compared with no specific treatment (70% vs. 13%)[7]. Conversely, a randomized trial with 150 patients found that HCQ administration did not result in a significantly higher PCR negative conversion.[14] Of note, two large observational studies of hospitalized patients with Covid-19 treated with HCQ at physician's discretion found no significant reduction in the risk of death/intubation compared with no specific treatment.[9,13]

Inhibitors of HIV protease have exhibited in-vitro antiviral activity against SARS and MERS viruses.[15,16] Based on these findings, LPVr and DRVc have been both proposed as candidates for treating Covid-19 and even included in local treatment protocols in a severe disease setting.[17,18] However, results from a randomized controlled trial that became available after the start of our study showed that LPVr added no benefits to standard treatment of seriously ill patients with Covid-19.[19] Specifically, treatment with LPVr failed to significantly reduce time to clinical improvement (primary endpoint; HR 1.31; 95% CI 0.95–1.80), mortality, or nasopharyngeal viral load in study participants.

The limited clinical evidence on dose-optimized therapies for Covid-19 stresses the need for high-quality randomized controlled trials. We hypothesized that HCQ and DRVc combined treatment would be more efficacious than no-treatment for patients with mild Covid-19. Owing to the emergence of clinical data indicating lack of efficacy of HIV protease inhibitors for Covid-19 treatment, the intervention was switched to HCQ alone. Analytical consequences of this change are discussed. We report herein the results of a randomized-controlled trial in which HCQ -alone or combined with DRVc was used to treat patients with mild Covid-19

METHODS

Study design and participants

This was an open-label, randomized, controlled trial conducted from March 17, 2020 (first patient in) to April 28, 2020 (last patient in) in three health regions in Catalonia covering 4,206,440 inhabitants (i.e., 60% of the Catalan population): *Catalunya central*, *Àmbit Metropolità Nord*, and *Barcelona Ciutat*. Study candidates were identified from the electronic registry of the Epidemiological Surveillance Emergency Service of Catalonia (SUVEC) of the Department of Health. During the Covid-19 epidemic in Catalonia, a public health ordinance required all patients tested positive for Covid-19 in any of the designated diagnostic laboratories to be notified to the SUVEC.[20] We invited candidates to participate in the trial by telephone call and sought verbal consent for a prescreening assessment for eligibility based on a telephone interview and an online review of the electronic medical records of the Catalan Health Institute. This trial was nested within the "BCN PEP CoV" RCT, aimed to investigate a post-exposure prophylaxis therapy for Covid-19 by mirroring the design of the ring vaccination trial "Ebola Ça Suffit!".[21] Following the ring design, we defined study clusters (called rings) of an index case and healthy individuals epidemiologically linked to them (contacts) and randomized them at a cluster level to one of the study arms. After the allocation of the study rings, we confirmed selection criteria for cases. Adult patients aged 18 years or more were eligible if they had mild symptoms of Covid-19 (fever >37·5, acute cough, shortness of breath, or sudden olfactory or gustatory loss) for less than seven days before enrollment, were non-hospitalized, and had a positive PCR test for SARS-CoV-2 in the pre-screening nasopharyngeal swab tested by the local designated laboratory. Patients were excluded if they had moderate-to-severe Covid-19 disease (respiratory distress with a respiratory rate higher than 30 breaths/minute or oxygen saturation less than 94%), presence of any condition that might preclude following the study procedures safely (e.g., mental disability), known allergy or hypersensitivity to study drugs, known retinal and severe liver or renal diseases, history of cardiac arrhythmia, known QT prolongation or other diseases that could be exacerbated by study drugs (e.g., psoriasis), use of medications that are contraindicated with study drugs, known HIV infection, or pregnant females (verbally declared or positive pregnancy test) or breastfeeding.

The study protocol and subsequent amendments were approved by the institutional review board of Hospital Germans Trias Pujol, (Badalona, Spain - Approval AC-20-029-HGT-CEIM) and the Spanish Agency of Medicines and Medical Devices (Madrid, Spain - Approval 66ZNRDJA98). Written informed consent was obtained from all patients. Full details about the trial design are provided in the protocol, available at thelancet.com. This trial was part of the Barcelona Postexposupre Prophylaxis Study against SARS-CoV-2 (BCN PEP CoV-2 Study) registered with ClinicalTrials.gov, NCT04304053 and EudraCT**,** 2020-001031-27 that is being published elsewhere [REF].

Randomization and masking

Rings of index cases and their corresponding contacts were cluster-randomized (1:1) using a computer-generated random-number list to either the control arm (no treatment aside from usual care) or the intervention arm: the index case received HCQ (Dolquine®, 800 mg on day 1, followed by 400 mg once daily for six days) combined with DRVc (Rezolsta®, 800/150 mg once daily for seven days). As of Apr 4, 2020, DRVc was withdrawn from the study protocol on the grounds of new clinical evidence proving the lack of efficacy of protease inhibitors for treating Covid-19.[19] Patients allocated to the intervention arm since that time point onward received HCQ only. The study medications were dispensed by the hospital pharmacy and provided free of charge to the patients at the first home visit by dedicated outbreak field teams of trained nurses aided with trained paramedical staff (Open Arms, Non-Governmental Organization). Random allocation was done remotely by a member of the study team not involved in participants' enrollment. Masking was not possible because a placebo could not be prepared due to the emergency nature of the trial. Laboratory technicians were unaware of participants' treatment allocation, treatment response, and previous PCR results at all times.

Procedures

Study procedures for the management of Covid-19 contacts are reported elsewhere [REF]. All Covid-19 index cases that gave oral consent to participate were assessed on days 1 (before antiviral treatment was administered), and days 3, 7, and 14. On day 1, patients were home visited for baseline assessment and patient enrollment. Outbreak field teams verified the selection criteria for eligibility, obtained patients' signed informed consent, assessed specific symptoms associated with Covid-19, conducted a physical examination to patients that did not present a good general state (temperature, oxygen saturation, respiratory rate, and blood pressure measurement), and collected relevant epidemiological information from a structured interview (place of exposure, use of personal protective equipment). Health status progression, safety, and treatment compliance were monitored by the Clinical Trials Unit (CTU) of Hospital Germans Trias Pujol at days 3 (home visit), 7, 14, and 21 (phone visits). Adverse events (AE) were defined as any new symptom or worsening of pre-existing symptoms and were followed until resolution or up to day 28 after enrollment. Serious adverse events (SAE) were defined as any medical event that required hospitalization or caused patient death; SAEs were graded for intensity, causality and expectedness and reported immediately to the Contract Research Organization (CRO) of the study sponsor and the trial pharmacovigilance consultancy (Asphalion, Barcelona, Spain) for independent adjudication of relatedness to study drug and/or need of expedited notification to the regulatory authorities. Clinical and safety data were recorded on paper case record forms by the outbreak field teams and the Clinical Trials Unit and then entered into an electronic openTIC database by the Data Entry team

of the sponsor. Data validation and cleaning were done by trial researchers with the support of the trial data management consultancy (Trial Form Support, Barcelona, Spain).

For each patient, serial oral and naso-pharyngeal swab samples were planned to be obtained on days 1 and 3. However, preliminary analyses revealed a possible delay for a significant viral load reduction beyond day 3; therefore, we amended the study protocol to extend the collection of an additional nasopharyngeal swab at home on day 7 or, when possible, on day 14. The presence of SARS-CoV-2 was investigated from nasopharyngeal swabs at SYNLAB Diagnostics, Barcelona, Spain. RNA was extracted using an automated workstation (Hamilton Star, Hamilton, US) and subsequently amplified by PCR using the TaqMan™ 2019-nCoV Assay Kit (Catalog no.: A47532, Thermo Fischer Scientific Inc.) according to the manufacturer's protocol. Positivity was recorded when an amplification curve with a Ct < 40 was detected. Viral load was quantified from nasopharyngeal swabs of all cases (all time points collected) at IrsiCaixa laboratory (Badalona, Spain). RNA extraction was performed by using Viral RNA/Pathogen Nucleic Acid Isolation kit, optimized for a KingFischer® instrument, following manufacturer's instructions (Catalog no.: 4462359, Thermo Fischer). PCR amplification was based on the 2019-Novel Coronavirus Real-Time RT-PCR Diagnostic Panel guidelines and protocol developed by the US Centers for Disease, Control and Prevention (CDC).[22] Briefly, a 20 μL PCR reaction was set up containing 5 μL of RNA, 1·5 μL of N3 primers and probe (2019-nCov CDC EUA Kit, cat num 10006770, Integrated DNA Technologies) and 5 μL of TaqPath 1-StepRT-qPCR Master Mix (Thermo Fischer). Thermal cycling was performed at 50ºC for 15min for reverse transcription, followed by 95°C for 2 min and then 45 cycles of 95°C for 3 sec, 55°C for 30 sec, in the Applied Biosystems 7500 or QuantStudio5 Real-Time PCR instruments (Thermo Fischer). For absolute quantification, a standard curve was built using 1/5 serial dilutions of a SARS-CoV2 plasmid (2019-nCoV_N_Positive Control, catalog number 10006625, $2x10^5$ copies/μL, Integrated DNA Technologies) and run in parallel in all PCR determinations. The viral load of each sample (in copies/mL) was extrapolated from the standard curve and corrected by the corresponding dilution factor.

Outcomes

The primary outcome was the reduction of viral RNA load in nasopharyngeal swabs at days 3, 7, and 14 after treatment start. The secondary outcomes were time from randomization to complete alleviation of symptoms at an extended 21-days follow-up and patient deterioration measured by a simplified scale: 1, not hospitalized with or without resumption of normal activities; 2, hospitalized, requiring supplemental oxygen; 3, hospitalized, requiring invasive mechanical ventilation; and 4, death. We also measured time from the onset of symptoms to their resolution. Safety outcomes included AE that occurred during treatment, SAE, and premature discontinuation of therapy. AE were classified according to the National

Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0. All unexpected SAE were notified through Eudravigilance.

Statistical analysis

The trial was conducted in a rapid response to the SARS-CoV-2 public health emergency in 2020. At the time the trial was designed (i.e., March 2020), there was insufficient information about clinical outcomes in non-hospitalized patients with Covid-19 for a robust estimate of the sample size. In this scenario, the target sample size was calculated using the same approach as in BCN PEP CoV-2 Study and taking into consideration that epidemiological rings formed with the patients of this trial and their contacts were to be analyzed. The sample size of 307 (142 and 165 in each arm) achieved in the present study would provide the trial with 80% power to detect a difference of 0·5 log10 in the mean viral load considering an intra-group variability of 1·6 units at a two-sided significance level of $\alpha = 0{\cdot}05$.

We selected the per-protocol (PP) population for the primary efficacy analysis in accordance to the study protocol. This population included all randomized individuals who completed the study procedures to Day 14 with no major protocol deviations. We also performed a sensitivity analysis with the intention-to-treat (ITT) population, which included all eligible participants.

We described the baseline characteristics and events at the individual level using the frequency and percentage for categorical variables and the mean and standard deviation (SD) or median and interquartile range (IQR) for continuous variables. For primary efficacy analysis of viral load reduction at different time points, specimens with undetectable viral load were assigned a value of 1 $\log_{10}$ copies per mL for the purpose of statistical analysis. The efficacy was determined by assessing the between-groups mean difference measured at 3, 7, and 14 days of the viral load reduction in nasopharyngeal swabs from baseline. The secondary clinical outcome regarding the time to clinical improvement was analyzed using Kaplan-Meier survival functions and HRs calculated by means of a Cox proportional hazards regression model. Patient deterioration was assessed in the per-protocol (PP) population with between-group difference estimated by means of a risk ratio (RR). We also performed a sensitivity analysis with the intention-to-treat (ITT) population, which included all eligible participants. Since we did not suspend enrollment when DRVc was withdrawn, comparative analyses were performed for each group of the intervention arm: HCQ plus DRVc and HCQ only. The significance threshold was set at a two-sided alpha level of 0·05 unless otherwise indicated, and all analyses were conducted in R version 3.6.2.[23]

Role of the funding source

The funder of the study had no role in the study design, data collection data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication,

RESULTS

Patients

Between Mar 17 and Apr 28, 2020, 651 confirmed Covid-19 patients were screened, and the case-contacts rings were randomized to either the control arm (n=326) or the intervention arm (n=325). 142 patients in the control arm and 156 in the intervention arm did not meet the inclusion criteria and were therefore not enrolled (figure 1, study profile). 184 patients assigned to the control arm were enrolled, 90/168 (53·6%) patients assigned to the intervention arm were enrolled and received HCQ+DRVc and 79/168 (47·0%) HCQ only. Twenty-six (7·4%) of the 352 participants were excluded during the first home visit because they presented with more than seven days of symptom onset, 4 (1·1%) were receiving a contraindicated medication, and 1 (0·3%) patient with oxygen saturation below 94% that was referred to the nearest hospital. During follow-up, 2 (0·6%) patients withdrew consent, 3 (0·9%) had treatment compliance under 80%, 4 (1·1%) were lost-to-follow-up, and 4 (1·1%) had other protocol deviations. The remaining 307 patients constituted the PP population.

