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(The information included in this review reflects the evidence as of the date posted in the document. Updates will be developed according to new available evidence)

COVID-19: Chloroquine and hydroxychloroquine research

Disclaimer

This document includes the results of a rapid systematic review of current available literature. The information included in this review reflects the evidence as of the date posted in the document. Recommendations were based on the evidence available and its quality (GRADE methodology) at the time the review was published. Yet, recognizing that there are numerous ongoing clinical trials, PAHO will periodically update these reviews and corresponding recommendations as new evidence becomes available.

COVID-19: Chloroquine and hydroxychloroquine research

Summary statement

In recent weeks, information on the potential use of chloroquine or hydroxychloroquine for the treatment of people with COVID-19 has been disseminated in academic journals and public media. Although there are now ongoing clinical trials testing the efficacy and safety of several medicines for COVID-19, **as of the date of this document, there is a lack of quality evidence to demonstrate chloroquine and/or hydroxychloroquine are effective in the treatment of COVID-19.** Evidence is recently emerging via small studies with sub-optimal methodologies that are conflicting.

In some countries in the Americas, chloroquine or hydroxychloroquine is readily available, in some cases as an over-the-counter medicine. National authorities should take measures to control the use of these medicines and prevent self-medication. The use of chloroquine and/or hydroxychloroquine outside of current guidelines and recommendations may result in adverse effects, including serious illness and death, and have a negative impact on other diseases where there is proven benefit. Public health authorities are urged to prioritize resources on those interventions that are currently recommended for standard of care.

En semanas recientes se ha estado difundiendo información en medios académicos y públicos sobre el posible uso de cloroquina o hidroxicloroquina para el tratamiento de las personas con COVID-19. Si bien se están haciendo ensayos clínicos de la eficacia y la seguridad de varios medicamentos para esta enfermedad, **a la fecha del presente documento no había evidencia de buena calidad que demostrara que la cloroquina o la hidroxicloroquina fueran eficaces para el tratamiento de la COVID-19.** Últimamente ha surgido evidencia, producto de pequeños estudios con metodologías subóptimas, que son conflictivos.

En algunos países de la Región de las Américas, la cloroquina se consigue fácilmente, en algunos casos incluso sin receta. Las autoridades nacionales deben tomar medidas para controlar el uso de estos medicamentos y prevenir la automedicación. El uso de cloroquina o hidroxicloroquina sin seguir las directrices y las recomendaciones vigentes puede tener efectos adversos, entre ellos una enfermedad grave y la muerte, así como efectos negativos en otras enfermedades para las cuales estos medicamentos son beneficiosos. Se insta a las autoridades de salud pública a que den prioridad a la asignación de recursos para las intervenciones recomendadas actualmente como tratamiento de referencia.

Abstract

There is currently a lack of strong evidence with strong trial design that this medication (chloroquine or hydroxychloroquine) works for COVID-19 patients. The former is used to prevent and treat malaria while the later (trade name Plaquenil) was first used to prevent and treat malaria, it is also used to treat rheumatoid arthritis, some symptoms of lupus erythematosis, childhood arthritis (or juvenile idiopathic arthritis) and other autoimmune diseases. The body of evidence thus far has been

largely *in vitro*, and methodological quality from the body of evidence is sub-optimal, and the studies have been poorly reported and largely confounded. Moreover, the recently emerging *in vivo* study evidence is thin and based on 2 studies, one of which is inconclusive (China) and the others (France) alludes to benefit but have very small sample sizes, small event numbers, sub-optimal methodology, and lack the depth of detail needed for us to draw any definitive conclusion on effectiveness (see extended details below of the recently emerging *in vivo* evidence). Studies have been judged to be at high risk of biased estimates after critical appraisal using relevant tools. The French research particularly (of hydroxychloroquine and azithromycin), which has garnered media attention is not the type of robust, high-quality, comparative effectiveness randomized controlled trial evidence that is needed to draw definitive conclusions on benefits (and harms).

Multiple clinical trials are underway to strengthen the data and better characterize effectiveness and PAHO is monitoring the situation carefully on a day to day basis. As such, while there is currently a lack of evidence for efficacy of pharmacological treatments, PAHO will immediately let countries know if/as that changes. At the same time, some are using medications in compassionate use settings/clinical trials. Moreover, compassionate use is based on the assumption that a medicine produces more benefit than harm. There is a concern of massive purchasing and possible shortages of these medicines (both chloroquine and hydroxychloroquine), which can take away from other disease programs where it is used in effective indications e.g. rheumatoid arthritis, lupus, childhood arthritis, and other autoimmune diseases.

Care must be exercised in extrapolating *in vitro* results to *in vivo*, and potential side effects, toxicities and interactions with other drugs must remain a key consideration. Moreover, evidence seems to suggest that chloroquine/ hydroxycloroquine have a direct role in the electrophysiology properties of the heart. Until the COVID-19 clinical trial evidence that rules out harm in this group of patients is available, then caution is urged in considering any use.

Summary of the evidence

The pandemic of COVID-19 disease caused by the coronavirus strain SARS-Cov 2 has provoked an intense focus on potential therapeutic options. To enhance the therapeutic armamentarium for COVID-19, repurposing of older, already established medications against COVID-19 warrants further consideration. One treatment of interest is the inexpensive anti-malarial drug, chloroquine, which has an established safety profile and has ongoing *in vitro* studies in China. Chloroquine and the 4-aminoquinoline drug hydroxychloroquine belong to the same molecular family and the latter differs from the former by having a hydroxyl group at the end of the side chain: the N-ethyl substituent is β-hydroxylated. Both are reported to have similar pharmacokinetics.

Chloroquine is a form of quinine which is a compound that is found in the bark of Cinchona tree indigenous to Peru. Currently, the evidence on chloroquine and hydroxychloroquine that is being reported comes largely from *in vitro* studies that are ongoing in China. Moreover, the reporting from the ongoing research has not been complete and the research has not yet been subjected to peer-review. In order to further enhance the therapeutic aroma for COVID-19, this consideration and re-contemplation of established therapies for other conditions, warrants serious and further consideration.

The needed research is comparative effectiveness, robust, high-quality, ethically approved RCT research, and the results have to be fully disclosed with the methods and findings subjected to scientific peer-review. Randomized clinical trials are urgently needed in COVID-19 and possible therapies should be evaluated in such clinical trials. Recently released study evidence has not yet been peer-reviewed and validated and are also of very poor methodological quality.

Two important issues must also be considered. One is that these drugs have not been optimally tested/used on COVID-19 patients directly and as such, caution is urged. There is no high-quality evidence on benefits and harms as of yet. What exists are two small studies (one RCT) whereby both raise many methodological concerns and are at best, low quality evidence. A full quality of evidence assessment of these two studies (using GRADE methods; url: https://gdt.gradepro.org/app/handbook/handbook.html) will most certainly place them at very low quality (certainty) of evidence due to the methodological shortcomings. Secondly, if this drug is effective in COVID-19, this may drive unavailability for malarial patients and other chronic diseases as a treatment and as a prophylaxis. Precautions will need to be in place to ensure that new uses do not drive unavailability.

Background

On 31 December 2019, WHO was informed of a cluster of cases of pneumonia of unknown cause detected in Wuhan City, Hubei Province of China. The coronavirus disease (COVID-2019) was identified as the causative virus by Chinese authorities on 7 January. On 30 January 2020, following the recommendations of the Emergency Committee, the WHO Director-General declared that the outbreak constitutes a Public Health Emergency of International Concern (PHEIC).
As a result, and as part of the heightened response, world scientists are currently initiating research studies to assess which therapeutic intervention can be optimally used as a treatment (or prophylaxis) for COVID-19. The present global crude mortality rate is approximately 3.4% and this is based on confirmed cases that comprise the denominator. A large portion of the research is presently ongoing in China.

