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. Yet, recognizing that there are numerous ongoing clinical studies, PAHO will periodically update these reviews and corresponding recommendations as new evidence becomes available.

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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 few studies, having 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.

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 Emergency / 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/ hydroxychloroquine 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 its 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 observational and small randomized controlled trials with high risk of bias. 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.

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 small studies whereby both raise many methodological concerns and are at best, low quality evidence. A full quality of evidence assessment of these studies (using GRADE methods; url: https://gdt.gradepro.org/app/handbook/handbook.html) 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. A large portion of the research is presently ongoing worldwide.

One treatment of interest is chloroquine (and hydroxychloroquine). 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]

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 (largely *in vitro*) 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]

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	$EC_{50} = 8.8 \pm 1.2\ \mu M$
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	$EC_{50} = 4.4 \pm 1.0\ \mu M$
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	Chloroquine: $EC_{50} = 1–4\ \mu M$ Chloroquine monophosphate: $EC_{50} = 4–6\ \mu M$ Chloroquine diphosphate: $EC_{50} = 3–4\ \mu M$
			BALB/c mice	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 $\pm$ S.D. of 5.4 $\pm$ 0.5 to 4.4 $\pm$ 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 kidney (CRFK) cells	Chloroquine: $EC_{50} = 6.5 \pm 3.2\ \mu M$ Chloroquine: $EC_{50} > 0.8\ \mu M$ Hydroxychloroquine: $EC_{50} = 28 \pm 27\ \mu M$
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	$EC_{50} = 0.306 \pm 0.0091\ \mu M$ 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

			transplacentall y and via maternal milk	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	$EC_{50} = 1.13\ \mu M$

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 6th 2020, the search date commencing in year 1996 (see Appendix for search strategy example). The search was not limited by study design as we wanted to examine all relevant published research, though the 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 May 6th 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.

Evidence was also considered from additional sources such as manuscript reference lists, clinical trials registers (such as the International Clinical Trial Registry Platform) and online trial portals that pre-publish studies not yet having completed the peer-review process. For example, the search includes the largest clinical medicine preprint repository, medRxiv.org, on a daily basis.

Results

The evidence presented is to May 6th 2020. The literature database search resulted initially in uncovering 557 published peer-reviewed studies directly and indirectly relevant to our update on chloroquine/hydroxychloroquine (MEDLINE=470, EMBASE=87). Twelve came from additional sources (e.g. published in pre-publications) and as such n=569 in total. In addition, there are over 200 registered studies in the WHO's International Clinical Trials Registry Platform (ICTRP) database (https://www.who.int/ictrp/en/), clinicaltrials.gov 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.

Key initial evidence relevant to COVID-19

Initial 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.

Initial in vivo

ii) The *in vitro* study was followed by the *in vivo* finding[2] that drove the initial 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 were reported to be based on roughly 100 patients who are participants in several ongoing studies in hospitals in China. The reporting was very sub-optimal and has not been clear as to the comparators to the interventions. Researchers reported that chloroquine showed 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, represented 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]

Further 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 this 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 (full reporting not yet available). This 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. However, this discussion, while not peer-reviewed or formally reported, provides an additional layer and characterization of the virus and disease sequelae.

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.[11] 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)

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] 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.

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.

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.

A 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.

Another 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.

They 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.

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.

Thus far, we have been able to identify and review 19 studies (where one must be considered as a publication of a RCT under journal review and we report what is publicly available; we do not offer officially) examining some role of hydroxychloroquine or chloroquine in COVID-19 patients (Table 2). Five have been RCTs[15, 17, 19, 20, 21] and 13 are observational studies[14, 16, 18, 23-32] (retrospective, prospective, and case-series).