The two study arms had similar characteristics at baseline, including age, gender, frequency of symptoms, and nasopharyngeal viral load (table 1). The mean age of patients was 42·0 years (SD 12·8), and 218 (71·0%) of them were women. The median time from symptom onset to enrollment was 3 days (IQR 2–4). Fever, cough, and anosmia were the most common presenting symptoms. The mean viral load in the nasopharyngeal swab at baseline was 7·93 (SD 1·81) Log_{10} copies/ml.

Primary outcome

For the primary outcome of reduction of the viral load in nasopharyngeal swabs on day 3 there were no significant differences between the control arm (mean -1·28 Log_{10} copies/ml, SD 1·68) and the intervention arm (-1.47, SD 1·50; difference [d] -0·18 [95% CI -0·59 to 0·22]; table 2 and figure 2A). The mean reduction of the viral load was also similar on day 7 (-2.93 [SD 1·90] in the control arm vs. -2·70 [SD 1·65] in the intervention arm; d 0.23 [95% CI -0.65, 1.12]), and on day 14 (-3·18 [SD 2·26] in the control arm vs. -2.75 [SD 2·70] in the intervention arm; d 0·43 [-2.85, 3.71]). The degree of intra-visit dispersion of viral load was similar in both groups (Day-0 IQR 6.40-9.19 Log_{10} copies/mL in the control arm, 6.87-9.48 in the intervention arm; figure 2B). The sensitivity analysis by ITT also showed no difference between groups (table S1, appendix). The sub-group analysis comparing the viral loads of patients treated with HCQ plus DRVc did not reveal differences compared with HCQ alone (table S2, appendix).

Secondary outcomes

For the clinical outcome, median (IQR) time from randomization to the resolution of Covid-19 symptoms was not significantly different in the control arm (19·0 days, IQR 7–22) and the intervention arm (17.0, IQR 7–22; log-rank-test for survival analysis p = 0·41; figure 3A). In the survival analysis of symptom

resolution, we observed a step function between day 20-22, partly related to Day-21 phone call visit. Median time from symptom onset to complete alleviation of symptoms was also similar in the control group (22·0, IQR 10–25) and the intervention group (20·5, IQR 10–25; log-rank-test for survival analysis $p = 0{\cdot}37$; figure 3B). Risk of hospitalization was similar in the two study arms (6·9% vs. 5·8%; RR 0·82, 95% CI 0·3 to 2·0; table 2). No patients required mechanical ventilation or died during the study period.

Safety

In the ITT population 17/184 (9·1%) patients in the control group and 121/168 (72·0%) in the intervention group experienced at least one AE during the 14 days of follow-up (table S3, appendix). Of participants given antiviral therapy, 90·9% (110/121) of AE were grade 1–2, 2·4% (3/121) were grade 3, and 6·6% (8/121) were grade-4 (table S4, appendix). SAE, all non-drug related were reported in 11 of 184 patients in the control group and 9 of 168 patients in the intervention group. All 20 patients required hospitalization due to disease progression of Covid-19 pneumonia without death events. The most frequent treatment-related AE were gastrointestinal (tableS5, appendix; diarrhea, nausea, and abdominal pain) and nervous system disorders (drowsiness, headache, and metallic taste). No cardiovascular or AE of special interest (syncope, palpitations, dizziness) were reported during treatment or for the entire-follow-up.

DISCUSSION

In this randomized trial of patients with mild Covid-19, we found that HCQ—alone, or in combination with DRVc—administered within seven days from symptom onset, was ineffective in reducing the shedding of SARS-CoV-2 in the nasopharynx compared with no antiviral therapy at 3, 7, and 14 days of follow-up. Furthermore, the treatment and control arms had similar mean viral load values in subgroups defined according to demographic and biological parameters at baseline. Both groups had similar median values of time to alleviation of symptoms and similar rates of patient deterioration requiring hospitalization. These results add to previous evidence of the inefficacy of the use of HCQ and DRVc to treat Covid-19. Previous studies reported on the failure of HCQ to reduce the virological load or improve clinical outcomes in hospitalized patients.[9,13,14] To our knowledge, this is the first trial that provides data on the lack of efficacy of HCQ in patients with mild Covid-19.

HCQ was well tolerated, and no major AE related to the study drug occurred. Of participants who were treated with HCQ and interviewed, 70% reported mild-to-moderate side-effects that were mainly gastrointestinal. Only eight patients presented a SAE within 14 days of HCQ treatment initiation, all related to disease progression, and no cardiac AEs were reported. Our findings in mild-Covid19 cases do

not corroborate—albeit they cannot rule out because of the small sample size for the safety outcome—the potential harm of HCQ due to electrical instability described in previous observational studies.[24,25]

Our study has several limitations. First, selection criteria in our study enrollment allowed for a longer time-lapse (i.e., seven days) from symptom onset to enrollment than other studies. [26,27] Previous research that had shown that early treatment with antiviral agents resulted in a superior clinical benefit in hospitalized Covid-19 patients than delayed treatment.[2819] However, time to enrollment in our study was generally short (median of three days), and we did not see an advantage in the subgroup of patients treated within three days after symptom onset. Second, owing to the urgency, the trial could not be masked with a placebo. The use of a placebo drug might have minimized the attrition bias of participants in the control arm who would be willing to take the study drug. Nonetheless, we did not identify a different drop-out rate between the two study arms. Moreover, to minimize the detection bias of the primary outcome (i.e., the viral load), the laboratory staff remained unaware of which group participants were assigned to. Third, although trained outbreak teams collected data using standardized procedures, some of the interactions with patients occurred under challenging circumstances related to the health crisis (e.g., nursing homes for older people heavily affected by the epidemic), thus increasing the risk of mistakes in data collection. Finally, the amendments introduced during the study conduct as knowledge on the inefficacy of protease inhibitors and viral load dynamics became available affected the actual sample size of various analyses. This was particularly relevant for the size of the intervention group, which was split into two subgroups of HCQ+DRVc (n = 90) and HCQ alone (n = 79).

HCQ and chloroquine have garnered unprecedented attention as potential therapeutic agents following inconclusive clinical trials in combination or not with azithromycin,[7,10] uncontrolled case series,[9] and public figure endorsements.[18] While there is a growing body of scientific data on HCQ as a candidate for treating Covid-19, there is also a concern for harm, particularly cardiovascular disease.[24,25]. To our knowledge, by the time of ending our trial, more than 50 research groups worldwide were conducting or planning studies to investigate the effectiveness of HCQ alone or in combination for treating Covid-19 mild to severe cases. Furthermore, it is worrisome that various countries have raised the off-label use of this drug.[18,29] The potential for treatment of mild Covid-19 with HCQ has been explored in this trial to provide definite evidence. DRVc has been less frequently investigated in the setting of Covid-19 than other protease inhibitors like LPVr. However, the more favorable safety profile of DRVc, particularly regarding the frequency of diarrhea, suggests better suitability for acutely infected patients. This feature, along with a similar mechanism of action as LPVr and its predicted inhibitory effect on the papain-like viral protease of SARS-CoV-2[30] prompted us to select it for combining with HCQ as a candidate therapy

for Covid-19. We have further evaluated the effect of post-exposure prophylaxis with HCQ of asymptomatic contacts of patients with Covid-19 along with treatment of patients with Covid-19 (The BCN PEP-CoV-2 Study) [REF]. Taken together, we considered of paramount relevance to provide high-quality evidence on the efficacy of these drugs in reducing viral shedding and onward transmission of SARS-CoV-2 to the population of contacts.

Our findings provide robust evidence on the lack of clinical efficacy of HCQ —administered either alone or in a combined regimen with DRVc—to treat patients with mild Covid-19 and warns on the limited capacity of HCQ to effectively reduce the SARS-CoV-2 shedding. These data provide the scientific community and policymakers with essential insights on the inefficacy of HCQ as a therapeutic candidate for SARS-CoV-2. Finally, in light of the negative results with LPVr in patients with severe disease along with our findings regarding the lack of effect of HCQ plus DRVc in patients with mild disease, the use of protease inhibitors does not seem a worthy therapeutic approach for SARS-CoV-2.

CONTRIBUTORS

OM, LB, VC, CV, RMV, MVM conceived, designed and wrote the manuscript,
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EB, PC, ER, LR did all laboratory tests
JM, MC, MS, SG directed and managed the planning and execution of the project
All authors reviewed and approved the final version of the manuscript

CONFLICTS OF INTEREST

We declare no conflicts of interest

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Figure legends

Figure 1. Flow diagram of individual selection and cluster allocation.

Figure 2. Change from baseline in SARS-CoV-2 viral RNA on nasopharyngeal swabs.

A) Mean viral load of participants in the control (blue line) and intervention arm (red line) at each assessment point (x-axis), determined by quantitative RT-PCR· Error bars show the 95% CI.
B) Box plot of viral load of participants in the control (blue box) and intervention arm (red box) at each assessment point (x-axis), determined by quantitative RT-PCR. Boxes represent median and IQR for each group, outliers are plotted as individual points.
Number of samples tested are: Day-0 265, Day-3 248· Day-7 68, Day-14 23

Figure 3. Time to clinical improvement in the per-protocol population.

A) Symptom resolution from randomization, B) Symptom resolution from symptom onset.

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Table

Table 1. Baseline characteristics of index cases in each study arm.

	Assigned to the control arm (N = 165)	**Assigned to the intervention arm** (N = 142)
Individuals' characteristics (N=307)		
Age (years), *mean (SD)*	42·0 (13·0)	41·9 (12·7)
Gender (female), *n (%)*	111 (67·3%)	107 (75·4%)
Time from onset of symptoms to PCR result (days), *median (IQR)*	2·0 (1·0; 3·0)	2·0 (1·0; 3·0)
Time from onset of symptoms to enrolment (days), *median (IQR)*	3·0 (2·0; 4·0)	3·0 (2·0; 4·0)
Symptoms at baseline (N=307)		
Dyspnea, *n (%)*	21 (12·7%)	29 (20·4%)
Fever, *n (%)*	98 (59·4%)	89 (62·7%)
Cough, *n (%)*	108 (65·5%)	86 (60·6%)
Sudden olfactory or gustatory loss, *n (%)*	65 (39·4%)	56 (39·4%)
Physical exam at baseline (N=307)		
Good general state	160 (97·0%)	140 (98·6%)
Poor health status*	5 (3·03%)	2 (1·41%)
Temperature (N=18), *mean (SD)*	36·9 (0·67)	37·3 (1·51)
Oxygen saturation (N=13), *mean (SD)*	96·7 (1·89)	97·3 (1·75)
Respiratory rate (N=6), *mean (SD)*	16·3 (2·52)	17·3 (2·31)
Laboratory data (N=306)		
PCR negative at baseline: n (%)	21 (12·8%)	18 (12·7%)
PCR positive at baseline: n (%)	144 (87·3%	123 (87·2%)
Viral load (RT-PCR Log_{10} copies/mL) (N=265),*mean (SD)***	7·80 (1·91)	8·09 (1·67)
Main risk factor of exposure to Covid-19 (N=306)		
Healthcare worker, *n (%)*	136 (82·4%)	112 (79·4%)
Nursing home worker, *n (%)*	10 (6·06%)	7 (4·96%)
Household contact of a case, *n (%)*	2 (1·21%)	3 (2·13%)
Unknown, *n (%)*	17 (10·3%)	19 (13·5%)

IQR: interquartile range. **SD:** standard deviation.
No statistically significant differences were found between groups.
* Patients who, according to the physician, showed poor health status in the general physical exam.
**Viral load was tested in 265/267 positive PCR specimens; sample volume was insufficient for testing in two specimens.

Table 2. Effects of the intervention on SARS-CoV-2 viral load and disease progression.