One treatment of interest is chloroquine (and hydroxychloroquine) (N4-(7-Chloro-4-quinolinyl)-N1, N1-diethyl-1,4 pentanediamine). The assumption is that both drugs yield the same anti-viral activity (capacity) and as such are discussed together in this report. Chloroquine phosphate is an old drug that has been used in the prevention and treatment of malaria and amebiasis.[1,2] Preliminary reporting is that it has apparent efficacy and acceptable safety against COVID-19 associated pneumonia in multicenter clinical trials conducted in China.[2-4] Given the preliminary research findings, chloroquine is being recommended to be included in the next version of the Guidelines for the Prevention, Diagnosis, and Treatment of Pneumonia Caused by COVID-19 issued by the National Health Commission of the People's Republic of China for treatment of COVID-19 infection in larger populations in the future.[2]

Prior research has revealed effectiveness of chloroquine, including against coronaviruses among which is the severe acute respiratory syndrome (SARS)-associated coronavirus (Table 1).[1,5-7] These prior anti-viral research results and the recent renewed research focus due to COVID-19 emergence and spread has heightened expectations for a beneficial effect. Moreover, the anticipation for the ongoing study results from China has dramatically increased due to reported efficacy in treatment of COVID-19 associated pneumonia in clinical studies.[2]

This document provides a rapid update of the present state of ongoing research on chloroquine and hydroxychloroquine for COVID-19, via in vitro or in vivo studies. We provide this update to assess if there is evidence to support the use of chloroquine / hydroxychloroquine in COVID-19.

Methods

We searched MEDLINE/PubMed and EMBASE electronic databases as of March 28th 2020, the search date commencing in year 1996 (see Appendix for search strategy). The search was not limited by study design as we wanted to examine all relevant published research, though our intent was to assess comparative effectiveness research principally (both RCT and observational evidence but with an intended focus on 'gold-standard' RCT evidence). We thus searched existing published studies as well as relevant study registries to gain a clearer picture of ongoing research as well as characterize existing findings. The search is updated daily in terms of including any publications released on a daily basis to March 27th 2020, noting that expected studies that would be published e.g. from China are emerging as pre-publications (not yet peer-reviewed) and are not located within the standard MEDLINE and EMBASE literature evidence repositories.

Results

The evidence presented is to March 30th 2020. The literature database search resulted in uncovering 557 published peer-reviewed studies directly and indirectly relevant to our update on chloroquine/hydroxychloroquine (MEDLINE=470, EMBASE=87). Seven have come from additional sources (e.g. published in pre-publications) and as such n=564 in total. In addition, we found over 30 registered studies in the WHO's International Clinical Trials Registry Platform (ICTRP) database (https://www.who.int/ictrp/en/) and the Chinese Clinical Trial Registry (ChiCTR) database (http://www.chictr.org.cn/searchprojen.aspxx) from among all studies initiated from January 1st 2020, examining chloroquine and hydroxychloroquine. The studies on chloroquine and hydroxychloroquine are all registered in a combination of China, Unites States and other nations.

There are more than 30 studies on this treatment chloroquine/hydroxychloroquine that are ongoing, as of March 11th 2020. See appendix Table 1 which lists an example of the results of prior published studies focusing on the activity of chloroquine or hydroxychloroquine in coronaviruses. We present the evidence that has the type of data that could inform the discussion on effectiveness.

Key evidence relevant to COVID-19

in vitro

i) A key preliminary finding thus far is the recent discovery in China of the *in vitro* activity of chloroquine against SARS-CoV-2, uncovered during culture tests on Vero E6 cells with 50% and 90% effective concentrations (EC_{50} and EC_{90} values) of 1.13 µM and 6.90 µM, respectively (antiviral activity being observed when addition of this drug was carried out before or after viral infection of the cells).[3] Researchers[3] reported that chloroquine blocks virus infection by increasing endosomal pH required for virus/ cell fusion, as well as interfering with the glycosylation of cellular receptors of SARS-CoV. The time-of-addition assay demonstrated that chloroquine functioned at both entry and at post-entry stages of the 2019-nCoV infection in Vero E6 cells.

in vivo

ii) This *in vitro* study was followed by the *in vivo* finding[2] that drove great interest and fervour when it was reported that chloroquine could reduce the length of hospital stay and improve the evolution of COVID-19 pneumonia.[2] These results have been reported to be based on roughly 100 patients who are participants in several ongoing studies in hospitals in China. The reporting has not been clear as to the comparators to the interventions. However, the claims are tantalizing such that chloroquine is showing reductions of exacerbation of pneumonia, duration of symptoms and delay of viral clearance, all in the absence of severe side effects. These findings as reported, represent the first successful use of chloroquine in humans for the treatment of an acute viral disease.

The *in vivo* findings in the roughly 100 patients[2] led to the recommendation of the administration of 500 mg of chloroquine twice a day for 10 days in patients with mild, moderate and severe forms of COVID-19 pneumonia. Specifically, the Guangdong Provincial Department of Science and Technology and the Guangdong Provincial Health and Health Commission's chloroquine treatment of new coronavirus pneumonia multi-center collaboration group developed the expert consensus after fully discussing the diagnosis of new coronavirus. This consensus is based on in vitro evidence and still unpublished data. The expert consensus applies only after chloroquine contraindications are ruled out. At such a dosage, a therapeutic concentration of chloroquine might be reached.[1,8]

in vitro

iii) In a more recent publication as of March 9th 2020,[9] researchers examined the immunomodulatory effect of hydroxychloroquine in controlling the cytokine storm that occurs late-phase in critically ill SARS-CoV-2 infected patients. The pharmacological activity of chloroquine and hydroxychloroquine was tested by utilizing SARS-CoV-2 infected Vero cells. In this study, physiologically-based pharmacokinetic models (PBPK) were implemented for both drugs separately by integrating their in vitro data, and using the PBPK models, hydroxychloroquine concentrations in lung fluid were simulated under 5 different dosing regimens to examine the most effective regimen whilst considering the drug's safety profile. Researchers found that hydroxychloroquine (EC50=0.72 μM) was more potent than chloroquine (EC50=5.47 μM) in vitro. They reported that based on a loading dose of 400 mg twice daily of hydroxychloroquine sulfate given orally, followed by a maintenance dose of 200 mg given twice daily for 4 days is recommended for SARS-CoV-2 infection, as it reached three times the potency of chloroquine phosphate when given 500 mg twice daily 5 days in advance.[9] This led researchers to propose that hydroxychloroquine is more potent than chloroquine in impeding and constraining SARS-CoV-2 in vitro.

Web-based discussion of an ongoing research study on COVID-19 in China

iv) As part of our research into the potential effectiveness of chloroquine or hydroxychloroquine in COVID-19, we also uncovered a link[10] that is essentially a description of a study conducted and ongoing in China in which chloroquine was given to patients. We decided to present this finding, and what we report here is not taken from any scientific, peer-reviewed manuscript/article. There is no comparison group and no mention of co-morbidities or more precisely what were the interventions.