Table 2: Studies examining the role of HCQ/CQ in COVID-19 patients

Chloroquine/hydroxychloroquine There is insufficient evidence to draw a conclusion on benefits and harms. The effectiveness is being evaluated in various randomized clinical trials. Cardiovascular adverse events should be closely monitored (see GRADE Table and Figure in appendix)			
RCT (clinical)			
Chen[15]; RCT; 2020	Hydroxychloroquine (HCQ) 400 mg per day for 5 days vs control (conventional treatment); 30 (15:15); 48.5 mean; 70%	None reported; nebulization with interferon alpha, and 80% patients in the experimental group received arbidol vs 66.7% in control, 2 received lopinavir / ritonavir.	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), 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, 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. Researchers concluded that the standard dose of hydroxychloroquine sulfate does not show clinical effects in improving patient symptoms and accelerating virological suppression. Note: sub-optimal randomization, allocation concealment, blinding, small sample size, small event number, and imbalanced co-treatment assignment.	High; Very low certainty[1] See Figure 1, Table 1
Chen[17]; RCT; 2020	5-day HCQ (n=31) (400 mg/d), control (n=31) received SoC; 62; 44.7 mean (SD 15.3); 46.8%	None reported; none reported	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. Note: 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. No accounting of whether patients were taking any other medications prior to study entry or during the study; sub-optimal randomization, allocation concealment, blinding, small sample size, small event number, and imbalanced co-treatment assignment.	High; Very low certainty[1]
Huang[19]; RCT; 2020	Twice-daily oral of 500 mg Chloroquine (n=10) versus 400/100mg Lopinavir/Ritonavir (n=12) for 10 days; 22; 44.0 mean (36.5 to 57.5); 59.1%	None reported; none reported	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. Note: this small RCT appeared to show better effectiveness of chloroquine over lopinavir/ritonavir in moderate to severely ill COVID-19 patients; plagued with sub-optimal randomization, allocation concealment, blinding, small sample size, small event number, and use of active comparator with uncertain treatment effectiveness against COVID-19.	High; Very low certainty[1]
Silva Borba[20];	CQ (600mg CQ twice daily	Hypertension 46.2%,	There were 11 deaths (13.5%) in high dose and low dose users;	Low-

RCT; 2020	for 10 days or total dose 12g); or low dose CQ (450mg for 5 days, twice daily only on the first day, or total dose 2.7g); 81 (41 high doses vs 40 low dose); 51; 75	diabetes 25.9%, alcoholism 26%, heart disease 9.2%, asthma 6.2%, CKD 7.5%, rheumatic disease 5.6%, liver disease 3.7%, TB 3.7%, HIV/AIDS 1.9%; corticosteroids 5.4%, ACE inhibitors 10.3%, oseltamivir 89.6%	the high dose CQ arm presented more QTc>500ms (25%), and a trend toward higher lethality (17%) than the lower dosage. Fatality rate was 13.5% (95%CI=6.9–23.0%), overlapping with the CI of historical data from similar patients not using CQ (95%CI=14.5-19.2%). In 14 patients with paired samples, respiratory secretion at day 4 was negative in only one patient; preliminary findings suggest that the higher CQ dosage (10-day regimen) should not be recommended for COVID-19 treatment because of its potential safety hazards. Note: sub-optimal randomization with randomization occurring before laboratory confirmation of SARS-CoV-2 infection, small sample size, small event number, and comparison of dose-comparison concurrent trial without a placebo control.	moderate; Moderate certainty[3]
Tang[21]; RCT; 2020	HCQ (a loading dose of 1, 200 mg daily for three days followed by a maintained dose of 800 mg daily for the remaining days) vs SoC; 150; mean 46.1±14.7; 54.7%	Diabetes 14.0%, hypertension 6%, others 31%; 80 patients used other drugs after randomization (not clearly reported)	The overall 28-day negative conversion rate was not different between SOC plus HCQ and SOC group (85.4% versus 81.3%, p=0.34). Negative conversion rate at day 4, 7, 10, 14 or 21. A significant efficacy of HCQ on alleviating symptoms was observed (HR, 8.83, 95%CI, 1.09 to 71.3). There was a significantly greater reduction of CRP (6.98 in SOC plus HCQ versus 2.72 in SoC, milligram/liter, p=0.045) conferred by the addition of HCQ, which also led to more rapid recovery of lymphopenia, albeit no statistical significance. Adverse events found in 8.8% of SoC and 30% of HCQ recipients with two serious adverse events in the HCQ group. Note: sub-optimal randomization, allocation concealment, no blinding, small sample size, small event number, and comparison of dose-comparison concurrent trial without a placebo control.	High; Low certainty[1]
Barbosa[22]; quasi-RCT; 2020 (submitted to NEJM for peer review, abstract form and available in the referenced blog)	HCQ + supportive care vs supportive care alone; 63 (32 HCQ vs 31 control);	Not reported; not reported	HCQ administration was associated with worse outcomes. Note: this paper was cited on a blog and appears to be a released paper submitted to NEJM; we felt the data is important as shed important light but we do not wish this reference or material to be cited out of regard to the originating authors; what we include we have taken from the blog as referenced (https://blogs.sciencemag.org/pipeline/about-derek-lowe)	High; Low certainty[1]

OBSERVATIONAL (clinical)