	N	Assigned to the control arm (N = 165) Mean (SD)	Assigned to the intervention arm (N = 142) Mean (SD)	d	(95% CI)
Primary endpoint					
Viral load in throat swabs (Log_{10} copies/ml)					
At day 0	265	7.80 (1·91)	8·09 (1·67)		
At day 3	248	6·59 (1·66)	6·64 (1·63)		
At day 7	68	4·88 (1·21)	4·98 (1·17)		
At day 14	23	4·60 (1·67)	5·07 (1·63)		
Viral load reduction in throat swabs from baseline (Log_{10} copies/mL) *					
At day 3	239	-1·28 (1·68)	-1·47 (1·50)	-0·18	(-0·59, 0·22)
At day 7	65	-2·93 (1·90)	-2·70 (1·65)	0·23	(-0·65, 1·12)
At day 14	23	-3·18 (2·26)	-2·75 (2·70)	0·43	(-2·85, 3·71)
	N	**Events (%)**	**Events (%)**	**RR**	**(95% CI)**
Secondary endpoints					
Not hospitalized with resolution of symptoms at home	297	147 (92·5)	130 (94·2)	0·75	(0·31, 1·77)
Hospitalization not requiring mechanical ventilation	298	11 (6·9)	8 (5·8)	0·82	(0·34, 1·99)
Hospitalization requiring mechanical ventilation	298	0 (0·0)	0 (0·0)		
Death	298	0 (0·0)	0 (0·0)		

None of the estimated mean differences and risk ratios were statistically significant.
d: mean difference. **RR:** risk ratio. **SD:** standard deviation.
* We provide viral load data among PCR-positive swabs.
Only the viral load of cases with positive PCR tests are shown. The number of positive/ negative/tested cases on Days 0, 3, 7, and 14 were 267/39/306, 244/36/280, 72/40/112, and 26/24/50, respectively.

Supplementary table 1. Effects of the intervention on SARS-CoV-2 viral load and disease progression in the intention-to-treat population.

	N	Assigned to the control arm (N = 184) Mean (SD)	Assigned to the intervention arm (N = 168) Mean (SD)	d	(95% CI)
Primary endpoint					
Viral load in throat swabs (Log_{10} copies/mL)					
At day 0	295	7·83 (1·89)	7·99 (1·74)		
At day 3	272	6·57 (1·68)	6·55 (1·66)		
At day 7	73	4·94 (1·23)	4·94 (1·16)		
At day 14	24	4·60 (1·67)	4·91 (1·52)		
Viral load reduction in throat swabs from baseline (Log_{10} copies/mL)					
At day 3	259	-1·32 (1·67)	-1·45 (1·53)	0·13	(-0·52, 0·26)
At day 7	70	-2·91 (1·87)	-2·69 (1·64)	-0·22	(-0·63, 1·07)
At day 14	23	-3·18 (2·26)	-2·75 (2·70)	-0·43	(-2·85, 3·71)
	N	**Events (%)**	**Events (%)**	**RR**	**(95% CI)**
Secondary endpoints					
Not hospitalized with resolution of symptoms at home	355	162 (93·1)	152 (94·4)	0·83	(0·36, 1·92)
Hospitalization not requiring mechanical ventilation	336	11 (6·9)	10 (6·1)	1·02	(0·45, 2·33)
Hospitalization requiring mechanical ventilation	336	0 (0·0)	0 (0·0)		
Death	336	0 (0·0)	0 (0·0)		

None of the estimated mean differences and risk ratios were statistically significant.
d: mean difference. **RR:** risk ratio. **SD:** standard deviation.

Supplementary table 2: Effects of the intervention on SARS-CoV-2 viral load and disease progression in patients treated with HCQ plus DRVc and HCQ only.

	N	Control arm (N=125) Mean (SD)	Treated with HCQ+DRV (N=49) Mean (SD)	Treated with HCQ (N=64) Mean (SD)
Primary endpoint				
Viral load in throat swabs (Log_{10} copies/mL)				
At day 0	265	7·80 (1·91)	8·52 (1·46)	7·57 (1·76)
At day 3	248	6·59 (1·66)	6·71 (1·53)	6·54 (1·76)
At day 7	68	4·88 (1·21)	5·32 (·)	4·97 (1·18)
At day 14	23	4·60 (1·67)	4·43 (0·28)	6·04 (2·72)
Viral load reduction in throat swabs from baseline (Log_{10} copies/mL)				
At day 3	239	-1·28 (1·68)	-1·74 (1·25)	-1·10 (1·73)
At day 7	65	-2·93 (1·90)	-3·64 (·)	-2·67 (1·67)
At day 14	23	-3·18 (2·26)	-3·46 (1·69)	-1·67 (4·44)
Secondary endpoints		**Events (%)**	**Events (%)**	**Events (%)**
Not hospitalized with resolution of symptoms at home	297	147 (92·5%)	58 (96·7%)	72 (92·3%)
Hospitalization not requiring mechanical ventilation	298	11 (6·88%)	2 (3·33%)	6 (7·69%)
Hospitalization requiring mechanical ventilation	298	0 (0·0)	0 (0·0)	0 (0·0)
Death	298	0 (0·0)	0 (0·0)	0 (0·0)

SD: standard deviation.

Supplementary table 3: Summary descriptive of participants with an Adverse Events by number of Adverse Events (intention to treat)

	Participants with AEs in the Control arm	**Participants with AEs in the Intervention arm**
	N=184	N=168
AE's per contact		
Nº adverse event per contact::		
None	167 (90.8%)	47 (28.0%)
1	15 (8.15%)	36 (21.4%)
2	1 (0.54%)	21 (12.5%)
3 or more	1 (0.54%)	64 (38.1%)

Supplementary table 4: Summary descriptive of participants with an Adverse Events by grade (intention to treat)

	Participants with AEs in the Control arm	**Participants with AEs in the Intervention arm**
	N=17	N=121
Grade:		
1	5 (29.4%)	80 (66.1%)
2	1 (5.88%)	30 (24.8%)
3	0 (0.00%)	3 (2.48%)
4	11 (64.7%)	8 (6.61%)
Serious:		
No	6 (35.3%)	112 (92.6%)
Yes*	11 (64.7%)	9 (7.44%)

*None of the serious adverse events (SAE) were adjudicated as related to HCQ by the pharmacovigilance consultants.

Supplementary table 5: Summary descriptive table of the total number of Adverse Events in the intention to treat population by type

	Number of AEs in the Control arm	**Number of AEs in the Intervention arm**
	N=23*	N=358**
Cardiac disorders	0 (0·00%)	0 (0·00%)
Ear and labyrinth disorders	0 (0·00%)	5 (1·40%)
Eye disorders	0 (0·00%)	6 (1·68%)
Gastrointestinal disorders	7 (30·4%)	208 (58·1%)
General disorders and administration site conditions	1 (4·35%)	30 (8·38%)
Infections and infestations	11 (47·8%)	10 (2·79%)
Injury, poisoning and procedural complications	0 (0·00%)	1 (0·28%)
Metabolism and nutrition disorders	1 (4·35%)	1 (0·28%)
Musculoskeletal and connective tissue disorders	1 (4·35%)	7 (1·96%)
Nervous system disorders	2 (8·70%)	70 (19·6%)
Psychiatric disorders	0 (0·00%)	2 (0·56%)
Renal and urinary disorders	0 (0·00%)	1 (0·28%)
Reproductive system and breast disorders	0 (0·00%)	1 (0·28%)
Respiratory, thoracic and mediastinal disorders	0 (0·00%)	2 (0·56%)
Skin and subcutaneous tissue disorders	0 (0·00%)	13 (3·63%)
Vascular disorders	0 (0·00%)	1 (0·28%)*

* Vascular AE was an hypertensive episode

Figure1

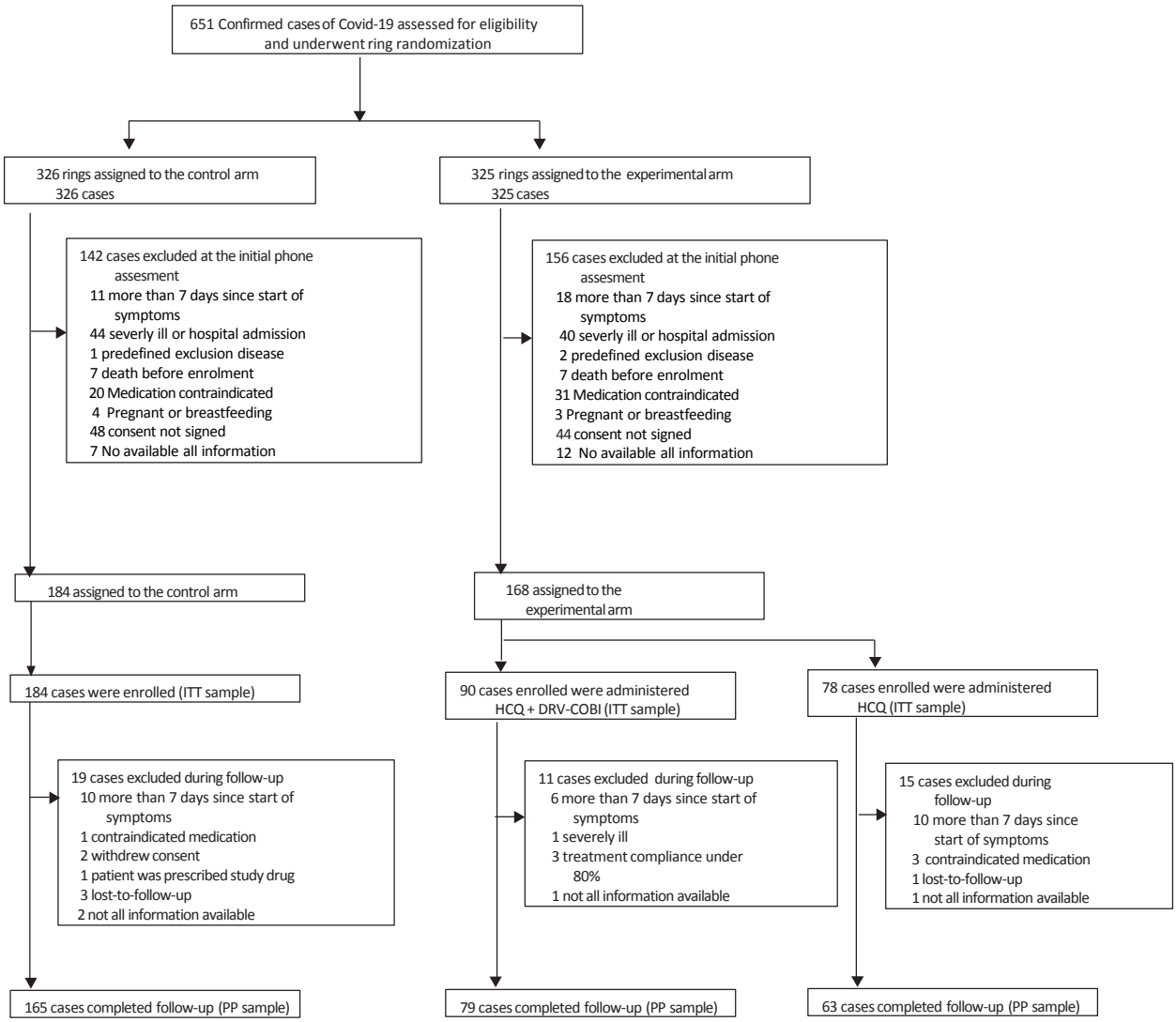
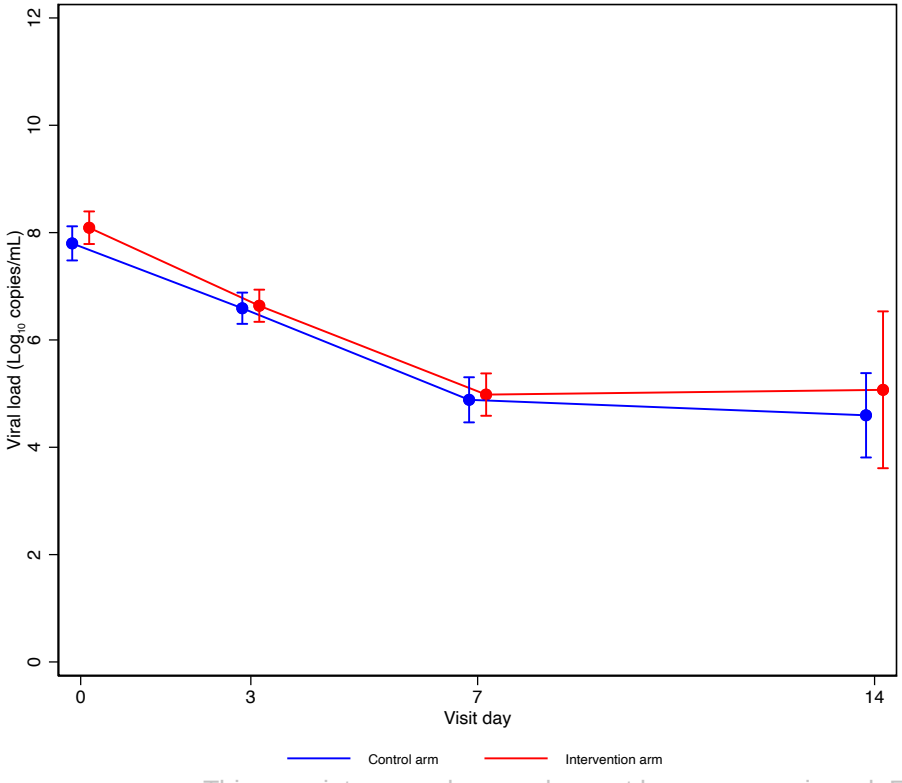