The link reported that "as of March 4th, 2020, there have been a total of 120 novel coronavirus patients enrolled in the chloroquine phosphate treatment experiment group. Among them were 9 mild cases, comprising 7.50% of all cases; 107 moderate cases, comprising 89.1% of all cases; and 4 severe cases, comprising 3.33% of all cases. Currently, patients in 110 cases have had NAT [nucleic acid test] by throat swab results become negative [presumably from positive]. Of these negative cases, 9 were mild, comprising 100% of observed cases (9/9); 97 were moderate, comprising 90.65% of observed cases (97/107); and 4 were severe, comprising 100% of observed cases (4/4). Cases became negative on average 4.4 days after taking medication. Of the 120 cases of patients who accepted chloroquine phosphate treatment, not a single case developed into a critical case. Currently 81 patients have already been discharged from their hospitals. Provisionally, we have yet to observe a severe unfavorable reaction during the course of treatment."

These results as indicated in the web-based discussion are indeed exciting on initial examination, but what we require are the full details and optimally conducted RCT studies. Short of peer-reviewed RCT evidence, we remain limited in any firm conclusions. The results and underlying methodology must be fully disclosed to the scientific community before we can make any conclusions and hopefully, these would be soon forthcoming.

Updated systematic review evidence

v) Researchers published a systematic review as of March 11th 2020 and included six articles (one narrative letter, one in-vitro study, one editorial, expert consensus paper, two national guideline documents) and 23 ongoing clinical trials in China (now 21 studies due to withdrawal of 2 trials).[11] We have reported on these studies earlier. In general, the review[11] concludes that pre-clinical evidence and expert opinions suggest potential use of chloroquine against SARS-CoV-2.

As part of the review,[11] authors refer to The Dutch Center of Disease control (CDC) and its public document on its website, suggesting treatment of severe infections requiring admission to the hospital and oxygen therapy or admitted to the ICU with chloroquine.[12] Authors[11] also were careful to mention that the Dutch document[12] also stated that treating patients only with optimal supportive care is still the core option, due to lack of underlying evidence. The suggested regimen in adults consists of 600 mg of chloroquine base (6 tablets A-CQ 100 mg) followed by 300 mg after 12 h on day 1, then 300 mg × 2/die per os on days 2–5 days. Also highlighted in the Dutch guidance was 1) the need for stopping the treatment on the 5th day to reduce the risk of side effects, considering the long half-life of the drug (30 h); 2) the need to differentiate between regimens based on chloroquine phosphate and chloroquine base since 500 mg of the first correspond to 300 mg of the second.[12]

Authors[11] also point to the Italian Society of Infectious and Tropical disease (Lombardy section) guideline on COVID-19,[13] which recommends the use of chloroquine 500 mg × 2/die or hydroxychloroquine 200 mg die for 10 days, although the treatment may vary from 5 to 20 days according to clinical severity. The guideline suggests that the target population ranged from patients with mild respiratory symptoms and comorbidities to patients with severe respiratory failure.

Recently emerging in vivo evidence in press (2020)

i) A study in press in France[14] looked at confirmed COVID-19 patients and included patients in a single arm protocol from early March to March 16th, to receive 600 mg of hydroxychloroquine daily and their viral load in nasopharyngeal swabs was tested daily in a hospital setting. Researchers enrolled 36 out of 42 patients meeting the inclusion criteria and had at least 6 days of follow-up at the time of analysis. Researchers reported that depending on their clinical presentation, azithromycin was added to the treatment. Untreated patients from another center and cases refusing the protocol were included as negative controls. Presence and absence of virus at Day 6-post inclusion was considered the end point. Researchers reported that 6 patients were asymptomatic, 22 had upper respiratory tract infection symptoms and eight had lower respiratory tract infection symptoms. Twenty cases were treated in this study and showed a significant reduction of the viral carriage at D6-post inclusion compared to controls, and much lower average carrying duration than reported of untreated patients in the literature. Azithromycin (Z-Pak) added to hydroxychloroquine was significantly more efficient for virus elimination. Researchers concluded that the preliminary results show that hydroxychloroquine treatment is significantly associated with viral load reduction/disappearance in COVID-19 patients and its effect is reinforced by azithromycin.

This French study[14] has received significant global focus and a critical appraisal quality assessment of this study was performed using the Guyatt et al. critical appraisal tool for non-randomized studies and using response options for a risk of biased estimates to be 'yes', 'probably yes', 'probably no', and 'no' (url: https://www.evidencepartners.com/wp-content/uploads/2017/09/Tool-to-Assess-Risk-of-Bias-in-Cohort-Studies.pdf, Accessed on March 25th 2020) (Table 1).

The study[14] raises particular methodological concerns (and lowers confidence in the estimates of effect) as this was an observational study (at risk of selection bias and residual confounding and there was no matching or appropriate statistical adjustment for most plausible prognostic variables) and not a randomized-controlled design, and endpoints were not the optimal type of patient-important outcomes and not clearly defined. Furthermore, an intent-to-treat analysis was not performed and there was attrition (patients appeared to have dropped out) in the treatment group as they got sick and were excluded (6 of 26 who got hydroxychloroquine "dropped out" early, 4 or 5 because of death/ICU admission, AEs). The reporting does not include clear data and accounting on the 6 of 26 patients that clearly could have impacted on virologic outcomes. The decisions as to who received azithromycin was not clear in the reporting. As such, based on a critical appraisal, we judged this study to be at high risk of biased estimates (Table 1). Importantly as to potential harms that should be considered, given there has been recent discussion of hydroxychloroquine possibly prolonging the QT interval and also leading to drug-induced torsade de pointes, a potentially lethal ventricular tachycardia. Similar findings have accumulated for azithromycin. This raises important questions that warrants urgent and acute study to exclude harms, for this is a dual medication approach.

ii) A recently published clinical trial out of China[15] (pre-publication) raises as serious or even more methodological concerns (Table 2 critical appraisal using the Guyatt et al. critical appraisal tool for randomized studies and using response options for a risk of biased estimates to be 'yes', 'probably yes', 'probably no', and 'no', url: https://www.evidencepartners.com/wp-content/uploads/2017/09/Tool-to-Assess-Risk-of-Bias-in-Randomized-Controlled-Trials.pdf, Accessed on March 25th 2020) as the French study[14] and the reporting was sparse (pre-publication), making assessment very difficult. The study was reported as a RCT that prospectively enrolled 30 treatment-naive patients with confirmed COVID-19 1:1 to hydroxychloroquine (HCQ) group and the control group. The primary endpoint was negative conversion rate of COVID-19 nucleic acid in respiratory pharyngeal swab on days 7 after randomization. Patients in HCQ group were given HCQ 400 mg per day for 5 days plus conventional treatments, while those in the control group were given conventional treatment only, with researchers also indicating bed rest, oxygen inhalation, and symptomatic supportive treatment. They also reported that viral drugs such as alpha interferon nebulization, oral lopinavir / ritonavir etc., and antibacterial drugs were given. Specifically, all patients received alpha interferon nebulization, while 12 (80.0%) of the experimental group received arbidol, 10 of the control group (66.7%) received arbidol, and 2 (13.3%) received lopinavir / ritonavir treatment.