Gautret[14]; observational (open-label non-randomized trial); 2020	HCQ 600 mg daily 6 d n=26 (AZ added depending on clinical presentation); 42; 26 HCQ, 16 control; 45.1 ± 22.0 (mean/SD); 41.7%	None reported; none reported	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 D 6-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. Note: clinical follow-up and occurrence of side-effects were not discussed in the paper; non-randomized, confounded, optimal adjustments and steps such as stratification and masking not applied, small sample size, small events, not optimally comparative, and sub-optimal reporting of methods and outcomes. This early data is to be considered hypothesis generating, calling for well-designed randomised clinical studies.	High; Very low certainty[1]
Gautret[16]; observational (uncontrolled non-comparative observational study); 2020	200 mg of HCQ three times per day for ten days combined with AZ (500 mg on D1 followed by 250 mg per day for the next four days); 80; 52.5	Cancer 6.3%, diabetes 11.2%, CAD 7.5%, hypertension 16.3%, chronic respiratory disease 10%, obesity	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	High; Very low certainty[1]

	median, 52.5%	5%; immune-suppressive treatment 5%, non-steroid anti-inflammatory treatment 2.5%	contagious wards with a mean length of stay of five days.

Note: 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. Patients may have recovered on their own; non-randomized, confounded, optimal adjustments and steps such as stratification and masking not applied, small sample size, small events, not optimally comparative, and sub-optimal reporting of methods and outcomes. This early data is to be considered hypothesis generating, calling for well-designed randomised clinical studies. | |
| Molina[18]; observational (narrative review); 2020 | HCQ 600 mg/d for 10 days and AZ 500 mg Day 1 and 250 mg days 2 to 5; 11; 58.7 mean, 64% | None reported; none reported | One patient, hydroxychloroquine and azithromycin were discontinued after 4 days because of a prolongation of the QT interval from 405 ms before treatment to 460 and 470 ms under the combination; They 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. 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".

Note: this was a small consecutive series of patients followed to describe the response to the treatment, high risk of biased estimates; non-randomized, confounded, optimal adjustments and steps such as stratification and masking not applied, small sample size, small events, not optimally comparative, and sub-optimal reporting of methods and outcomes. This early data is to be considered hypothesis generating, calling for well-designed randomised clinical studies. | High; Very low certainty[1] |
| Lane[23]; network cohort and case-series; 2020 | Network cohort and self-controlled case series study that involved 956,374 and 310,350 users of HCQ and sulfasalazine, and 323,122 and 351,956 users of HCQ-azithromycin and HCQ-amoxicilin. | ARDS 58%, COPD 5%, depression 14.5%, diabetes 13.2%, hyperlipidemia 30%, pneumonia 5.7%, renal impairment 4.2%, UTI 14.2% | Data comprised 14 sources of claims data or electronic medical records from Germany, Japan, Netherlands, Spain, UK, and USA. Researchers found no excess risk of SAEs was when 30-day hydroxychloroquine and sulfasalazine use were compared. However, when azithromycin was added to hydroxychloroquine, researchers reported an increased risk of 30-day cardiovascular mortality HR 2.19 (95% CI 1.22-3.94), chest pain/angina HR 1.15 (95% CI 1.05-1.26), and heart failure HR 1.22 (95% CI 1.02-1.45)). The conclusion was that short-term hydroxychloroquine treatment was safe, but when azithromycin is added, it can induce heart failure and cardiovascular mortality, likely due to synergistic effects on QT length. Researchers urged caution in the use of this combination in COVID-19.

Note: very confusing methods, non-randomized, confounded, not optimally comparative (e.g. comparison of hydroxychloroquine compared to hydroxychloroquine with azithromycin was not reported), sub-optimal reporting of methods and outcomes. | High; Very low certainty[1] |
| Chorin[24]; observational (retrospective cohort study); 2020 | HQC plus azithromycin; 84; mean 63 ±15; 74% | CAD 11%, hypertension 65%, CKD 7%, diabetes 20%, COPD 8%, congestive heart failure 2%; Levofloxacin, | 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 malignant arrhythmia and sudden cardiac death.