***Figure 1*: Trial profile**

Figure2



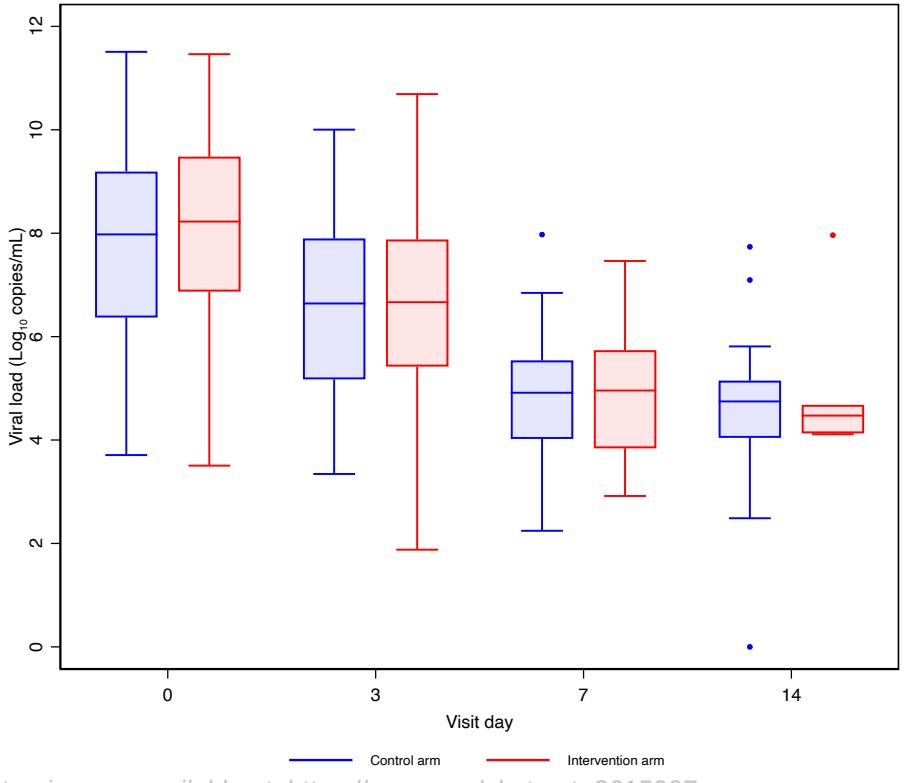
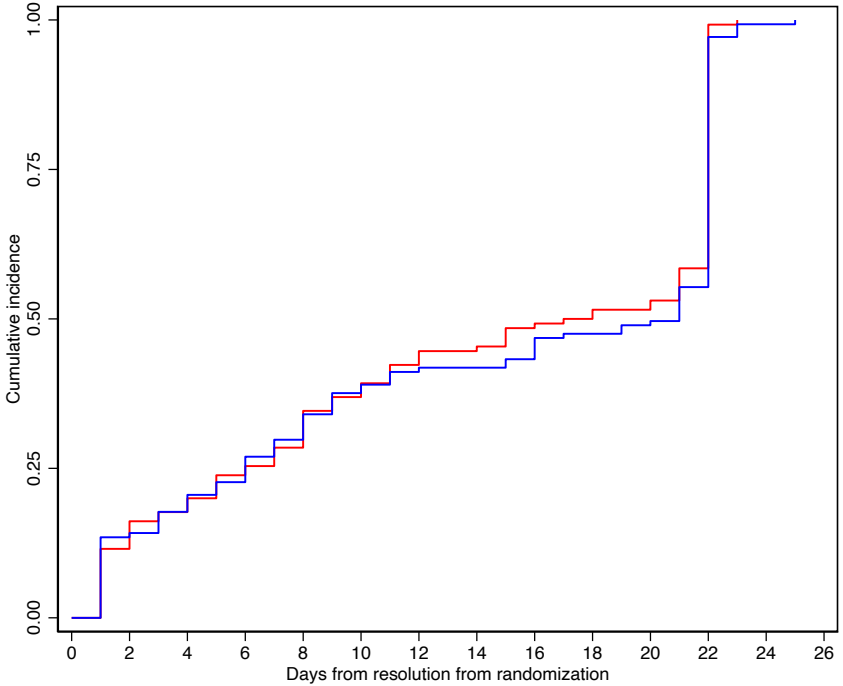
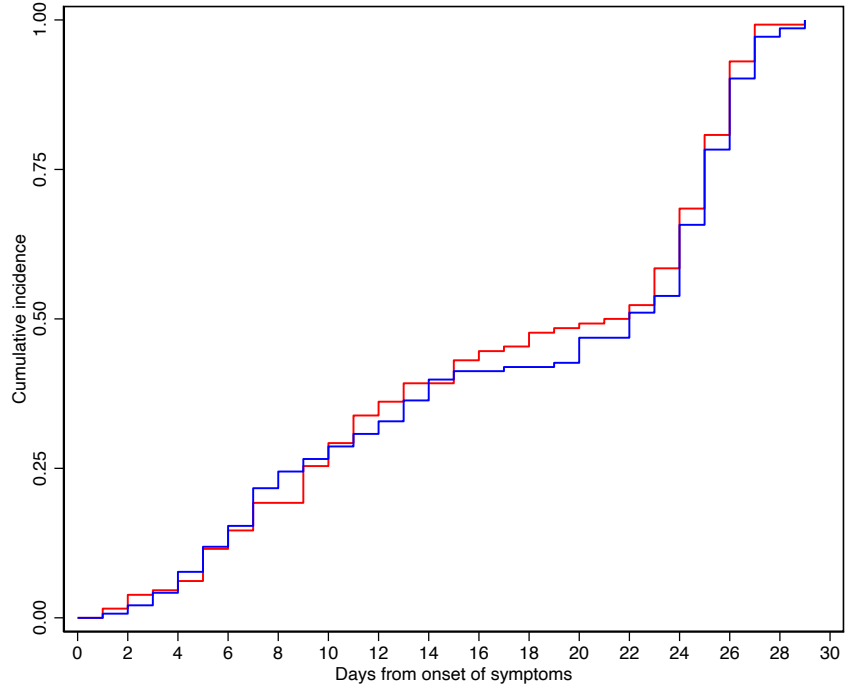


Figure3





Treatment of non-severe confirmed cases of COVID-19 and chemoprophylaxis of their contacts as prevention strategy: a Cluster Randomized Clinical Trial (BCN PEP CoV-2 Study)

Short name: Treatment of COVID-19 Cases and Chemoprophylaxis of Contacts as Prevention
ID: CQ4COV19
Protocol: v15.0, 12/05/2020

It includes:
Modification Number 1, version 1, 17 March 2020 substantial (Protocol version 12, 17/03/2020)
Modification Number 2, version 1, 30 March 2020 substantial (Protocol version 13, 30/03/2020 and version 14.0 after additional information requested by AEMPS)
Modification Number 3, version 1, 13 May 2020 substantial (Protocol version 15, 12/05/2020)

EudraCT: 2020-001031-27
ClinicalTrials.gov Identifier: NCT04304053

Study Type: Community based targeted screening and treatment – interventional randomized open label clinical trial

Sponsor Name:
FUNDACIÓN FLS DE LUCHA CONTRA EL SIDA, LAS ENFERMEDADES INFECCIOSAS Y LA PROMOCIÓN DE LA SALUD Y LA CIENCIA

Funding partners:
- Fundación FLS de Lucha contra el Sida, las enfermedades infecciosas y la promoción de la salud y la ciencia
- Institut Català de la Salut, Generalitat de Catalunya
- Departament de Salut, Generalitat de Catalunya
- Laboratorios Rubió SA
- Laboratorios Gebro pharma SA

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List of investigators and Key Roles:

PARTNER/PRINCIPAL INVESTIGATOR (PI) /INVESTIGATOR (I)	**KEY ROLE**
Hospital Universitari Germans Trias i Pujol	
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Marti Vall, (I) Infectious Diseases Department,	
Roger Paredes, (I) Infectious Diseases Department,	
Jordi Ara, (I) Nephrology Department,	
Lurdes Matas, (I) Microbiology Department,	
Ventura Clotet, (I) Infectious Diseases Department,	
Maria Ubals, (I) Infectious Diseases Department	
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Departament de Salut	
César Velasco, **(PI)** Agencia de Qualitat i Avaluació Sanitàries de Catalunya (AQuAs), Barcelona.	Patient identification and enrolment Contacts listing Patient examination, randomization, treatment and follow-up Data collection Creating research design
Rosa M Vivanco Hidalgo, (I) Agencia de Qualitat i Avaluació Sanitàries de Catalunya (AQuAs), Barcelona	
Servei d'Urgències de vigilancia epidemiológica de Catalunya (SUVEC),	
Joan Guix, (I) Secretaria de Salut Pública del Departament de Salut, Generalitat de Catalunya	
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Centre for Epidemiological Studies on Sexually Transmitted Diseases and HIV/AIDS of Catalonia (CEEISCAT)	
Jordi Casabona**, (PI)**	Building an online survey supervision of data entry Data cleaning and coding Participating in the research design and in the interpretation of statistical analyses
Alexis Sentís, (I)	

1. Brief Summary:

This study is a cluster randomized trial to evaluate the efficacy of a preventative strategy on reducing transmission and consequently, the COVID-19 incidence in the target population during an outbreak. The intervention entails treating non severe confirmed cases with hydroxychloroquine and administering prophylactic hydroxychloroquine same treatment to all contacts. Treatment of patients can reduce viral shedding in respiratory secretions to undetectable levels resulting in a reduction on the probability of onward transmission of SARS-CoV-2. Prophylactic hydroxychloroquine treatment administered to all contacts of confirmed index cases aims to protect all potential individuals that could become infected and develop the disease. Such approach will be in line with the current prospective surveillance to assess the population-level effect of this transmission prevention strategy.

Condition or disease	Intervention/treatment	Phase
Confirmed non severe case of COVID-19: SARS-CoV-2 positive by PCR plus mild respiratory symptoms (index case)	Pharmacological intervention: treat with hydroxychloroquine	Proof of concept (Phase III)
Contact of non severe COVID-19 case (index case confirmed with PCR)	Pharmacological intervention: treat with hydroxychloroquine	Proof of concept (Phase III)

2. Background:

As people with the SARS-CoV-2 infection arrive in countries or areas without ongoing transmission, efforts are being made to halt transmission, and prevent potential outbreaks. The standard public health interventions are firstly to identify the cases and make sure they are isolated, and secondly to identify their contacts to be monitored and quarantined. Mathematical models by Hellewell's and colleagues show that case isolation and contact tracing could contribute to reducing the overall size of an outbreak but will still be insufficient to achieve control of transmission of SARS_CoV-2 when the basic reproduction number (Ro) is higher than 1.5 or the proportion of contacts traced is below 80% (1). The Ro is the average number of cases generated by one case. In the early stage of this outbreak the Ro was estimated to be around 2 in China and the objective of control measures is to get the number below 1 which means that on average each case generates less transmission (1). Transmission before symptom onset could only be prevented by tracing contacts of confirmed cases and quarantining those contacts.

Enhanced treatment is required based on mathematical models

One of the main assumptions of the model is that isolation of cases and contacts is 100% effective to stop transmission from infected patients to other subjects not affected by the disease. If that isn't true, then an ancillary method which provides additive benefit on transmission would be good. Home confinement of infected individuals is challenging and efficacy is variable; (2) the person shares a common space with household members, no visitors should be allowed in the home, and the person should not be allowed to use any form of public transportation or stay at public spaces. Naturally, if infected individuals do not comply with the public health departments' instructions, this strategy is less effective. Similarly, rigorous tracking and quarantine of contacts requires considerable amount of public health resources and patient's compliance, but even in a best scenario some contacts continue to have household exposure to the patient with confirmed SARS-CoV-2 infection during the patient's isolation period. Thus, other strategies are needed to contain, delay or mitigate the outbreak.

The use of chemoprophylaxis of contacts as prevention is common practice in Infectious Diseases

The current COVID-19 emergency warrants the urgent development of potential strategies to protect high risk subjects (close contacts, health care workers, and others). The reason is that secondary attack rate of households (SARh) is ~15%, and that of close contacts (SARc)~ 10%. (3,4) This means that the risk of becoming infected after contact with a COVID-19 case is very high. The SARc is like influenza (10%) and much higher than meningococcal disease (<1%).