One patient in HCQ group declined as reported. On day 7, COVID-19 nucleic acid of throat swabs was negative in 13 (86.7%) HCQ cases and 14 (93.3%) cases in the control group (P>0.05). The median duration from hospitalization to virus nucleic acid negative conservation was 4 (1-9) days in HCQ group, which is comparable to that in the control group [2 (1-4) days, (U=83.5, P>0.05)]. The median time for body temperature normalization in HCQ group was 1 (0-2) after hospitalization, which was also comparable to that in the control group 1(0-3). Radiological progression was shown on CT images in 5 cases (33.3%) in the HCQ group and 7 cases (46.7%) in the control group, and all patients showed improvement in follow-up examination. Four cases (26.7%) of the HCQ group and 3 cases (20%) of the control group had transient diarrhea and abnormal liver function. Researchers concluded that the standard dose of hydroxychloroquine sulfate (400 mg, 1 time / day) does not show clinical effects in improving patient symptoms and accelerating virological suppression.

iii) A recent publication out of France[16] that builds on the initial French hydroxychloroquine and azithromycin study[14] describes a larger observational case series of 80 COVID-19 patients admitted to hospital and treated with hydroxychloroquine and azithromycin (the 80 patients included 6 patients from the prior reporting[14]). The included patients were PCR-documented SARS-CoV-2 RNA from a nasopharyngeal sample (6 of the 80 were reported on in the prior study[14]). Among the patients, 5% were asymptomatic, 41.2% had upper respiratory tract infection symptoms, and 53.8% had lower respiratory tract infection symptoms. A scoring system of risk of deterioration was used (NEWS score) on admission whereby a risk score of 0 – 4 (low risk) was documented in 92%, a score of 5 – 6 (medium risk) in 5.3%, and a score ≥ 7 (high risk) in 2.7%. The median age was 52.5 (IQR 42-62, min 20, max 88), 42 were males (52.5%), and there were co-existing condition in patients as follows: cancer (6.3%), diabetes (11.2%), CAD (7.5%), hypertension (16.3%), chronic respiratory diseases (10%), obesity (5%), and immune-suppressed (5%). Patients with no contraindications were offered a combination of 200 mg of oral hydroxychloroquine sulfate, three times per day for ten days combined with azithromycin (500mg on D1 followed by 250mg per day for the next four days), and in patients with pneumonia and NEWS* score≥5, a broad-spectrum antibiotic (ceftriaxone) was added to hydroxychloroquine and azithromycin.

Researchers reported that there was 1 death (86-year old patient) from among the 80 patients. Nasopharyngeal viral load tested by qPCR and negative on day 8 was found in 93.7% of patients, not contagious (with a PCR Ct value<34) at day 10 was found in 98.7%, negative virus cultures on day 5 was found in 98.7%, and length of stay in ICU (days) was a mean 4.6 days ± 2.1 SD (n=65). Researchers reported that patients were rapidly discharged from highly contagious wards with a mean length of stay of five days. This study was judged to be at high risk of biased estimates due to it being a case-series observational study with no control group. Based on reporting, the cohort appears to be younger and the NEWS risk scoring system placed them all at very low risk of deteriorating, leaving one to speculate on if they would have recovered on their own. This group appears to be COVID-19 patients with mild illness. Researchers were unclear as to what happened with the 3 who were transferred to MICU. The adverse events were mild, and the period of hospitalization was brief again underscoring that this was not necessarily a severely ill group of COVID-19 patients to begin with. Patients may have recovered on their own.

iv) A recently published RCT[17]conducted in China sought to establish the efficacy of hydroxychloroquine (HCQ) in the treatment of 62 patients with COVID-19 (n=31 hydroxychloroquine, and n=31 control). Included patients were >18 years, were laboratory (RT-PCR) positive for SARS-CoV-2, had a chest CT with pneumonia, and had SaO2/SPO2 ratio > 93% or PaO2/FIO2 ratio > 300 mmHg under the condition in the hospital room (mild illness). Severe or critically ill patients were excluded. The primary end-points were time to clinical recovery (TTCR) which was defined as the return of body temperature and cough relief, maintained for more than 72 h. The absorption of pneumonia was also measured as well as adverse event data was also sought. The mean age was 44.7 (SD 15.3) and 46% were male. Researchers reported that the body temperature recovery time and the cough remission time were significantly shortened in the HCQ treatment group (mean days and SD was 2.2 (0.4) in the HCQ groups vs 3.2 (1.3) in the control, p=0.0008. They also reported a greater proportion of patients with improved pneumonia (on chest CT) in the HCQ treatment group (80.6%, 25 of 31) relative to the control group (54.8%, 17 of 31). Four patients in the control group developed severe illness (none in the treatment group) and there were 2 mild adverse events in the HCQ group. The study group was generally younger, and the illness was mild on entry, suggestive that this was not an overly ill group to begin with and patients may have recovered on their own. Researchers did not provide an accounting of whether they were taking any other medications prior to study entry or during the study.

v) A very recently published small consecutive case series in France (n=11)[18] seems to contradict the emerging *in vivo* evidence of benefit and particularly the recently published French evidence that has driven considerable global interest in the combination hydroxychloroquine and azithromycin.[14,16] Researchers questioned the rapid and full viral clearance in the French research[14,16] as it was "quite unexpected".[18] They looked at 11 consecutive patients hospitalized in their hospital department who were administered hydroxychloroquine (600 mg/d for 10 days) and azithromycin (500 mg Day 1 and 250 mg days 2 to 5) using the same dosing regimen reported in the French research.[14,16] The patients were 7 men and 4 women who had mean age of 58.7 years (a range of 20-77), and 8 patients had substantial underlying comorbidities linked with poor outcomes e.g. obesity, cancer, and HIV-infection. As the treatment was started, 10 of the 11 had fever and their received nasal oxygen therapy. Researchers reported that within a 5 days period, 1 patient died and 2 were moved to the ICU. They also found that for one patient, hydroxychloroquine and azithromycin had to be discontinued after 4 days due to a prolongation of the QT interval from 405 ms before treatment to 460 and 470 ms under the treatment combination. The report that in the 10 living patients, repeated nasopharyngeal swabs were positive for COVID-19 RNA in 8 of the 10 patients (80%) at days 5 to 6 following treatment initiation. The virologic results contradict the results reported by Gautret et al.[14,16] and calls into question the strong antiviral efficacy of the reported combination. Researchers also questioned the one death and 3 ICU transfers[14] that suggest a worsening clinical outcome. They conclude that there is "no evidence of a strong antiviral activity or clinical benefit of the combination of hydroxychloroquine and azithromycin for the treatment of our hospitalized patients with severe COVID-19".[18] This was a small consecutive series of patients followed to describe the response to the treatment, high risk of biased estimates.

vi) Researchers[19] reported on a RCT (n=22 patients) that examined the efficacy of chloroquine versus lopinavir/ritonavir (control group) in hospitalized COVID-19 patients. Ten patients were randomized to chloroquine (500 mg orally twice-daily for 10 days moderate/severe cases) and 12 were randomized to lopinavir/ritonavir 400/100mg orally twice-daily for 10 days. Using RT-PCR, on day 13, all patients in the chloroquine group were negative, and 11 of 12 in the control group (lopinavir/ritonavir) were negative on day 14. Via lung CT on day 9, 6 patients in chloroquine group achieved lung clearance versus 3 in the comparison group. At day 14, the rate ratio based on CT imaging from the Chloroquine group was 2.21, 95% CI 0.81-6.62) relative to the control group. Five patients in the chloroquine group had adverse events versus no patients in the control group. This small RCT appeared to show better effectiveness of chloroquine over lopinavir/ritonavir in moderate to severely ill COVID-19 patients. This was a small sample study, small event number, very poor methodology and judged to be at high risk of bias.