Note: nonrandomized, confounded, optimal adjustments and | High; Very low certainty[1] |

		Lopinavir/Ritonavir, or Tacrolimus 8%, Norepinephrine, Phenylephrine, or Vasopressin 13%, Amiodarone 7%	steps such as stratification and masking not applied, small sample size, small events, not optimally comparative, sub-optimal reporting of methods and outcomes. This early data is to be considered hypothesis generating, calling for well-designed randomised clinical studies.	
Mahévas[25]; observational (retrospective cohort study); 2020	HCQ at a daily dose of 600 mg in the first 48 hours after hospitalisation vs no HCQ; 181; median 60 years (IQR 52 to 68 years); 71.1% Note: in the HCQ group, 20% received concomitant azithromycin	Respiratory disease 11%, heart failure 3.3%, hypertension (cardiovascular illnesses) 51.9%, diabetes 8.3%, CKD 5%, immuno-depression 11.6%; none reported	In terms of deaths or transfer to the ICU, 19% vs 21.6% occurred in the HCQ vs no HCQ groups respectively (RR 0.93 (0.48 to 1.81)), for day 7 mortality, 3.6% died in HCQ group vs 4.1% in the no-HCQ group (RR 0.61 (0.13 to 2.90)), occurrence of acute respiratory distress syndrome, 28.6% occurred in HCQ group vs 24.1% in no HCQ group (RR 1.15 (0.66 to 2.01)); in the 84 patients receiving HCQ within the first 48 hours, 8 (9.5%) experienced ECG modifications requiring HCQ discontinuation at a median of 4 days (3-9) after it began. Note: one of the stronger methodologies from among COVID-19 research releases; inverse probability of treatment weighting (IPTW) approach was used to closely approximate randomisation and try to balance the differences in baseline prognostic variables between treatment groups; some potentially important prognostic variables were not balanced in the modelling; overall, nonrandomized, confounded, optimal adjustments and steps such as masking not applied, small sample size, small events, and not optimally comparative. This early data is to be considered hypothesis generating, calling for well-designed randomised clinical studies.	Low-moderate; Very low certainty[1]
Magagnoli[26]; observational (retrospective analysis study); 2020	One of three cohorts based on medication exposure to hydroxychloroquine (HC) and azithromycin (AZ): 1) HC-treated (97); 2) HC- and AZ-treated (113); or 3) HC-untreated (158), all received standard support care; 368; median age (IQR) HC 70 (60-75), HC + AZ 68 (59-74), no HC 69 (59-75); 100%	Hyperlipidemia 15.7%, asthma 5.9%, 4.9%, congestive heart failure 20.4%, peripheral vascular disease 17.4%, cerebrovascular disease 12.8%, COPD 19.6%, diabetes 67.6%, renal disease 25%, cancer 16%, liver disease 1.1%; ACE inhibitor 13.9%, ARBs 8.9%	27 deaths (27.8%) HC group, 25 deaths (22.1%) HC+AZ group, 18 deaths (11.4%) no HC group, mechanical ventilation in 13.3% HC group, 6.9% HC+AZ group, and 14.1% no HC group (Table 4). Relative to the no HC group, there was higher risk of death from any cause in HC group (adjusted HR, 2.61; 95% CI, 1.10 to 6.17; p=0.03) but not in HC+AZ group (adjusted HR, 1.14; 95% CI, 0.56 to 2.32; P=0.72), no significant difference in the risk of ventilation in either the HC group (adjusted HR, 1.43; 95% CI, 0.53 to 3.79; p=0.48) or the HC+AZ group (adjusted HR, 0.43; 95% CI, 0.16 to 1.12; p=0.09), compared to the no HC group; no evidence that HCQ, with or without AZ, reduced the risk of mechanical ventilation and an association of increased overall mortality in HCQ alone. Note: adjusted for a large number of confounders including comorbidities, medications, clinical and laboratory abnormalities; however, even with propensity score adjustment for a large number of relevant confounders, one cannot discount the potential of selection bias or residual confounding; 100% male with median age was over 65 years, so not applicable directly to women or younger hospitalized populations; most were black; small sample size, small events number, though reporting was an improvement over COVID-19 reporting in general. This early data is to be considered hypothesis generating, calling for well-designed randomised clinical studies.	High; Very low certainty[1]
Ramireddy[27]; observational case-series; 2020	HCQ 10%, Azithromycin 28%, both 62%; 98; mean age 62±17; 61% Note: 73 patients COVID-19 positive and 25 suspected	Heart failure 20%, hypertension 60%, diabetes 22%, CKD 14%, COPD 26%; none reported	Significant prolongation was observed only in males (18±43 ms vs -0.2±28 ms females, p=0.02); researchers reported 12% of patients reached critical QTc prolongation, multivariable logistic regression, age, sex, Tisdale score, Elixhauser score, and baseline QTc were not associated with critical QTc prolongation (p>0.14). HCQ + AZ revealed the greatest changes in QTc relative to each drug; changes were highest with combination treatment relative to either drug, with many-times greater prolongation using combination vs. azithromycin	High; Very low certainty[1]