Postexposure prophylaxis (PEP) using antimicrobial agents is effective in preventing illness after potential or documented exposure to a variety of microbial pathogens and in reducing the risk of secondary spread of infection. PEP should be given to persons exposed to index cases of invasive meningococcal infection (Rifampin 600 mg orally, twice daily for two days), pertussis (Azithromycin 500 mg orally), necrotizing Streptococcal infection (Penicillin G benzathine 1.2MUI), and Tuberculosis in tuberculin skin test positives (Isoniazid for 6 months). Also, high risk people exposed to Influenza (oseltamivir 75mg, twice daily for five days) or HIV (raltegravir twice daily for 28 days) may benefit of PEP.

The most similar situation to SARS-CoV-2 infection is influenza infection. Previous research on influenza has indicated that antiviral drugs administered before o short after symptom onset can reduce infectiousness to others by reducing viral loads in the respiratory secretions of patients and targeted prophylactic use of contacts reduce the risk of becoming infected (5,6). The measure of providing antiviral treatment to patients and prophylaxis to the close contacts of influenza patients has been recommended by the World Health Organization as a principle of early aggressive measures to prevent pandemic influenza (7) and the strategy was shown highly effective in reducing the incidence of secondary cases. The same principle could be applied to all type of respiratory infections with epidemic potential spread by droplet transmission, including SARS-CoV-2.

We consider that this approach might be successful also if performed during the current SARS-CoV-2 epidemic due to the similarities of both infections.

Current knowledge of the efficacy of drugs to treat COVID-19 based on in vitro and clinical data

There are some reports and clinical trials that describe and investigate the efficacy of different drugs, among which the following ones that are included in some protocols, without robust data yet to support thist:

1. Protease Inhibitors:

Lopinavir/ritonavir, a protease inhibitor used to treat HIV/AIDS, was found to inhibit the *in vitro* cytopathic effect of SARS-CoV and MERS-CoV at concentrations (Half maximal effective concentration EC50 ~4.0μg/ml) achievable in humans. (6,7) Lopinavir/ritonavir 400/100, pharmacokinetic parameters for lopinavir are as follows: Cmax 9.6μg/ml, T1/2 5h, AUC24 186 μg*h/ml.

In addition, preliminary results show that this drug, either alone or with various combinations could provide some clinical benefit to the treatment of hospitalized patients with SARS-CoV-2 infection.(8) China's guidelines were set up in January 2020 and recommended treatment of hospitalized patients with lopinavir/ritonavir. Some countries, including Spain, provide similar recommendations (9). However, the drug is so far offered to sick patients only and we believe

that it should also be evaluated in mild cases in which it could contribute to halt transmission. Common side effects include diarrhea, nausea, abdominal pain in about 27% of patients treated.

Darunavir (DRV)/Cobicistat, is also a protease inhibitor used to treat and prevent HIV/AIDS. Its mechanism of action is very similar to Lopinavir/ritonavir. This drug combination was shown to be as effective as lopinavir/ritonavir for the treatment of HIV/AIDS. However, this combination is better tolerated tan lopinavir/ritonavir because the adverse effects rate is lower (diarrhea 2% vs 27%). Besides, the drug is being trialled currently for COVID-19 and preliminary seem promising (clinicaltrials.gov/ct2/show/NCT04252274
In last week DRV has been shown to exert no activity on the SARS-CoV-2 clinical studies and is therefore withdrawn for futility. Although LPVr shows in vitro efficacy against SARS-CoV-2 at elevated total drug concentrations, human clinical trials have not demonstrated superiority of LPVr vs Placebo. It has been considered that the magnitude of the possible clinical benefit of LPVr if it is started in the early stages of the disease is small and does not compensate for the gastrointestinal and renal toxicity of the drug.

2. Hydroxychloroquine

In vitro studies

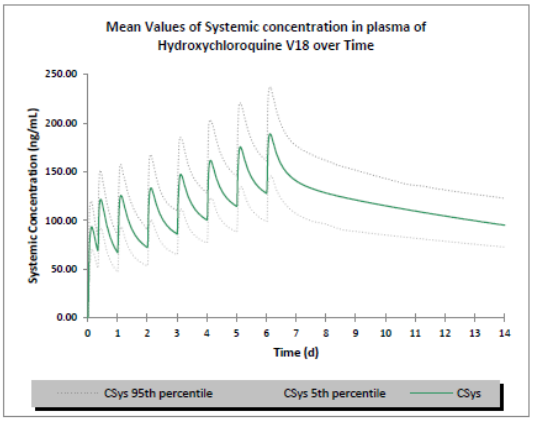
Hydroxychloroquine (HCQ) is a drug that has been extensively used for the prevention of malaria. HCQ showed excellent in vitro results and strong antiviral effects on SARS-CoV-2 infection of primate cells at low concentration. The EC50's were 0.72uM and 6.1 at 48 and 24 hrs incubation, respectively.(8) In SARS-CoV and MERS infections, an IC50 of approximately 5uM provides a reasonable and achievable target concentration to reach in plasma and lung (9,10). HQQ was found to be more potent than chloroquine (EC50 5.47uM [7], and 1.1uM in a previous study [11]) to inhibit SARS-CoV-2 in vitro. This family of drugs appears to interfere with terminal glycosylation of the cellular receptor, angiotensin-converting enzyme 2 which is the main host cell receptor of SARS-CoV-2.(11) This may negatively influence the virus-receptor binding and abrogate the infection, with further ramifications by the elevation of vesicular pH, resulting in the inhibition of infection and spread of SARS-CoV at clinically admissible concentrations.

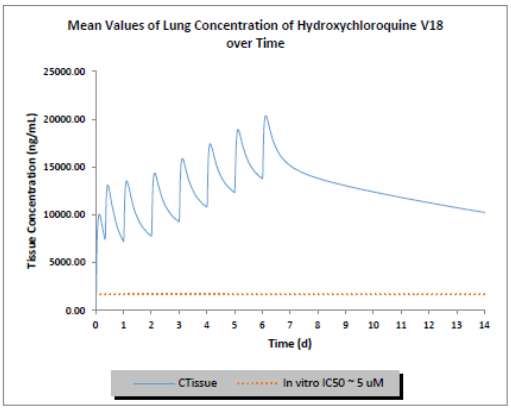
In vivo studies

An open-label non-randomized controlled trial in 36 patients diagnosed of SARS-CoV-2 reported that hydroxychloroquine alone or in combination with azithromycin reduced detection of SARS-CoV-2 RNA in upper respiratory tract specimens compared with a non-randomized control group but did not assess clinical benefit (12). The results showed that patients in the treatment group were significantly more likely to test negative for the virus on Day 6 than patients in the control group (70% vs 12.5% virologically cured, $p<0.001$). Moreover, all the six patients who were treated with a combination of HCQ and azithromycin tested negative on Day 6. The authors argue that this finding speaks to the effectiveness of HCQ and a potential synergistic effect of its combined treatment with azithromycin. A study in China reported that chloroquine treatment of COVID-19 patients had clinical and virologic benefit versus a comparison group,(13) and chloroquine was added as a recommended antiviral for treatment of COVID-19 in China.

Pharmacological aspects

According to pharmacological modelling conducted (Figure - Scott Miller, 16/03/2020) higher dose regimen (OHCQ 800mg d1, 400mg d2-7 (total dose 3,2g) will give good plasma levels and corresponding lung levels. Plasma troughs will be nearer to 100ng/ml, compared to 70ng/ml for a lower dose regimen OHCQ 800mg d1, 400mg d2-4 (total dose 2,0g). Lung concentrations will be much higher (2-2.5 log higher), but the free log concentration in lung epithelial cells are what will matter (which is not known).





Side Effects

Hydroxyhloroquine has a good safety profile (60% reduction of AEs compared to chloroquine) with a 3-day treatment course (Total dose (adults): 2.0 hydroxychloroquine sulfate in 3 days. (drug dataheet) Gastrointestinal upset has been reported with HCQ intake. Retinal toxicity has been described with long-term use of CQ and HCQ, and may also be related to over-dosage of these medications (daily doses of hydroxychloroquine sulfate greater than 6.5 mg/kg (5 mg/kg base) of actual body weight, durations of use greater than five years). Isolated reports of cardiomyopathy and heart rhythm disturbances caused by treatment with CQ have been reported. Chloroquine should be avoided in patients with psoriasis andporphyria. Both CQ and HCQ are metabolised in the liver with renal excretion of some metabolites, hence they should be prescribed with care in people with liver or renal failure.

Monitoring efficacy of treatment

- Upper respiratory tract (URT) and lower respiratory specimen (LRT) specimens: The virus is detected from URT specimens on day 2 (10*7 copies/ml)of symptom onset, increasing levels peak on day 5-7 (10*8 copies/ml) and then become spontaneously negative by day 14.
- Serum, plasma, urine, and stool samples: The virus is detected at very low levels in these type of specimens

In a study including the 66 confirmed cases, (18) the median time from the onset of symptoms to first negative RT-PCR results for oropharyngeal swabs in convalescent patients was 9.5 (6.0-11.0) days, for stool samples was 11 (9-16) days. Positivity of urine samples was low (7%) and all blood specimens were negative. Exceptionally, 4 cases have been found to have positive rt-PCR after clinical and molecular cure (2 consecutive negative tests) (19).

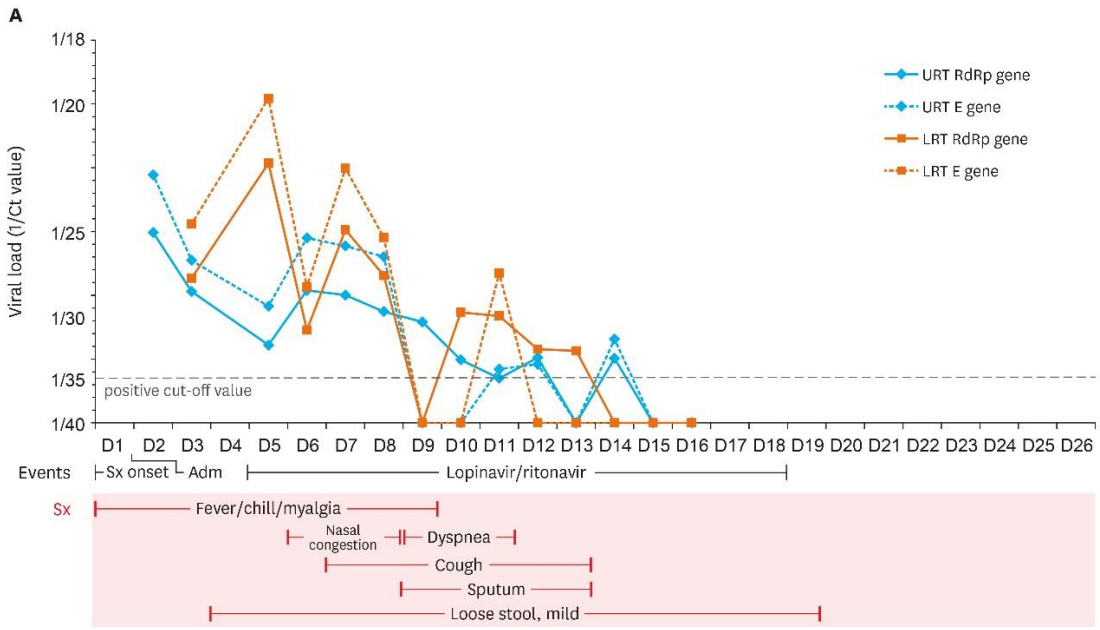


Figure 1. Viral load kinetics of respiratory specimen presented by reverse Ct value.

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3. Study sites:

The study will be conducted over the course of a COVID-19 outbreak in Catalonia and for the selection and case definitions of the participants we will follow the current Catalan/Spanish protocols in line with WHO. The detection and notification of confirmed cases and contacts is centralized by the Catalan epidemiological surveillance system (SU-VEC). Thus, for the purpose of this study randomization will be performed by a member of this team.

The study outbreak team, consisting of 20 health care workers, will visit all cases and contacts at home for baseline assessment, administration of intervention drugs (in the experimental arm) and follow-up assessments to explore the effect of the intervention.

Individuals who choose not to participate in the study will be managed following the current protocols.