Table 1: Risk of bias assessment observational study designs[14,16,18]

Study author, year, design, location	Was selection of exposed and non-exposed cohorts drawn from the same population?	Can we be confident in the assessment of exposure?	Can we be confident that the outcome of interest was not present at start of study?	Did the study match exposed and unexposed for all variables that are associated with the outcome of interest or did the statistical analysis adjust for these prognostic variables?	Can we be confident in the assessment of the presence or absence of prognostic factors?	Can we be confident in the assessment of outcome?	Was the follow up of cohorts adequate?	Were co-interventions similar between groups?	Overall risk of bias
Gautret, 2020[14] Observational study design, France	Probably no (patients unsure presenting at same points of care over the same time frame; used patients from other center as controls e.g. who refused treatment were the controls and from another center that is problematic)	Probably yes	Probably yes	No (there was no matching or stratification or appropriate statistical adjustment for most plausible prognostic variables)	No (there is no Prognostic information from data base with no available documentation of quality of abstraction of prognostic variables)	Probably yes (however, the reporting is very sparse and this assessment is being generous as no clear evidence is given on how this was done e.g. was it blinded assessment)	Probably no (unclear as to reasons for drop-outs in terms of if related to outcome; not balanced and many concerns of why the '6' dropped out)	Probably yes (however, we remain uncertain given the limited reporting)	High risk of bias
Gautret, 2020[16] Observational study design, France	NA; one group case-series; however, most patients came from one institution	Probably yes	Probably yes	NA as only one COVID-19 group described and exposed	No (not a randomized study and thus multiple factors could account for the results; there is no control group; seems young in age MEDIAN 52, not at elevated risk of death (70% 20-60 yrs), the outcome is confounded by co-morbid conditions, seemingly low risk to begin based on low NEWS; differential in upper and lower respiratory tract infection)	Probably yes (however, this is a case series at best and not comparative and ideally the adjudicator of outcomes should be blinded; no indications here)	No, not comparative; no dropouts reported; no firm description of the 3 who went to MICU	NA; not comparative	High risk of bias

Molina, 2020[18] Observational consecutive series, France	Probably yes (11 consecutive patients hospitalized in our department)	Probably yes	Probably yes	No, one consecutive group	No	Yes	Probably yes	NA	High risk of bias

https://www.evidencepartners.com/wp-content/uploads/2017/09/Tool-to-Assess-Risk-of-Bias-in-Cohort-Studies.pdf

Table 2: Risk of bias assessment RCT design[15,17,19]

Study author, year, design, location	Was the allocation sequence adequately generated?	Was the allocation adequately concealed?	Blinding: Was knowledge of the allocated interventions adequately prevented (patients, health care personnel, data collectors)?	Blinding: Was knowledge of the allocated interventions adequately prevented (adjudicators of outcome, analysts)?	Was loss to follow-up (missing outcome data) infrequent?	Are reports of the study free of selective outcome reporting?	Was the study apparently free of other problems that could put it at a risk of bias?	Overall risk of bias
Chen 2020[15] RCT study design, China	Probably no (not clearly reported as to how the sequence was generated e.g. reported as "the subjects were randomly assigned to the experimental group and the control group"	Probably no (no indication was provided of how the randomization sequencing was concealed)	No (no indication provided in any manner)	No (no indication provided in any manner)	Probably yes	Probably yes	Probably no	High risk of bias; also a concern in the findings was that viral drugs such as alpha interferon nebulization, oral lopinavir / ritonavir (clepivir), and arbidol etc., and antibacterial drugs were administered beside the interventions, distorting the treatment effect
Chen Z, 2020, RCT, China[17]	Probably yes, though the description was inadequate	No, no explanation how allocation was concealed	Probably no, unclear	Probably no, unclear	Yes	Probably no	Serious methodological concerns	High risk of bias
Huang, 2020, RCT, China	Probably no (no reporting)	Probably no (no reporting)	No	Probably no	Yes	Probably no	Serious methodological concerns	High risk of bias

https://www.evidencepartners.com/wp-content/uploads/2017/09/Tool-to-Assess-Risk-of-Bias-in-Randomized-Controlled-Trials.pdf

Discussion

What is known thus far? Presently, mainly *in vitro* data suggests that chloroquine (and hydroxychloroquine) mitigates and inhibits the replication of SARS-CoV-2.[3] Results have been coming from China but with little access to the actual and complete data. Medical research and practice changing decisions must be made only when there has been access to the data as well as a peer-reviewed examination to assess the potential benefits (and harms) of chloroquine or hydroxychloroquine (any intervention). The peer-review is critical and this has not yet occurred in some of these studies and significant decisions are being made based on few patients. The research and medical community eagerly await all ongoing studies on these drugs.

There are major challenges to the medical community since no approved treatment (or prophylaxis) for COVID-19 disease exists. This underscores the urgency. There is evidence that chloroquine has been used successfully to treat malaria and the adverse effects are known in this patient group.[20] Chloroquine is known to inhibit virus infection by increasing the endosomal pH required for virus/cell fusion, and also upsets the glycosylation of cellular receptors of SARS-CoV.[21] Researchers did report that chloroquine is potent in preventing the spread of SARS CoV in cell culture. They also report favorable inhibition of virus spread when the cells were either treated with chloroquine prior to or after SARS CoV infection.[21]

In summary, there is not the definitive type of evidence that is needed in COVID-19 patients and thus one must be cautious making recommendations based on this limitation. Studies are ongoing. The *in vitro* results from researchers in China[3,22] (and recently emerging from France[14,16,18] and from China,[15,17,19] all *in vivo*) are methodologically weak and of low quality based on critical appraisal. The type of robust comparative research needed and the methodologies as reported, are poor thus far based on what has been released.[23] A major concern is the small sample sizes and the lack of optimal methods in randomization, allocation concealment, and blinding/masking. Furthermore, the types of strategies to be used in the weaker observational study designs such as a comparative group and matching (propensity score matching), restriction, stratification, and statistical adjustment were not used in these studies where needed, so as to allow for more confidence in the estimates of effect.[14-19] The *in vivo* body of evidence in COVOD-19 patients thus far is weak and the robust, high-quality research is lacking that could underpin confidence and definitive discussions on effectiveness.

Research priorities

Research examination of chloroquine type drugs in COVID-19 patients should assess issues around i) whether the treatment is chloroquine only or in combination with other interventions (or hydroxychloroquine) and what is the drug-drug interaction (issues around co-interventions) ii) specific age-groups whereby the drug may have differential effects iii) differential impact of the drug based on stage of illness iv) differential impact of the drug based on severity of illness and v) how chloroquine works in the company of co-morbid conditions.

Toxicity

Toxicity of chloroquine/hydroxychloroquine is critical as we consider its effectiveness and is given extended consideration here. The safety profile is known with over 50 years of use in malaria and for rheumatic illnesses and even when used continuously for several years.[24] Reported adverse events thus far have been[24-28] macular retinopathy that was based on the cumulative dose rather than on the daily dose, and researchers reported that lasting damage can be mitigated and even stopped with routine visual monitoring during the treatment.[25-28] One study used a high dose of up to 500 mg base per day and in pregnancy and the results were encouraging as to safety.[28]

However, while there are indications of low level adverse effects, there has been evidence[29-31] of *torsades de pointes* (TdP) and a growing body of evidence that has been derived from post-marketing surveillance, that azithromycin may be linked to arrhythmia-related adverse cardiac events including pronounced QT interval prolongation and associated TdP that provide the substrate for potentially life-threatening arrhythmias such as ventricular fibrillation (VF).[29-32] This is an important consideration along with recent discussion of the potential of hydroxychloroquine to prolong the QT interval and also lead to drug-induced torsade de pointes, a potentially lethal ventricular tachycardia.[33] A recent description of the clinical characteristics of 138 hospitalized patients with COVID-19 in China,[34] reported that the common complications among the 138 were shock (8.7%), ARDS (19.5%), arrhythmias (17.2%), and acute cardiac injury (7.2%).