			alone (17±39 vs. 0.5±40 ms, p=0.07); researchers reported that no patients experienced torsades de pointes. Note: pre-publication and not yet peer-reviewed, nonrandomized, potentially confounded even with adjustments, small sample size, sub-optimal reporting. This early data is to be considered hypothesis generating, calling for well-designed randomised clinical studies.	
Mathian [28]; case-series; 2020	HCQ treatment in SLE patients; 17; median age 53.5 (26.6–69.2); 23%	CHD 12%, cerebrovascular disease 18%, hypertension 35%, cancer 6%, COPD 12%, CKD 47%; prednisone 71%, ACE inhibitors 35%, anticoagulants 29%	HCQ did not prevent COVID-19 in severe forms, in patients with SLE. Note: nonrandomized, confounded, optimal adjustments and steps such as stratification and masking not applied, small sample size, small events, not optimally comparative, sub-optimal reporting of methods and outcomes. This early data in this SLE patient group with SARS-CoV-2 infection is to be considered hypothesis generating, calling for well-designed randomised clinical studies.	High; Very low certainty[1]
Yu [29]; observational (retrospective); 2020	HCQ for 7–10 days (200 mg twice per day) vs no HCQ (basic treatment); all 568 critically ill COVID-19 patients who were confirmed by pathogen laboratory tests; median 68 (57-76); 63% Note: HCQ age 68 (60-75) vs 68 (57-77)	Hypertension 44%, CHD 10.4%, COPD 2.8%, diabetes 17.1%;	Died=247 patients, 8 in HCQ and 238 in non-HCQ; time of hospital stay before patient death was 15 (10 to 21) days and 8 (4 to 14) days for the HCQ and NHCQ groups, respectively (p<0.05). The level of inflammatory cytokine IL-6 was significantly lowered from 22.2 (8.3 to 118.9) pg/mL at the beginning of the treatment to 5.2 (3.0 to 23.4) pg/ml (p<0.05) at the end of the treatment in the HCQ group but there is no change in the NHCQ group; researchers concluded that HCQ seemed to play a role in decreased mortality in critically ill patients with COVID-19 via a role in mitigating the inflammatory cytokine storm. Note: nonrandomized, small sample sized and events (especially in HCQ group), not optimally comparative; conducted adjusted analysis (Cox regression) including baseline drugs, but still cannot account for all known and unknown confounders; methods were sub-optimal but an improvement over the general methods across COVID19 and the reporting was not optimal but still an improvement.	Moderate to high; Very low certainty[1]
Chorin [30]; observational case-series; 2020	HCQ/Azithromycin combination; 251; 64 +-13; 75% Note: HCQ orally at 400 mg BID for one day (loading dose) followed by 200 mg BID for 4 days. Azithromycin orally at a dose of 500 mg daily for 5 days.	CAD 12%, hypertension 54%, CKD 115, diabetes 27%, COPD 7%, congestive heart failure 3%	Researchers reported that QTc was prolonged in parallel with increasing drug exposure and incompletely shortened following its completion; of concern was the extreme new QTc prolongation to > 500 ms which is an established marker of high risk for TdP and this developed in 15.9% of patients; reporting suggested that 1 patient developed TdP requiring emergent cardioversion and 7 patients required premature termination of therapy; HCQ combined with azithromycin macrolide significantly prolonged the QTc in patients with COVID-19 and the prolongation may be responsible for life threating arrhythmia in the form of TdP. Note: nonrandomized, confounded, some logistic regression adjustments employed but optimal adjustments and steps such as stratification and masking not applied, small sample size, small events, not optimally comparative, sub-optimal reporting of methods and outcomes; weaker evidence but raises concern about the combination of HCQ and AZ. Note, adjusted analysis is an improvement over unadjusted analysis whereby the estimates are very unreliable but still is unable to adjust for all unknown confounders.	High; Very low certainty[1]
Mallat [31]; observational retrospective cohort; 2020	HCQ; 34 (23 HCQ vs 11 non-HCQ); median age 37; 73.5% male	Asthma 8.8%, diabetes 5.9%, hypertension, 14.7%, malignancy 8.8%,	Researchers reported that HCQ treatment was independently associated with longer time to SARS-CoV-2 test negativity; at day 14, virologic clearance was significantly higher in patients who did not receive HCQ, and HCQ treatment did not result in	High; Very low certainty[1]