4. Study Design

Study Type:	Interventional (ring treatment trial)
Estimated Enrolment:	712 COVID-19 cases (356 each arm) and 2850 contacts (4 per index case)
Allocation:	Cluster-randomized
Masking:	Open-label
Index Cases	Those individuals diagnosed of mild COVID-19 (SARS-CoV-2 PCR positive plus symptoms)
Contacts:	As defined by the current protocol of the Catalan epidemiological surveillance system

Intervention:	Pharmacological ((Hydroxychloroquine in index cases and in contacts)
Primary Purpose:	Prevention at population level
Actual Study Start Date:	March 16, 2020
Estimated Primary Completion Date:	May 13, 2020
Estimated Study Completion Date:	May 13, 2020
Site:	Catalonia

5. Design considerations

The design intervention is based on the design used during the vaccination trial developed for Ebola in 2015 (ref1). This was a cluster randomised controlled trial with the aim of evaluating vaccines against the disease in Guinea, West Africa. In the ring vaccination trial, a person newly diagnosed with the disease becomes the index case around whom an epidemiologically defined ring is formed. This ring is then randomised to either immediate vaccination (intervention) or delayed vaccination (control) in a 1:1 ratio on an open label basis. The incidence of disease is compared between the two arms over equivalent time periods measured from the time of randomisation of each ring. Comparing the hazard ratio in those enrolled in the study allows estimation of vaccine efficacy, while overall vaccine effectiveness can be estimated by comparing incidence across all members of the rings, including those not eligible for vaccination in the study. This design permits to track the epidemic, recruiting individuals at increased risk of infection due to their connection to a case and thus, may both contribute to transmission interruption and have a higher power to detect vaccine efficacy than other study designs.

In our scenario, after the Catalan epidemiological surveillance system detects a person newly diagnosed with the COVID-19 with non-severe symptoms, this individual will be considered the index case and an epidemiological ring of contacts will be formed. These rings of contacts will include all the index case contacts on day 1, as are defined in the Catalan/Spanish protocol and eventually new cases that could be also linked with the index case a posteriori taking into account the incubation period. In our intervention, the index case will be randomised (experimental arm vs control arm). The ring assigned to the index case receiving experimental intervention will be treated too.

If the index case is identified but he/she does not meet the inclusion criteria (for example, due to hospital admission), we will recruit and randomize his/her ring of contacts in the clinical trial. The ring will receive the experimental intervention or not according to the group assigned to the index case.

Additionally, we will enhance passive recruitment by conducting an Information, Education, Communication (IEC) campaign in the social media (Twitter, etc). The material posted will explain information about the study (describing the aims, details, risks and benefits), describe the criteria for inclusion, and provide contact information with the research team for those people who meet the criteria and are willing to participate. This procedure will allow to reach the estimated sample in the contacts group.

*[Ref1]: Ebola ça Suffit Ring Vaccination Trial Consortium. The ring vaccination trial: a novel cluster randomised controlled trial design to evaluate vaccine efficacy and effectiveness during outbreaks, with special reference to Ebola. BMJ. 2015 Jul 27;351:h3740. doi: 10.1136/bmj.h3740.

6. Interventions

Arm	Intervention/treatment
CONTROL ARM **No treatment. Standard surveillance.** Index case completes a survey collecting demographic, epidemiological and clinical data and provides a swab for RT-PCR testing at baseline and on days 3 and 7. Contacts will also complete a survey collecting demographic, epidemiological and clinical data and provides a swab for RT-PCR testing at baseline and day 14. Rescue therapy with Hydroxychloroquine will be administered when a contact in the control arm develops symptoms consistent with COVID-19 and positive PCR. Isolation of patient and contact tracing as per national guidelines. **1. Rescue treatment of COVID-19 (contacts):** Contacts in the control arm who develop symptoms of COVID-19 and have a positive PCR during the 14-day study period will be offered rescue treatment with Hydroxychloroquine 800 mg (620 mg base) followed by 400 mg daily for 6 days [OHCQ 800mg d1, 400mg d2-7 (total dose 3,2g)]. Follow-up of symptom diaries will be collected by phone from participants 3 and 7 days (contacts) and 14 days (cases) after baseline visit.	
EXPERIMENTAL ARM **Treatment of SARS-CoV-2 PLUS prophylaxis to contacts.** Index case completes a survey collecting demographic, epidemiological and clinical data and provides a swab for RT-PCR testing at baseline and on days 3 and 7. Isolation of patient and contact tracing as per national guidelines. Index case receives Hydroxychloroquine. Contacts receive Hydroxychloroquine prophylaxis. Index case contacts will also complete a survey collecting demographic, epidemiological and clinical and provides a swab for RT-PCR testing at baseline and day 14. **1.Treatment of COVID-19 (index case):** Eligible individuals will offered Hydroxychloroquine 800 mg (620 mg base) followed by 400 mg daily for 6 days [OHCQ 800mg d1, 400mg d2-7 (total dose 3,2g)] . Follow-up of symptom diaries will be collected by phone from participants 3 and 7 days (contacts) and 14 days (cases) after receiving treatment at baseline visit. **2.Prophylaxis of contacts:** Contacts will be offered a prophylactic regimen of Hydroxychloroquine 800 mg (620 mg base) followed by 400 mg daily for 6 days [OHCQ 800mg d1, 400mg d2-7 (total dose 3,2g)]. Follow-up symptom diaries will be collected for 14 days.	

Supply, packaging, and storage

All treatments will be stored at and administered by the Pharmacy Department of Hospital Universitari Germans Trias i Pujol (HUGTIP).

Hydroxychloroquine and will be stored in a safe place during the study, in accordance with conditions defined in its Summary of Products Characteristics (SmPC). Being marketed medication, specific temperature control for the study will not be performed.

The medication will be supplied in blister packs and the primary packaging will not be altered by the Pharmacy office. An information sheet with the relevant information on instructions for use, pharmaceutical form, dosage and safety aspects will be attached.

The distribution of the treatments will be performed through field teams consisting of health care workers. The treatments will be prepared for each participant.

To check compliance with study treatment, the investigators will ask the subject about treatment adherence and this data is to be written in the database. Pills will not be counted to assess compliance.

7. Aim and Outcome Measures

Hypothesis: Our primary hypothesis is that implementation of an early antiviral treatment intervention among confirmed cases with COVID-19 presenting mild symptoms and their contacts, detected by the Catalan epidemiological surveillance system will reduce the transmissibility of SARS-CoV-2 within the study population over the course of the outbreak. A process evaluation will also be conducted to explore the effect of the intervention on patient individual parameters (cure or reduction of symptoms).

Overall Aim: We aim to evaluate the effectiveness to reduce transmissibility and disease progression of antiviral treatment of all who are found to be infected and chemoprophylaxis of close contacts assessed by secondary attack rate of COVID-19 among contacts in the control and experimental arm.

Objectives:
- Evaluate the transmissibility of SARS-CoV-2 and reduction of disease progression within the study population over the course of the outbreak.
- Explore the effect of the intervention on patient individual parameters.

Outcome Measures [Population level]:
1. Ring prophylaxis effectiveness to reduce development of disease assessed by Incidence of secondary cases (basic case reproduction number) among contacts of a case [Time Frame: Up to 14 days after start of treatment]
2. Ring prophylaxis effectiveness to reduce transmissibility assessed by PCR conversion to positive of contacts that are negative at baseline [Time Frame: Up to 14 days after start of treatment]
3. Ring prophylaxis effectiveness to reduce transmissibility assessed by SARS-CoV-2 IgM/IgG positivity at day 14 [Time Frame: Up to 14 days after start of treatment]
4. Feasibility of implementation of treatment strategy [Time Frame: Up to 6 months]
5. Cost effectiveness of test-and-treat intervention [Time Frame: Up to 6 months]
   Assessed using health resource utilization (including emergency department visits)

Outcome Measures [COVID-19 case Individual outcomes]:

1. Symptom type, duration and severity among SARS-CoV-2 positive cases [Time Frame: Up to 14 days after start of treatment]
   Descriptive statistics of clinical manifestations of illness, stratified by demographic, comorbidity
2. The virological clearance rate of throat swabs, sputum, or lower respiratory tract secretions at days 3 and 7 of index case [Time Frame: 3-7 days after start of treatment]
3. The mortality rate of subjects at weeks 3 [Time Frame: 21 days after start of treatment]
4. Proportion of participants that drop out of study [Time Frame: 14 days after start of treatment]
   Measured as dropping out after providing consent
5. Proportion of participants that show non-compliance with study drug [Time Frame: 14 days after start of treatment]
6. Proportion of participants that show non-compliance with public health measures [Time Frame: 14 days after start of treatment]
7. Drug levels and biomarkers of severity of infection [Time Frame: Up to 14 days after start of treatment]

8. Eligibility Criteria for Index Cases

Inclusion Criteria:

1. Patients who meet the requirements of the New Coronavirus Infection Diagnosis (Acute - ≤7 days - respiratory infection symptoms, fever, cough, shortness of breath, acute olfactory loss and positive PCR)
2. Aged ≥18 years male or female
3. In women of childbearing potential[1], negative pregnancy test and commitment to use contraceptive method[2] throughout the study.
4. Willing to take study medication
5. Willing to comply with all study procedures, including repeat nasal swab at day 3
6. Able to provide oral and written informed consent

[1]A woman will be considered of childbearing potential if not permanently sterilized nor postmenopausal. Permanent sterilization methods include tubal ligation, hysterectomy and bilateral oophorectomy. Postmenopausal is defined as 12 months with no menses without an alternative medical cause.

[2]Contraceptive methods: male or female condom with or without spermicide, cap, diaphragm or sponge with or without spermicide, intrauterine device, bilateral tubal occlusion, vasectomized partner, sexual abstinence during the study.

Exclusion Criteria:

1. Hospital admission
2. Serious condition meeting one of the following: (1) respiratory distress with respiratory rate >=30 breaths/min; (2) oxygen saturation<=93% on quiet status; (3) Arterial partial pressure of oxygen (PaO2)/oxygen concentration<=300mmHg;
3. Critically ill patients meeting one of the following: (1) Experience respiratory failure and need to receive mechanical ventilation; (2) Experience shock; (3) Complicated with other organs failure and need intensive care and therapy in ICU;
4. Participants under treatment with medications likely to interfere with experimental drugs
5. Unable to take drugs by mouth;
6. With significantly abnormal liver function (Child Pugh C)
7. Need of dialysis treatment, or GFR≤30 mL/min/1.73 m2;

8. Participants with psoriasis, myasthenia, haematopoietic and retinal diseases,CNS-related hearing loss or glucose-6-phosphate dehydrogenase deficit
9. Participants with severe neurological and mental illness;
10. Pregnant or lactating women;
11. Inability to consent and/or comply with study protocol;
12. Individuals with known hypersensitivity to the study drugs;
13. Persons already treated with any of the study drugs during the last 30 days.
14. Any contraindications as per the Data Sheet of Hydroxychloroquine.

Eligibility Criteria for contacts

Inclusion Criteria:

1. Asymptomatic individuals exposed to a PCR confirmed COVID19 case within 7 days as either a healthcare worker or household contact
2. Aged ≥18 years male or female;
3. In women of childbearing potential, negative pregnancy test and commitment to use contraceptive method throughout the study.
4. Willing to take study medication;
5. Willing to comply with all study procedures;
6. Able to provide oral, informed consent and/or assent.

Exclusion Criteria:

1. With known history of cardiac arrhythmia (or QT prolongation syndrome);
2. Unable to take drugs by mouth;
3. With significantly abnormal liver function (Child Pugh C)
4. Need of dialysis treatment, or GFR≤30 mL/min/1.73 m2;
5. Participants with psoriasis, myasthenia, haematopoietic and retinal diseases,CNS-related hearing loss or glucose-6-phosphate dehydrogenase deficit; 6. Persons already treated with any of the study drugs during the last 30 days;
7. Pregnant or lactating women;
8. Any contraindications as per the Data Sheet of Hydroxychloroquine.

If a contact is symptomatic at the time of the baseline visit, he/she will be classified as a co-primary case, and we will collect epidemiological information but will not be enrolled in the study as a contact participant.

9. Randomization and statistical analysis

Sample size calculation: Approximately 712 rings (95 per arm) of size 4 are required to have 90% power to reject the null hypothesis (10% difference in incidence of secondary cases among contacts, expected 15% in control arm, and 5% in intervention arm) The final sample size achieved will depend on the number of new index cases accumulating during the study period

Stratified randomization: Random allocation of intervention (the ring includes the index case plus its contacts) is done remotely, by a member of the study team not involved in the definition of rings. We will use block randomization to achieve balanced sample size in each group. Stratified randomisation by province is achieved by performing a separate randomisation procedure within each of the participant provinces (example, if the index case is in Tarragona, incidence will be estimated and compared in this province).

Allocation: The study is open label. Oral preinformed consent is obtained before randomization in order to ask willingness to participate in the trial. With a positive answer to participate,

informed consent and eligibility are done after randomisation. Communicable disease control measures other than ring treatment are identical in the two groups.