These results[34] raised serious questions for the COVID-19 patient since evidence[35] suggests that patients with arrhythmias (atrial-fibrillation) have poorer outcomes, with respect to all-cause mortality. Researchers report that atrial-fibrillation can cause a shortening of action potential duration along with attenuation of APD rate-adaptation[36] What is known? i) published and anecdotal reports seeming to indicate cases of acute onset heart failure, myocardial infarction, myocarditis, and cardiac arrest ii) as with any acute illness, higher cardio-metabolic demand can precipitate cardiac complications iii) current reporting does not yet describe prevalence of cardiac complications in CVD naïve versus cardiac co-morbid patients and iv) cardiac complications of COVID-19 are approximately commensurate with SARS, MERS, and influenza analogues.

The initial reporting[34] indicates that patients with COVID-19 frequently develop arrhythmia and myocarditis. Moreover, evidence seems to suggest that chloroquine has a direct role in the electrophysiology properties of the heart.[37] For example, it was reported that in isolated hearts of three mammalian species, intracoronary chloroquine perfusion played a role in reductions in fibrillatory frequency (both atrial or ventricular). Researchers looked at the role of chloroquine in terminating stretch-induced atrial fibrillation (SAF) relative to flecainide in the sheep heart (n=30 sheep hearts).[38] They reported that chloroquine is more optimal in terminating SAF via "significantly increasing core size and decreasing re-entry frequency". There is also evidence[37] that chloroquine reduces ventricular ectopy. Researchers examined 6 patients and found antiarrhythmic action via administration of 500 mg chloroquine for 9 weeks, whereby in 4 patients, "there was a reduction in ventricular ectopy, which recurred when the drug was discontinued, while a fifth patient reverted to sinus rhythm from atrial fibrillation previously resistant to other antiarrhythmic medication". Research published as early as 1988 was showing a role of chloroquine and hydroxychloroquine in the treatment of cardiac arrhythmias.[39]

Researchers reported on a case of hydroxychloroquine-induced cardiomyopathy that presented as pulmonary hypertension in a 63-year old female.[40] The patient was diagnosed with rheumatoid arthritis and was treated with hydroxychloroquine at a cumulative dose of 164 g. On follow-up she was diagnosed with pulmonary hypertension due to left heart disease and complete atrioventricular block that resulted from hydroxychloroquine toxicity. Researchers reported that an insertion of a permanent pacemaker and the discontinuance of hydroxychloroquine significantly improved the disease condition. They concluded that hydroxychloroquine may play a role in cardiac complications despite a small cumulative dose relative to doses reported in other cases.[40]

Researchers reported on hydroxychloroquine-induced cardiotoxicity in a 39-year-old woman with systemic lupus erythematosus and systolic dysfunction.[41] Ventricular endomyocardial biopsy was performed and light microscopy showed diffuse myocyte vacuolization without myocarditis, and transmission electron microscopy demonstrated sarcoplasmic myelinoid and curvilinear bodies, leading to a diagnosis of hydroxychloroquine toxicity.

Similarly, researchers reported on 2 cases of hydroxychloroquine-induced cardiomyopathy.[42] They discuss how hydroxychloroquine- or chloroquine -induced cardiomyopathy is an infrequent but potentially catastrophic condition that can be fatal. These drugs are typically used for long-term treatment of rheumatic diseases and for malaria prophylaxis and hydroxychloroquine- and chloroquine-induced cardiomyopathy have well-described microscopic features, with the classic electron microscopic findings of myelin figures (myeloid bodies). They focused on 2 cases, one in a patient with systemic lupus erythematosus, who was found to have megamitochondria as well as myelin figures under electron microscopy. The other was a case of hydroxychloroquine cardiomyopathy in a patient with scleroderma, these 2 cases adding to the existing knowledge base of hydroxychloroquine-induced cardiomyopathy.[42]

In a similar light, researchers reported on hydroxychloroquine-induced cardiomyopathy.[43] Their focus is on the drug-induced cardiac damage that could result and advise that ongoing clinical monitoring is critical and early recognition of toxicity and harm is a central management strategy in patients who are undergoing long-term treatment with hydroxychloroquine. They emphasize that along with retinal toxicity and neuromyopathy,[43] cardiac disease is a potent adverse result of hydroxychloroquine use. They urge for instant withdrawal of hydroxychloroquine should any toxicity emerge or is suspected due to the possibility of reversing the cardiomyopathy if recognized early enough. As such, experts provide guidance for "regular screening with 12-lead electrocardiogram and transthoracic echocardiography to detect conduction system disease and/or biventricular morphological or functional changes" in hydroxychloroquine-treated patients.[43] They also suggest that cardiac magnetic resonance imaging and endomyocardial biopsy could be critically important in yielding analytical and prognostic insights to "confirm the diagnosis of hydroxychloroquine-induced cardiomyopathy". Researchers caution the medical community regarding hydroxychloroquine use and particularly long-term, as it can result in "an acquired lysosomal storage disorder, leading to a drug-induced cardiomyopathy characterized by concentric hypertrophy and conduction abnormalities associated with increased adverse clinical outcomes".[43] Researchers also warned that this could result in death.

Moreover, researchers[44] reported on a case of a male in his 60s who presented to their clinic for worsening exercise capacity, dyspnoea on exertion for 18 months and chest pain not associated with exercise. The patient had medical history of rheumatoid arthritis (RA), Sjogren's syndrome, Raynaud's phenomenon, gastro-oesophageal reflux, dyslipidaemia and Parkinson's disease and was on hydroxychloroquine for RA. The researchers discussed that while hydroxychloroquine is an antimalarial, it is also used as a RA treatment and for systemic lupus erythematosus. The patient underwent various tests and "endomyocardial biopsy showed cardiac myocytes with fibre enlargement, fibre size variation, endocardial fibrosis, perivascular fibrosis and vacuoles containing brown pigment, suggestive of lipofuscin raising possibility of hydroxychloroquine toxicity under light microscopy. Electron microscopy showed clear vacuoles in myocytes with patchy accumulation of glycogen in myocytes and lipofuscin-like material in some vacuolated areas as described in hydroxychloroquine toxicity".[44] They stopped the hydroxychloroquine and initiated a standard heart failure (HF) treatment. They went on to outline that the clinical manifestations of antimalarial-induced cardiotoxicity can manifest as "restrictive cardiomyopathy, dilated cardiomyopathy, or conduction abnormalities such as bundle branch and atrioventricular block. Patients usually present with HF symptoms. However, non-specific chest discomfort may be a presenting or coexisting feature".[44]

Additionally, researchers reported on suspected hydroxychloroquine-associated QT-interval prolongation in a patient with systemic lupus erythematosus.[45] They report on a 41-year old woman who presented to the cardiology clinic as follow-up for recent congestive heart failure (CHF) with systolic left ventricular dysfunction. Her compliance on medications was poor based on her reporting and she had been on hydroxychloroquine for 3 prior years, stopped, and re-started after consulting with the rheumatologists. The CHF was suitably managed and she was discharged. On subsequent follow-up testing, there was significant prolongation of QT and corrected QT (QTc) intervals of 586 and 614 milliseconds, respectively[45] and the patient was admitted to the coronary care unit and the decisions was to stop the hydroxychloroquine. The researchers reported that "review of outpatient medications did not suggest other potential causes of QT-interval prolongation. Oral furosemide was continued, but the (beta)-blocker carvedilol was held to avoid bradycardia. During the course of admission, the dose of nifedipine was increased to achieve adequate blood pressure control".[45] They reported that "serial ECGs revealed gradual shortening of the QTc interval"[45] and nuclear stress testing revealed normal myocardial distribution of activity and no indications of ischemia. After 72-hours she was sent home and at her follow-up visit, there was continued improvement in the QTc interval. Researchers reported that at a one-year follow-up visit, with the continued cessation of hydroxychloroquine, the "QTc was relatively normal (473 milliseconds)".