		chronic heart failure 2.95, chronic kidney disease 29%; immunosuppressive 2.9%, NSAID 11.8%	improvement of inflammatory markers or lymphopenia rate. Note: nonrandomized, confounded, steps such as masking not applied, small sample size, small events, adjustment could not control for all unknown confounders and did not adjust for key prognostic variables, sub-optimal reporting of methods and outcomes.	
Huang [32]; observational prospective; 2020	197 CQ patients and 176 patients as historical controls; 373; mean age 44.78; 46.9% male	Hypertension 6.4%, diabetes 2.4%; not reported	53 adverse events in CQ vs 57 in non-CQ group; time to undetectable viral RNA, median no. of days (IQR) CQ 3.0 (3.0, 5.0) vs non-CQ 9.0 (6.0, 12.0) (absolute difference in medians -6.0 days; 95% CI -6.0 to -4.0); length of hospital stay, median no. of days (IQR) CQ 19.0 (16.0, 23.0) vs non-CQ 20.0 (15.8, 24.0). Note: nonrandomized, confounded, sub-optimal reporting of methods and outcomes.	High; Very low certainty[1]
Membrillo [70]; observational cohort; 2020	166 patients, HCQ 123 and 43 no HCQ; 166; mean age HCQ 61.5 (16.2) vs 68.7 (18.8) non HCQ; 62% male	Hypertension 42.7%, diabetes 17.4%, cardiopathy 22.2%, malignancy 13.8%, pulmonary disease 14.4%, dyslipidaemia 28.3%; none reported	Hydroxychloroquine treatment was associated with an increase in the mean cumulative survival; HCQ group 22% vs 48.8%; mean hospital stay days mean 6 (SD 5) HCQ vs 5 (7) non HCQ group; median (IQR) from symptoms begin to the start of treatment with HCQ: 7(6) days. Note: nonrandomized, confounded design, small sample sized, small number of events, plagued by selection bias, residual confounding bias.	High; Very low certainty[1]
Geleris [71]; observational prospective; 2020	HCQ (n=811) vs no HCQ (n=565), HCQ 600 mg twice on day 1, then 400 mg daily for a median of 5 days; n=118 <40 yrs, n=287 40-59 yrs, n=485 60-79 yrs, and n=206 >=80 yrs, 58.5% males (propensity score matched HCQ 811 vs 274 matched controls	Chronic lung disease 17.9%, diabetes 36.4%, hypertension 50.1%, cancer 13.2%, chronic kidney disease 17.8%, transplantation, HIV infection, or immune-suppressive medications 4.7%; statin 38.5%, ACEi or ARBs 29.5%, corticosteroid 23.7%, anticoagulant 9.2%, azithromycin 54.1%, antibiotic 72.5%, tocilizumab 6.2%, remdesivir 2.5%	Primary end point of respiratory failure developed in 346 patients (25.1%); 180 patients were intubated; 166 died without intubation; in unadjusted analysis, patients who had received hydroxychloroquine were more likely to have had a primary end-point event than were patients who did not (HR 2.37; 95% CI 1.84 to 3.02); there was no significant association between hydroxychloroquine use and the composite primary end point (HR 1.04; 95% CI 0.82 to 1.32); there was no significant association between treatment with azithromycin and the composite end point (HR 1.03; 95% CI 0.81 to 1.31). Researchers concluded that results do not support the use of hydroxychloroquine unless within confines of randomized clinical trials testing. Note: nonrandomized, potentially confounded design, decent sample sized though control group markedly smaller, small number of events, compositive end-point (time to intubation or death), plagued by selection bias, residual confounding bias even with propensity-score matching and adjustment (these steps strengthen the weaker nonrandomized design but still is unable to correct for selection and residual confounding/confounded by indication biases).	Low-moderate; Very low certainty[1]

Notes and considerations:

*ratings are high vs moderate-low vs low RoB; note, high risk for RCTs would be for serious flaws in randomization, allocation concealment, blinding, severe data loss, baseline imbalances etc. and for observational non-randomized studies (single or two-arm), there could be no adjustment for confounders, no masking, stratification etc.

**ratings are high, moderate, low, very low certainty (GRADE); note using GRADE, RCTs start as high certainty/quality evidence, observational studies start as low certainty/quality; for imprecision, the focus is on sample size, number of reported events, width of confidence intervals (if reported); note also that the use of GRADE in this application for RCTs and observational studies focuses mainly on risk of bias and imprecision given we are dealing with single studies and domains of consistency (heterogeneity), indirectness, and publication bias are not ideally applicable. We would consider the magnitude of effect, dose-response, and plausible residual confounding for observational designs.