Analysis: We will analyse outcomes at the cluster level using the cumulative incidence for each cluster. If no cases of SARS-CoV-2 virus disease occurs in one group, we will derive a 95% CI for the intervention effect by fitting a β-binomial distribution to the cluster-level numerators and denominators. For comparisons in which cases of SARS-CoV-2 virus disease occurred in both groups, we will fit a Cox proportional hazards model using a cluster-level frailty term to adjust for clustering within rings. The primary analysis will be per protocol. We will conduct secondary analysis adjusted for baseline values of delay between symptom onset and isolation/treatment.

Planned interim analysis: We plan an interim analysis for possible early trial termination for superiority or futility of the experimental therapy. The trial is open-label and does not need unblinding. The interim analysis will be performed by an independent statistician. The analysis will be performed on the primary endpoint when 25% (n=48) of patients have been randomized and have completed 14 days follow-up. Randomization will be done by blocks, so we expect similar numbers in each group at interim analysis. We will look at the 95% CI for the difference between groups. The Peto approach will be used: the trial will be ended using symmetric stopping boundaries at P<0,01, both in case of superiority or futility.
Interruption criteria: Incidence of secondary cases among contacts of a case is < 2% (stop for futility) or >8% (stop for superiority)

10. Procedures

- Active surveillance, laboratory confirmation of cases of COVID-19, and the list of contacts is independently undertaken by Catalan epidemiological surveillance system (SUVEC).
- After notification of the disease the SUVEC will process the data and notify the researchers team
- The researcher's team will call the positive cases in order to offer people diagnosed with coronavirus to participate in a clinical
- An oral inform consent will be obtained by phone. The researcher will inform about the trial to individuals that fulfil inclusion criteria (on the basis of online medical records and clinical history taken by phone) and the randomization process will start (for index cases and their contacts).
- Dedicated outbreak field-teams will visit candidates and verify the inclusion criteria eligibility on day 1.
-The SUVEC will provide the list of contacts. The same procedures as described for cases will be done for each contact.
-Kits will be numbered to ease traceability

1. Baseline visit (cases)
    - Nasopharyngeal swab (or sputum for patients with productive cough) will be collected and sent to the microbiology department for testing.
        - Nasopharyngeal: Use only synthetic fibre swabs with plastic shafts. Insert a swab into the nostril parallel to the palate. Leave the swab in place for a few seconds to absorb secretions. Place swabs immediately into sterile tubes containing 2-3 ml of viral transport media.
        - Sputum: Have the patient rinse the mouth with water and then expectorate deep cough sputum directly into a sterile, leak-proof, screw-cap sputum collection cup or sterile dry container.

- Everyone will be asked and examined when needed for signs of COVID-19 infection to identify the severity signs (Temperature, Oxygen saturation, Respiratory rate, Blood Pressure) Patients on the experimental arm will be offered treatment according to regimen in Fig1.

Fig 1. Treatment schedule for a COVID-19 mild case

Days	1	2	3	4	5	6	7	14
AM	♣♣♣♣ †	♣♣	♣♣ †	♣♣	♣♣	♣♣	♣♣ †	*

♣ Hydroxychloroquine 200 mg ; † Home visit
* Telephone check

2. Baseline visit (contact):
   - Nasopharyngeal swab (or sputum if possible) will be collected and sent to the microbiology department for testing as above for cases.
   - Everyone will be asked and examined when needed for signs of COVID-19 infection as above for cases:
     - Symptoms of acute respiratory infections (cough, odynophagia, rhinorrhoea). Severe (any duration) or mild (lasting at least 48h - two nights)
     - Dyspnoea of any duration
     - Fever (> 37.5) of any duration
     - Diarrhea accompanied by 1, 2, or 3
   -
   - Epidemiological investigation will include questions about:
     - number of days that has been in contact with the index case,
     - place of contact (home, work, nursing care facility, hospital),
     - use of mask (both case and contact)
   - Contacts on the experimental arm will be offered prophylactic treatment as per regimen in Fig 2.

Fig 2. Treatment schedule for contacts of a COVID-19 case

Days	1	2	3	4	5	6	7	14
AM	♣♣♣♣ †	♣♣	♣♣*	♣♣	♣♣	♣♣	♣♣*	†

♣ Hydroxychloroquine 200 mg ; *Telephone check; † Home visit

3. Follow up day-3 of cases (cases only) -home visit-
- A nasopharyngeal swab will be collected at home by the outbreak team
- Everyone will be examined for signs of COVID-19 infection and will be asked about Adverse Events and Compliance to treatment.

4. Follow up day-7 (cases home visit and contacts by telephone call)
- Evaluation of health status, adverse events, and compliance to treatment
- A nasopharyngeal swab will be collected from the case.

5. Follow up day-14 (cases and contacts) -home visit- (telephone call to cases if home visit not possible)
- Evaluation of health status
- A nasopharyngeal swab will be collected from the contact.
- A SARS-CoV-2 rapid test IgM/IgG/Ag will be conducted by fingerprick (contacts)

6. At any follow visit, if a participant presents with a clinical condition that might need a detailed medical evaluation (including, but not only respiratory distress with respiratory rate >=30 breaths/min; Temperature >38ºC, Blood pressure <90/60mmHg) will be referred to the reference hospital for further management. In a less severe symptomatic situation not requiring hospitalization we will take a nasopharyngeal swab at this time from contacts at home.

Trial withdrawal due to medical condition

If at any time during the 14-day follow-up a participant requires hospital admission due to COVID-19 OR is prescribed additional antiviral drugs by the treating physician at the hospital, then the participant will be withdrawn of the study.

Evaluation of primary outcome - health status of contacts for 14 days follow-up-

- The research team will initiate active surveillance of any asymptomatic person who meets the definition of contact, following the protocols of the Catalan epidemiological surveillance system (SUVEC).
- If during the 14 days after the exposure the contact develop symptoms, he/she is asked to immediately contact the research team.
- The research team will investigate that contact to rule out infection by SARS-CoV-2 including:
- Clinical examination
    - Symptoms of acute respiratory infections (cough, odynophagia, rhinorrhoea). Severe (any duration) or mild (lasting at least 48h - two nights)
    - Dyspnoea of any duration
    - Fever (> 37.5) of any duration
    - Diarrhea accompanied by 1, 2, or 3
- Nasopharyngeal swab

Evaluation of Adverse Events

See definitions and procedures below (point 11).

Evaluation of Compliance to treatment

We will use a self-reported questionnaire for assessment of adherence to treatment (Brief Medication Questionnaire – BMQ).

The tool includes a 5-item Regimen Screen that asks patients how they took each medication in the past week, a 2-item Belief Screen that asks about drug effects and bothersome features,

Fig 3a. Workplan timeline for a case.

	Baseline	**Day 3**	**Day 7**	**Day 14**
Written Informed Consent	X			
Pregnancy test	X			
Inclusion criteria checks	X			
Clinical examination	X	X		
Nasopharyngeal Swab	X	X	X	
Adverse events assessment		X	X	X

Compliance assessment		X	X	
Follow up assessment			X	X

Fig 3b. Workplan timeline for a contact

	Baseline	**Day 3**	**Day 7**	**Day 14**
Written Informed Consent	X			
Pregnancy test	X			
Clinical examination	X			
Inclusion criteria checks	X			
Nasopharyngeal Swab	X			X
Blood sample (Rapid Test)				X
Adverse events assessment		X	X	X
Compliance assessment		X	X	
Follow up assessment			X	X

11. Adverse events

11.1. Definitions:

Adverse event (AE): Medical event presented by a patient or clinical research subject administered a pharmaceutical product, and which does not necessarily have a causal relation to the treatment.

Serious adverse event (SAE): Medical event classified as such and which, regardless of the dose involved:

- Causes patient death.
- Produces a life-threatening situation for the patient.
- Requires or prolongs in hospital admission.
- Produces important or persistent incapacitation/handicap or constitutes a congenital defect or anomaly.
- Needs action to prevent any of above situations.
- Is considered medically significant (examples of such events are intensive care in an Emergency Service or at home in a patient with allergic bronchospasm; blood dyscrasias or seizures not giving rise to hospital admission, or the development of drug dependency or abuse).

Unexpected adverse event (UAE): AE related to the product in investigation the nature or intensity of which does not coincide with the information available on the product administered (IB or SmPC).

Serious Unexpected Adverse Reaction (SUSAR): SAE related to the product in investigation the nature or intensity of which does not coincide with the information available on the product administered (IB or SmPC).

11.2. Adverse Events Assessment

11.2.1. Seriousness

An SAE is any medical event that meets the criteria of SAE of section 11.1.
Events not considered to be SAEs are hospitalizations for:

- A standard procedure for protocol therapy administration. However, hospitalization or prolonged hospitalization for a complication of therapy administration will be reported as an SAE.
- Routine treatment or monitoring of the studied indication not associated with any deterioration in condition.
- A procedure for protocol/disease-related investigations (e.g., surgery, scans, endoscopy, sampling for laboratory tests, bone marrow sampling). However, hospitalization or prolonged hospitalization for a complication of such procedures remains a reportable SAE.
- Hospitalization or prolongation of hospitalization for technical, practical, or social reasons, in absence of an AE.
- A procedure that is planned (i.e., planned prior to starting of treatment on study); must be documented in the source document and the CRF. Hospitalization or prolonged hospitalization for a complication remains a reportable SAE.
- An elective treatment of a pre-existing condition unrelated to the studied indication.
- Emergency out participant treatment or observation that does not result in admission, unless fulfilling other seriousness criteria above.

11.2.2. Intensity

The following scale will be used:

- Grade 1 (mild): Symptoms causing no or minimal interference with usual social and functional activities.
- Grade 2 (moderate): Symptoms causing greater than minimal interference with usual social and functional activities.
- Grade 3 (severe): Symptoms causing inability to perform usual social & functional activities.
- Grade 4 (potentially life-threatening): Symptoms causing inability to perform basic self-care functions or medical or operative intervention indicated to prevent permanent impairment, persistent disability, or death.
- Grade 5 (death): Any AE where the outcome is death.

11.2.3. Causality

All AEs must have their relationship to study intervention assessed by the physician who examines and evaluates the subject based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below. In a clinical trial, the study product must always be suspect.

- Related – The AE is known to occur with the study intervention, there is a reasonable possibility that the study intervention caused the AE, or there is a temporal relationship between the study intervention and event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study intervention and the AE.
- Not Related – There is not a reasonable possibility that the administration of the study intervention caused the event, there is no temporal relationship between the study intervention and event onset, or an alternate aetiology has been established.

11.2.4. Expectedness

An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study intervention. Risk information of study interventions may be found in the SmPC of each study drug.
The assessment of the expectedness between an AE and the administration of treatment is a decision to be made by the principal investigator OM or co-investigator MV, who are qualified physicians.
Expectedness will be assessed in relation to the AE being previously documented as per attached Technical Data Sheet – Ficha técnica point 4.8-). A serious unexpected adverse reaction (SUSAR) is a suspected adverse reaction (AR) whose nature, severity or outcome is not consistent with the Technical Data Sheet.
All unexpected serious ARs will be notified through Eudravigilance. For a suspicion of AR considered to be expected only for one of the two treatments (darunavir-cobicistat or hydroxychloroquine, we will consider question 7.25 (The rules governing medicinal products in the European Union VOLUME 10 - Guidance documents applying to clinical trials) on how should SUSARs of combination IMPs be reported? The question and answer document, section 7 of which includes relevant aspects of AR assessment to be considered.

11.2.5. Duration

For both AEs and SAEs, the Investigator will provide a record of start and stop dates of the event (expressed in the shortest time unit possible). Changes in the severity of an AE or SAE will be documented in the clinical record.

11.2.6. Action taken

The Investigator will report the action taken with study intervention as a result of an AE or SAE, as applicable (e.g., discontinuation or reduction of dose, as appropriate) and report whether concomitant and/or additional treatments were given for the event.

11.2.7. Outcome

Any AE or SAE will be followed preferably until:

- Resolution of the event;
- Stabilization of the event; or
- Resetting the baseline situation of the event, in case baseline situation is available.

Otherwise, they will continue until:

- The event can be attributed to products other than the study medication or factors unrelated to the study; or
- It is unlikely to obtain further information

In the event that the subject dies from a SAE, the rest of AE or SAE that are active will be recorded as "not recovered".

11.3. Timeframe for adverse events collection

The investigator must collect all the AE and SAE that occur from the moment the subject signs the informed consent until the last study visit.

11.4. Documentation related to adverse events

Each AE and SAE to take place during the study should be documented in the medical records of the participant in accordance with standard clinical practice of the investigator. For each SAE, an independent set of SAE form will be used independently. Only if there are multiple SAE at the time of the initial report and these are temporary and / or clinically interrelated can be registered on the same set of SAE form.
The investigator should try to make a diagnosis of the event based on the signs, symptoms and / or other clinical information. An AE diagnosis must be recorded per line, or a sign/symptom if

the diagnosis is not available. If a diagnosis subsequently becomes available, this then should be entered, and the sign/symptom crossed out.
SAE pages found in the investigator's file shall be completed as precisely as possible and shall be signed by the investigator before being sent to the sponsor. In the initial page of the SAE form, the investigator must provide his/her opinion in regard to the relationship of the event to the study intervention.