This led researchers to discuss reasons why the QTc interval did not go back to normal values until one year following complete cessation of hydroxychloroquine treatment. They offered that this may be due to the "long half-life of hydroxychloroquine potentiated by the patient's renal impairment, permitting potential toxic effects even after discontinuation of therapy".[45]

Similar findings were reported by Chen et al. (2006)[46] surrounding chronic hydroxychloroquine use that is associated with QT prolongation and refractory ventricular arrhythmia. On the other hand, a report by Teixeira et al. (2014)[47] suggested cardiac safety and antiarrhythmic potential of chloroquine in systemic lupus erythematosus. They conducted a comprehensive evaluation of heart rhythm disorders and the influence of disease/therapy factors in a large systemic lupus erythematosus (SLE) cohort (n=317 patients) and reported that chloroquine appears to have a protective role in the unexpected high rate of cardiac arrhythmias and conduction disturbances observed in systemic lupus erythematosus. In a similar beneficial light, researchers[48] reported on their retrospective cohort study, whereby one million participants were included being sampled from 23 million beneficiaries (2000-2013). They report a significantly decreased hazard ratio for CAD in lupus patients with an elevated usage of hydroxychloroquine for at least 318 days (HR 0.31, 95% CI: 0.12-0.76).

Results were however unclear in a systematic review[49] that sought to examine the arrhythmogenic cardiotoxicity of the quinoline and structurally related antimalarial drugs. The review was underpinned by the background on antimalarial drugs being linked to cardiovascular side effects, and especially hypotension and electrocardiographic QT interval prolongation (QT interval prolongation reported as a risk marker for the development of Torsade de Pointes which is a potentially lethal polymorphic ventricular tachyarrhythmia).[49] The review thus sought to describe "clinical and electrocardiographic cardiovascular side effects of quinine, mefloquine, lumefantrine, piperaquine, halofantrine, chloroquine, sulfadoxine-pyrimethamine, amodiaquine, and primaquine". They judged 177 of the studies to be eligible but reported that there was too much missing information and what was reported was too heterogeneous to allow for any meaningful meta-analytical pooling of the evidence regarding QT interval changes.

Researchers also examined chloroquine for an extended period of 7 months in patients with systemic lupus erythematosus to assess the influence on arrhythmia, heart rate variability and repolarization parameters.[50] They found after 7 months of use in 28 systemic lupus erythematosus patients treated with chloroquine as a monotherapy at 250 mg daily, that it did not cause conduction disturbances or an increment in cardiac arrhythmias. Tobón et al. (2019)[51] studied whether blocking I_{K1} and I_{KACh} with chloroquine would decrease the burden of persistent atrial fibrillation. They used molecular modeling in order to simulate "the interaction between chloroquine and Kir2.1 and Kir3.1, the molecular correlates of I_{K1} and I_{KACh}". Following this, they administered oral chloroquine to one patient with persistent atrial fibrillation, finding that (based on mathematical simulations of persistent atrial fibrillation in a 3D model of human atria) chloroquine was having the effect of prolonging the action potential duration, and "leading to failure of re-entrant excitation, and the subsequent termination of the arrhythmia".[51]

Atrial fibrillation and various cardiomyopathy present ongoing cardiac challenges to the patient and it appears that COVID-19 status, chloroquine, hydroxychloroquine, as well as azithromycin, has some impact on the cardiovascular and electrophysiological system of the heart.[29-34, 37-52] The concern here is drug-induced heart pathology that we are unsure of. Is it a deleterious effect or protective effect? The issue importantly, is what is the role in the COVID-19 patient's heart of either of these drugs (chloroquine or hydroxychloroquine) or when used with azithromycin or other drugs such as antiarrhythmics? What is the role in atrial fibrillation or any cardiac malfunction? The robust and methodologically rigorous comparative effectiveness *in vivo* research in COVID-19 patients has not yet been done. Comprehensive reporting is also critical for a clearer understanding of the role in cardiac electrophysiology and particularly in COVID-patients and what this means in terms of increased or decreased morbidity and/or mortality.

Furthermore, and perhaps the closest comparative effectiveness data we have on how hydroxychloroquine may operate in COVID-19 comes from a well-conducted robust RCT in Singapore[53] that included 1,516 patients (randomised, double-blind, placebo-controlled trial) who were allocated to chloroquine phosphate (500 mg/day for 1 week, then once a week to complete 12 weeks) or matching placebo. Researchers found it was well tolerated but reported that chloroquine did not prevent infection with influenza. Similar findings emerged on viral replication whereby there was a lack of virologic or clinical benefit when chloroquine was used (either as a therapeutic or prevention).[54-56]

A more recent pre-publication (not yet peer-reviewed) of *in vitro* activity reported on the lethality of hydroxychloroquine or chloroquine in combination with metformin in mice.[57] Researches reported that when hydroxychloroquine or chloroquine was combined with metformin as a possible anti-cancer drug, 30-40% of all mice died. In addition, researchers[58] recently reported that they looked at

84 COVID-19 infected patients who were administered a hydroxychloroquine/azithromycin combination. They found that the QTc was prolonged maximally from baseline (days 3-4) and in 25 patients, the QTc increased more than 40ms. They also found that in 9 patients (11%), the QTc increased to >500 ms, indicative of a high-risk group for arrhythmia.

Therefore, one cannot conclusively say it is safe to use these drugs in the COVID-19 patient and they should be used in ethically approved RCTs testing benefits and harms and with precaution in patients with CV disease. Caution is urged at this time. Understanding (and ruling out) the potential harm is critical at this stage. Overall, these findings raise important questions that warrants study to exclude harms. In this light, one clinical trial in n=40 patients (ClinicalTrials.gov Identifier: NCT02932007) seeks to examine the efficacy of chloroquine in terminating persistent atrial fibrillations and assess the potential role as a pharmacological agent for the management of atrial fibrillation. While not in the COVID-19 patient directly, this may provide some initial clarity to emerging questions around the use of chloroquine and hydroxychloroquine. Another ongoing trial (NCT04308668) seeks to discern the clinical efficacy of hydroxychloroquine as a postexposure prophylaxis.

A matter of dosing

One critical and invaluable issue in repurposing medications such as hydroxychloroquine if it is shown to be beneficial, is the issue of optimal dosing for COVID-19 patients. In this light, researchers[59] evaluated the hydroxychloroquine antiviral activity when administered before (pre-adsorption), after (post-adsorption) or before and after (full-time) virus adsorption in order to simulate its possible prophylactic, therapeutic and prophylactic/therapeutic clinical use. In brief, they used Vero E6 (Vero C1008, clone E6 – CRL-1586; ATCC) cells and a clinical isolate hCoV-19/Italy/UniSR1/2020 was proliferated in Vero E6 cells, and the viral titer was determined by 50% tissue culture infective dose (TCID50) and plaque assay for confirming the obtained titer. They focused on evaluating hydroxychloroquine activity virus adsorption at 37 and 4°C. They report that at 37°C the virus enters the intended host cell in a more physiological context and at 4°C virus it can dock to the cell receptor of the host, but that its internalisation is much more constrained. They found in their study that there is constrained activity of HCQ when it is administered only prophylactically or therapeutically. On the other hand, they detected substantial antiviral activity when in the prophylactic/therapeutic (full-time) experimental setting both at 37°C and 4°C, and they thus conjecture on the need of a combined prophylactic and therapeutic clinical use of HCQ so as to maximise its antiviral effects.[59] This *in vitro* research is preliminary to help guide the set up of clinical trials and it is not yet peer-reviewed.