[1]risk of bias (potentially selection bias and residual confounding bias if observational and not randomized in design) and imprecision (small sample sizes, small event numbers), downgrade one level each (one may argue that since observational studies start as low certainty that the risk of bias due to lack of randomization etc. is already accounted for and no need to downgrade for risk of bias; in any case, one downgrade for imprecision still leads to very low; in some sense in the use of the ROBINS-I tool for risk of bias in

nonrandomized studies that is suggested to start at high certainty, eventually, certainty will become low due to the challenges of nonrandomization, selection bias, confounding bias etc.).

[2]risk of bias for in vitro studies uses OHAT risk of bias tool/NTP
url: Handbook for Conducting a Literature-Based Health Assessment Using OHAT Approach for Systematic Review and Evidence Integration. Available online: http://ntp.niehs.nih.gov/ntp/ohat/pubs/handbookjan2015_508.pdf whereby questions such as i) was administered dose or exposure level adequately randomized ii) was allocation to study groups adequately concealed and iii) can we be confident in the exposure characterization, were answered. Rating are definitely high, probably high, probably low, definitely low.
[3]imprecision downgrade one level due to small sample size and/or events.
[4]risk of bias downgrade due to open-label and imprecision due to small sample size and events; down-grade of two levels
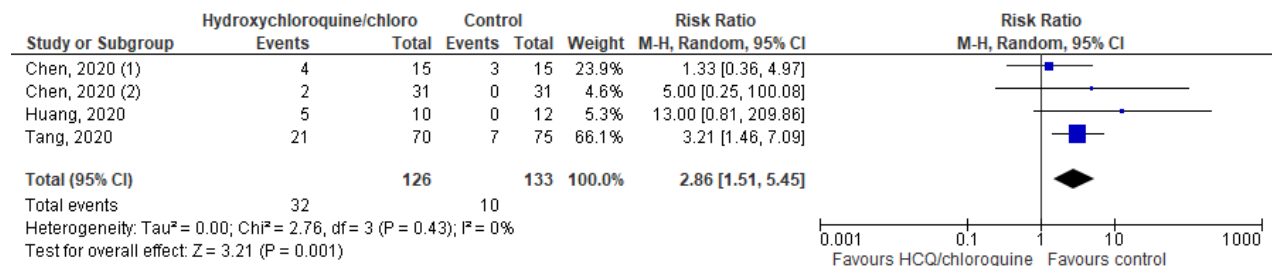[5]Low risk of bias based on application of AMSTAR II tool (url: https://amstar.ca/Amstar_Checklist.php).
[6]Very low RCT due to single downgrade risk of bias and double for imprecision
[7]AMSTAR II critical appraisal of systematic review and/or meta-analysis, url: https://amstar.ca/docs/AMSTAR-2.pdf (Accessed on April 1st 2020); citation: Shea BJ, Reeves BC, Wells G, Thuku M, Hamel C, Moran J, Moher D, Tugwell P, Welch V, Kristjansson E, Henry DA. AMSTAR 2: a critical appraisal tool for systematic reviews that include randomised or non-randomised studies of healthcare interventions, or both. BMJ. 2017 Sep 21; 358: j4008.

A risk of bias assessment was applied to RCTs as well as observational studies in Table 2 focusing on randomization, allocation concealment, blinding, attrition, or other relevant biases to the estimates of effect, as well as selection bias, residual confounding bias, statistical adjustment, matching (propensity score), stratification, or restriction, respectively.[33-37] The GRADE 'outcome-centric' method was applied to individual outcomes per study to derive a certainty/quality of evidence rating to establish how much confidence one could have in the estimates of effect. These are principally single studies and the approach was to consider the outcomes per study in a rapid manner to establish some sense of GRADE 'lite' rating per outcome and then to derive an overall rating. The overall rating is based on the lowest rating from among the critical/important patient outcomes. The reporting in these studies was very poor, scarce, and the general methodologies were very weak. This has been a rapid, albeit sub-optimal application of GRADE methods, while seeking to apply as much rigor to a flawed body of evidence emerging from the current reporting across COVID-19 research in general.[33-37]

Figure 1 and Table 3 presents a meta-analysis of adverse events combined in use of RCT on HCQ / CQ. There was no available data to perform meta-analysis for other critical outcomes (i.e. mortality, need of ICU).