11.5. Pregnancy

Cases of pregnancy shall be recorded as AE and should only be considered as SAE only if they meet any seriousness criteria. Pregnancy is also a protocol deviation requiring premature termination of the subject. The investigator will provide medical support to the pregnant subject.
No special measures are required in relation to the pregnancy of a partner of a male participant.

11.6. Procedure for adverse event reporting

11.6.1. Investigator

All AEs and SAEs will be recorded, regardless of the causality, in the corresponding AE form.
The investigator will immediately notify the study sponsor of any SAE. The notification will be performed within 24 hours of first knowledge by the investigator.
Contact details for Sponsor
safety@fls-rs.com
The recording of AEs and SAEs is the responsibility of the trial investigator team, which should indicate the time of appearance of the event (expressed in the shortest time unit possible), its serious / not serious status, and in case it is considered related to investigational products, whether it was expected or unexpected. The intensity of the event (grade 1 to 5) is to be specified, along with the measures adopted (none, treatment, temporal or permanent discontinuation of investigational product), course (complete remission, partial remission, persistence) and causality based on the criteria indicated in section 11.2.3.

11.6.2. Sponsor

A group of researchers designated by the sponsor, will review the list of AEs, SAEs and SUSARs reported by the investigators in the CRF. The objective of this revision is the proper adjudication and notification, if needed, to the Spanish Agency of Medicines and Medical Devices (AEMPS) through the notification to Eudravigilance database, competent authorities of the autonomous region and the Ethics Committee implicated in the clinical trial.

The sponsor will inform the AEMPS, the competent authorities of the autonomous region and the Ethics Committee implicated in the clinical trial about any important information of security of the investigational medicinal product.
The sponsor will inform the Spanish AEMPS of any SUSAR which may be related to the study treatment.
The sponsor will inform competent authorities of the involved autonomous region of any SUSAR which may be related to the study treatment, and that have been happened in subjects in its autonomous region.
The deadlines to notify a SUSAR is, from the first knowledge by the investigator:

- 15 days
- 7 days if the SUSAR has resolved in death or has been life-threatening. Relevant follow-up information for these cases will be subsequently be submitted within an additional 8 days.

If the notification is sent in electronic form, it will not necessary to notify the competent authorities of the autonomous region.

The sponsor will keep a detailed register of all the AE notified by the investigators.
All AE will be notified in table form in the final report of the clinical trial.

12. Data collection

The CRF will be administered to all selected participants. Data will be collected using face-to-face questionnaires (paper CRF) by the field clinical teams and data will be entered in a standardized electronic questionnaire (digital CRF) to be accessed online, and which will be merged in a secured web site that uploads data in real time. The chief investigator will be responsible for keeping a subject identification log of all subjects enrolled into the study, their corresponding study number and sample IDs. This information will be kept on a secure server in a password protected file and will only be available to the chief investigator and the study personnel who are directly obtaining clinical data.

Identifying information of a SARS-CoV-2 PCR result of some participants will be extracted from the Epidemiological Repository of Catalonia (REC), which is the data platform that aggregates and manages data of Catalan surveillance systems of notifiable diseases including epidemic outbreaks (all are of mandatory declaration), and which is coordinated by the general sub-directorate for public health emergencies surveillance and response of the Public Health Agency of Catalonia, Health department, Government of Catalonia. Subjects will be assigned a linked-anonymised study number to ensure subject confidentiality throughout the duration of the study.

13. Data management

The clinical trial has created a data management system and procedures to warrant homogenization, traceability, and data quality. Paper CRF will be used to collect the CRF's data during home visits, and electronic CRF for telephone visits. Data will be entered in a digital CRF. Quality control procedures will be put in place for data checking by an external data management group. Rigorous consistency checks will be created in order to reduce errors during data entry. The data management group and statisticians will be responsible for the final analysis of the data.
Study data will be sent from paper CRF to a central FLS database. This database will enter and store the final data and will be on a server hosted at a secure Data Center with appropriate series of protocols to test and maintain network security, and to provide access management policies for network drives, databases and remote access.

For data safety purposes each person entering data in the digital CRF will be required to define clear data access. Data management team and researchers will be the only ones to access the database. The backup of the data will be done on a timely basis. The final stored data will be placed on the FLS server and will be anonymous; the tools used to identify individuals may have individual identifiers, but this information will only be associated to a numerical identification number. This information will uniquely identify project participants will be associated with the rest of the captured sensitive information. If information that could enable to identify individuals has to be stored, used or shared, it will be encrypted. Consequently, those receiving the final data for analysis will not have access to any information that might help to physically identify individuals.

14. Data Quality inspection team

People from FLS will be selected to constitute a data quality inspection team in order to undertake periodic quality reviews of the entered data. The Data Quality inspection team will identify potential data entry errors, inconsistencies and missing data.

15. Direct Access to Source Data and Documents

Data will be stored in accordance with the Data Protection Law (LOPD, the organic Law 3/2018 of 5 December on the Protection of Personal Data and the Guarantee of Digital Rights complementary to the Regulation (EU) 2016/679 of the European Parliament and of the Council of 27 April 2016, on the protection of natural persons with regard to the processing of personal data and on the free movement of such data). The chief investigator will have overall control of, and act as the custodian for all data for the full duration of the study. The data will be available for internal monitoring (verification of data using paper CRF validate by research team against the information recorded in the CRF).

16. Monitoring and good clinical practice

We will carry out risk-adjusted monitoring since the trial is performed in a clinical care practice setting, with follow-up of the subjects treated in the community or primary care setting.

Data will be entered directly into the application, and it will be considered source data, as the contacts do not have a care episode opened.

Data monitoring tasks defined:

- Verification of the study master file (authorizations, protocol, drug information and other essential documents, pursuant to section 8 of ICH Guide E3),
- Verification of signature of informed consent,
- Checking the dates of visit and verification of absent data not entered in the application
- Verification of the values of serological results
- Detection of unreported adverse effects from the review of data from the medical records with open episodes during the course of the study

17. Ethics:
GENERAL CONSIDERATIONS
The clinical trial will be conducted according to the principles of the Declaration of Helsinki, (amended Fortaleza, Brazil, October 2013).
This study will be conducted according to Spanish regulations regarding clinical trials (Royal Decree 1090/2015) and biomedical investigations (Organic Law 14/2007 of biomedical investigation and the Royal Decree 1716/2011), which develop the European Directive on clinical trials (Regulation EU No 536/2014). The required documentation prior to the start will be:

- Protocol acceptance by the Sponsor and the Coordinating Investigator
- Protocol approval by the Ethics Committee
- Protocol authorization from the Spanish Drug Agency (Ministry of Health)

All subjects will be guaranteed continued medical and nursing supervision throughout the duration of the study.

This study will conform to the standards of GCP published by ICH (E6 R2).

DATA HANDLING

The processing of the data will be subject to current legislation as regards data protection (LOPD, The Organic Law 3/2018 of 5 December on the Protection of Personal Data and the Guarantee of Digital Rights complementary to the Regulation (EU) 2016/679 of the European Parliament and of the Council of 27 April 2016, on the protection of natural persons with regard to the processing of personal data and on the free movement of such data).

Cases are registered by the Epidemiological Surveillance Emergency Service of Catalonia (SUVEC). The SUVEC is under the Public Health Agency of Catalonia (ASPCAT), which is within the Departament of Salut (Public Health Secretariat).
The sponsor and the *Departament of Salut* have signed a collaboration agreement where, among other points, the access of the study investigators to COVID-19 patient data is specified for the conduct of this clinical trial, preserving the confidentiality of personal data, through of the *Sub-direcció General de Vigilància i Resposta a Emergències de Salut Pública. So, the study team will contact with participants after to review the SUVEC's* register oof COVID-19 data.

The participant will be identified in the records by the corresponding unique code number. The participant is to be guaranteed anonymity and is to be informed that all communication will take place between him/her and the investigator and not the sponsor of the study.

The SUVEC aim is to respond quickly to the diseases of urgent declaration and epidemic outbreaks declared by doctors in the healthcare network of Catalonia through research and control of urgent declaration diseases, outbreaks and public health alerts, as well as the application of prevention and control measures (chemoprophylaxis, vaccination, detection of risk contacts, isolation measures).

On the other hand, the ASPCAT is regulated by Law 5/2019, July 31, of the Public Health Agency of Catalonia and modification of the Law 18/2009, October 22, on public health. Its functions are expressly attributed to "Fostering research in public health and promoting the training of professionals engaged in it, in collaboration with other competent bodies, universities and research centers."

According to the data protection regulations, the ASPCAT, and therefore the SUVEC can process the data of patients (and their contacts) diagnosed with SARS-CoV-2 coronavirus to invite them to participate in the clinical trial. In this sense, Organic Law 3/2018, on the Protection of Personal Data, establishes through its Additional Provision seventeenth in section 2. B that "b) the health authorities and public institutions with powers to monitor the public health can carry out scientific studies without the consent of those affected in situations of exceptional relevance and seriousness to public health. "

Therefore, and understanding that given the current situation of coronavirus, and that ASPCATB, and therefore SUVEC, is a public institution with powers in public health surveillance, and that it specifically has the function of promoting health research public, would be enabled to process the data in order to offer people diagnosed with coronavirus to participate in a clinical trial, without prejudice to the need to sign the subsequent informed consent document to participate in the trial.

ORAL INFORMED CONSENT AND PARTICIPANT INFORMATION SHEET AND WRITTEN INFORMED CONSENT

The investigator will inform the candidates of the nature, duration and purpose of this study and, in addition, of all the inconveniences and obstacles that, if any, can be expected. In addition, information will be provided to the participant. Subjects must have the legal capacity to give their consent and exercise their freedom of decision. If the subject wishes to participate in the study, his/her oral consent will be obtained by phone.

The consent will be given orally during the enrolment telephone call and will be obtained before starting the participation in the study. The investigator will keep a call recording of the informed consent process.

At the Baseline home-visit, a Written Informed Consent will be obtained.

INSURANCE POLICY

In accordance with the Royal Decree 1090/2015, of 4th December, the trial sponsor has a policy of liability insurance. The sponsor shall extend this policy or another with equivalent coverage until the end of the trial. This policy also covers the responsibilities of the sponsor, the principal and his/her collaborators, as well as the hospital or site where they carry out the clinical trial.

18. Risk mitigation

After analysis of the safety of drugs and the current evidence of the efficacy, we do not consider choosing another drug. . As of March 26, 2020, the Catalan Infectious Diseases Departments involved in the treatment of COVID-19 have agreed to withdraw LPVr, DRVc from the treatment protocol. DRV has been shown to exert no activity on the SARS-CoV-2 clinical studies and is therefore withdrawn for futility. Although LPVr shows in vitro efficacy against SARS-CoV-2 at elevated total drug concentrations, human clinical trials have not demonstrated superiority of LPVr vs Placebo. It has been considered that the magnitude of the possible clinical benefit of LPVr if it is started in the early stages of the disease is small and does not compensate for the gastrointestinal and renal toxicity of the drug. The same applies to azithromycin in line with AEMPS that has questioned the use of azithromycin in patients with COVID-19 based on the limitations of a single trial. And after in vitro studies with azithromycin no activity against the virus is detected

We have considered that a reduction in the glomerular filtration rate can alter the safety profile of hydroxychloroquine and might be an appropriate reason to exclude patients from participation in trials. We have also decided to exclude patients with a positive history of arrythmia or QT prolongation or use of QTc prolonging medication.

We will advise patients to call the investigator team if they present any adverse event. The patients will be advised of frequent adverse events related to study drugs including those of hydroxychloroquine (blurred vision, nausea, vomiting, abdominal cramps, headache) and darunavir (gastrointestinal).

Ref.
1) The Janssen UK team. Lack of evidence to support use of darunavir-based treatments for SARS-CoV-2. 2020 March (https://www.janssen.com/uk/sars-cov-2-treatment)

2) Cao B, Wang Y, Wen D et al. A Trial of Lopinavir-Ritonavir in Adults Hospitalized with Severe Covid-19. N Engl J Med. 2020 Mar 18. doi: 10.1056/NEJMoa2001282.

3) AEMPS (https://www.aemps.gob.es/la-aemps/ultimainformacion-de-la-aemps-acerca - del-covid% e2% 80% 9119 / treatments-available-for-the-management-of-respiratory-infection-by-sars-cov-2 /)