Conclusion

In sum, the medical research community has to urgently examine chloroquine/hydroxychloroquine as stand-alone treatments or in combination with azithromycin (or any other treatments), within robust RCT research in COVID-19 patients specifically to definitively establish benefits and harms. Evidence is emerging via small studies with sub-optimal methodologies that are conflicting. These emerging *in vivo* studies do add to the evidence base and may be indicative that there could be some role in COVID-19. Exactly what it is remains to be clarified. There could be some benefit of these anti-malarias but the level of certainty that is needed in the resulting estimates is not there with this body of emergent evidence. As well, proper clinical research may show that there is real harm with use in this patient group. Research is ongoing to clarify which. The reporting thus far is very thin and confusing, the research methodologies used thus far are very poor, and the type of patient-important clinical outcomes needed for decision-making are not clear or even reported. At this time, the type of high-quality robust evidence is not available to optimally inform on the safe use of chloroquine or hydroxychloroquine (with azithromycin) in COVID-19 patients.

Ongoing studies

We await the findings from studies that have been registered worldwide. Specifically, there are more than 30 different trials for SARS-CoV-2 already registered in different Clinical Trial Registries e.g. ChiCTR2000029939, ChiCTR2000029935, ChiCTR2000029899, ChiCTR2000029898, ChiCTR2000029868, ChiCTR2000029837, ChiCTR2000029826, ChiCTR2000029803, ChiCTR2000029762, ChiCTR2000029761, ChiCTR2000029760, ChiCTR2000029741, ChiCTR2000029740, ChiCTR2000029609, ChiCTR2000029559, ChiCTR2000029542 NCT04303507 NCT04323527 NCT04318444 NCT04316377 NCT04321278 NCT04307693 NCT04322123 NCT04315896 NCT04318015 NCT04319900 NCT04303299 NCT04323631 NCT04308668 NCT04324463 NCT04286503 NCT04261517 NCT04321616 NCT04322396 NCT04321993 NCT04322396 RBR-9d8z6m ISRCTN83971151, which list the use chloroquine or hydroxychloroquine in the treatment of COVID-19.

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Appendix

MEDLINE search strategy
Database: Ovid MEDLINE(R) <1996 to March 26, 2020>
Search Strategy:

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1 exp Betacoronavirus/ or exp Coronavirus/ or exp Coronavirus Infections/ or COVID-19.mp. or exp Retrospective Studies/ or exp SARS Virus/ (728446)
2 chloroquine.mp. or exp Chloroquine/ (12447)
3 1 and 2 (407)
4 hydroxychloroquine.mp. or exp Hydroxychloroquine/ (3363)
5 1 and 4 (339)
6 2 or 4 (13432)
7 1 and 6 (521)
8 limit 7 to yr="2000 -Current" (470)

EMBASE search strategy
Database: Embase <1996 to 2020 March 26>
Search Strategy:

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1 Betacoronavirus/ (140)
2 Coronavirus.mp. or exp Coronavirinae/ (16037)
3 Coronavirus Infections.mp. or exp Coronavirus infection/ (11351)

4 COVID-19.mp. (186)
5 SARS Virus.mp. or exp SARS coronavirus/ (4634)
6 1 or 2 or 3 or 4 or 5 (21128)
7 chloroquine.mp. or chloroquine plus hydroxychloroquine sulfate plus mepacrine/ or chloroquine/ (25872)
8 exp hydroxychloroquine/ or hydroxychloriquine.mp. (20883)
9 7 or 8 (44358)
10 6 and 9 (87)
11 limit 10 to yr="2000 -Current" (87)

Note: searches updated to March 27th 2020 and on a daily basis

Table 1: Main results of studies on the activity of chloroquine or hydroxychloroquine on coronaviruses

Reference	Compound (s)	Targeted virus	System used for antiviral activity screening	Antiviral effect
E Keyaerts, L Vijgen, P Maes, J Neyts, M Van Ranst**In vitro inhibition of severe acute respiratory syndrome coronavirus by chloroquine** Biochem Biophys Res Commun, 323 (2004), pp. 264-268, 10.1016/j.bbrc.2004.08.085	Chloroquine	SARS-CoV	Vero (African green monkey kidney) E6 cells	EC_{50} = 8.8 ± 1.2 µM
MJ Vincent, E Bergeron, S Benjannet, BR Erickson, PE Rollin, TG Ksiazek, *et al.***Chloroquine is a potent inhibitor of SARS coronavirus infection and spread** Virol J, 2 (2005), p. 69, 10.1186/1743-422X-2-69	Chloroquine		Vero E6 cells	EC_{50} = 4.4 ± 1.0 µM
DL Barnard, CW Day, K Bailey, M Heiner, R Montgomery, L Lauridsen, *et al.***Evaluation of immunomodulators, interferons and known in vitro SARS-coV inhibitors for inhibition of SARS-coV replication in BALB/c mice** Antivir Chem Chemother, 17 (2006), pp. 275-284, 10.1177/095632020601700505	Chloroquine, chloroquine monophosphate, chloroquine diphosphate	SARS-CoV (four strains)	Vero 76 cells BALB/c mice	Chloroquine: EC_{50} = 1–4 µM Chloroquine monophosphate: EC_{50} = 4–6 µM Chloroquine diphosphate: EC_{50} = 3–4 µM Intraperitoneal or intranasal chloroquine administration, beginning 4 h prior to virus exposure: 50 mg/kg but not 10 mg/kg or 1 mg/kg reduced for the intranasal route (but not the intraperitoneal route) viral lung titres from mean ± S.D. of 5.4 ± 0.5 to 4.4 ± 1.2 in log_{10} $CCID_{50}$/g at Day 3 (considered as not significant)
C Biot, W Daher, N Chavain, T Fandeur, J Khalife, D Dive, *et al.***Design and synthesis of hydroxyferroquine derivatives with antimalarial and antiviral activities** J Med Chem, 49 (2006), pp. 2845-2849, 10.1021/jm0601856	Chloroquine, hydroxychloroquine	SARS-CoV Feline coronavirus	Vero cells Crandell–Reese feline	Chloroquine: EC_{50} = 6.5 ± 3.2 µM Chloroquine: EC_{50} > 0.8 µM

			kidney (CRFK) cells	Hydroxychloroquine: EC_{50} = 28 ± 27 μM
M Kono, K Tatsumi, AM Imai, K Saito, T Kuriyama, H Shirasawa**Inhibition of human coronavirus 229E infection in human epithelial lung cells (L132) by chloroquine: involvement of p38 MAPK and ERK** Antiviral Res, 77 (2008), pp. 150-152, 10.1016/j.antiviral.2007.10.011	Chloroquine	HCoV-229E	Human epithelial lung cells (L132)	Chloroquine at concentrations of 10 μM and 25 μM inhibited HCoV-229E release into the culture supernatant
E Keyaerts, S Li, L Vijgen, E Rysman, J Verbeeck, M Van Ranst, *et al.***Antiviral activity of chloroquine against human coronavirus OC43 infection in newborn mice** Antimicrob Agents Chemother, 53 (2009), pp. 3416-3421, 10.1128/AAC.01509-08	Chloroquine	HCoV-OC43	HRT-18 cells Newborn C57BL/6 mice; chloroquine administration transplacentally and via maternal milk	EC_{50} = 0.306 ± 0.0091 μM 100%, 93%, 33% and 0% survival rate of pups when mother mice were treated per day with 15, 5, 1 and 0 mg/kg body weight, respectively
M Wang, R Cao, L Zhang, X Yang, J Liu, M Xu, *et al.***Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro** Cell Res (2020 Feb 4), 10.1038/s41422-020-0282-0	Chloroquine	SARS-CoV-2	Vero E6 cells	EC_{50} = 1.13 μM

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