Figure 1: Adverse events combined in use of HCQ / CQ (RCT pre-publications, non-peer review)

Study or Subgroup	Hydroxychloroquine/chloro Events	Total	Control Events	Total	Weight	Risk Ratio M-H, Random, 95% CI
Chen, 2020 (1)	4	15	3	15	23.9%	1.33 [0.36, 4.97]
Chen, 2020 (2)	2	31	0	31	4.6%	5.00 [0.25, 100.08]
Huang, 2020	5	10	0	12	5.3%	13.00 [0.81, 209.86]
Tang, 2020	21	70	7	75	66.1%	3.21 [1.46, 7.09]
Total (95% CI)		126		133	100.0%	2.86 [1.51, 5.45]
Total events	32		10			

Heterogeneity: Tau² = 0.00; Chi² = 2.76, df = 3 (P = 0.43); I² = 0%
Test for overall effect: Z = 3.21 (P = 0.001)

Table 1: GRADE certainty hydroxychloroquine/chloroquine adverse events (all combined)

№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	hydroxychloroquine/chloroquine	no HCQ/CQ or control	Relative (95% CI)	Absolute (95% CI)	Certainty	Importance
Certainty assessment							**№ of patients**		**Effect**			
Adverse outcomes (all combined)												
4	randomised trials	serious [a]	not serious	not serious	serious [b]	none	32/126 (25.4%)	10/133 (7.5%)	**RR 2.86** (1.51 to 5.45)	**140 more per 1,000** (from 38 more to 335 more)	⊕⊕◯◯ LOW	CRITICAL

CI: Confidence interval; **RR:** Risk ratio

Explanations
a. unclear/absent randomization, concealment, blinding, sub-optimal outcomes, imbalanced co-treatment assignment
b. small sample size, small number of events (OIS not met)

Discussion

What is known thus far? Initially, mainly *in vitro* data suggested that chloroquine (and hydroxychloroquine) mitigates and inhibits the replication of SARS-CoV-2. Results however, have began to emerge mainly from China but of very poor methodological and reporting quality. Medical research and practice changing decisions must be made only when there has been access to the full 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 many of the released studies. The concern is that 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.[38] Chloroquine is known to inhibit virus infection by increasing the endosomal pH required for virus/ host cell fusion, and also upsets the glycosylation of cellular receptors of SARS-CoV. 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.[39]

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. Aa indicated, the initial *in vitro* results (recently emerging from France[14, 16, 18] and from China,[15, 17, 19, 21] 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. 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. These are major concerns since the resulting estimates of effect become very unreliable. The overall *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.[40] Reported adverse events thus far have been[40-44] 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.[41-44] 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.[44]

However, while there are indications of low level adverse effects, there has been evidence[45-47] 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).[45-48] 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.[49] A recent description of the clinical characteristics of 138 hospitalized patients with COVID-19 in China,[50] 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[50] raised serious questions for the COVID-19 patient since evidence[51] 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[52] 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 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.[53] 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).[54] They reported that chloroquine is more optimal in terminating SAF via "significantly increasing core size and decreasing re-entry frequency". There is also evidence 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.[55]

Researchers reported on a case of hydroxychloroquine-induced cardiomyopathy that presented as pulmonary hypertension in a 63-year old female.[56] 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.[56]

Researchers reported on hydroxychloroquine-induced cardiotoxicity in a 39-year-old woman with systemic lupus erythematosus and systolic dysfunction.[57] 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.[58] 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.[58]

In a similar light, researchers reported on hydroxychloroquine-induced cardiomyopathy.[59] 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,[59] 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.[59] 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".[59] Researchers also warned that this could result in death.

Moreover, researchers[60] 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".[60] 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".[60]

Additionally, researchers reported on suspected hydroxychloroquine-associated QT-interval prolongation in a patient with systemic lupus erythematosus.[61] 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[67] 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".[61] They reported that "serial ECGs revealed gradual shortening of the QTc interval"[61] 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".[61]

Similar findings were reported by Chen et al. (2006)[62] 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)[63] 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[64] 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[65] 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).[65] 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.

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[66] 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).[67-69]

The evidence on hydroxychloroquine (with or without azithromycin) continues to be conflicting, of very poor methodology, and poorly reported. Therefore, based on the accumulated body of evidence present above (both *in vitro* but principally *in vivo*), one cannot conclusively say it is safe to use these drugs in the COVID-19 patient (nor is it effective) and they should be used in ethically approved (patient consented) 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.

Conclusion

In sum, the medical research community has to urgently examine chloroquine/hydroxychloroquine as stand-alone treatments, within robust RCT research in COVID-19 patients specifically to definitively establish benefits and harms. Equipoise still exists. 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 in COVID-19 patients.

Ongoing studies

We await the findings from several studies that have been registered worldwide which list the use chloroquine or hydroxychloroquine in the treatment of COVID-19.
https://clinicaltrials.gov/ct2/results?term=Hydroxychloroquine&cond=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)