Ther Adv Infectious Dis
2020, Vol. 7: 1–14
DOI: 10.1177/2049936120947517

© The Author(s), 2020.
Article reuse guidelines: sagepub.com/journals-permissions

Chloroquine and hydroxychloroquine as a repurposed agent against COVID-19: a narrative review

Yogesh Acharya[1] and **Abida Sayed**[2]

***Abstract*:** The predicament arising from the coronavirus disease 2019 (COVID-19) pandemic has become one of the most significant modern public health challenges. Despite uncertainties in the viral determinants and pathogenesis, it is crucial to accurately inspect all available evidence to construct accurate clinical guidelines for optimised patient care. This study aims to discuss the available evidence for the use of chloroquine (CQ) and hydroxychloroquine (HCQ) against COVID-19. Early *in vitro* studies of CQ/HCQ against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) are convincing. But contradictory evidence exists on the clinical use of CQ/HCQ, either alone or in combination with azithromycin. As of now, there is no compelling clinical evidence on CQ, HCQ, and azithromycin in COVID-19 and the available evidence is limited to methodologically inferior non-randomised studies. Studies have also shown detrimental drug reactions to CQ and 'HCQ plus azithromycin', mainly cardiac side effects in hospitalised patients with coexisting cardiovascular comorbidities. Therefore, we recommend that physicians avoid high doses and exercise extreme caution in the compassionate use of CQ/HCQ, either alone or in combination with other antiviral drugs.

Keywords: chloroquine, COVID-19, evidence, hydroxychloroquine, review

Received: 19 May 2020; revised manuscript accepted: 8 July 2020.

Introduction

The sudden outbreak of Coronavirus Disease 2019 (COVID-19), stemming from a novel coronavirus originating from Wuhan, China, has grown into a global pandemic as the third major outbreak of the virulent coronavirus family after Severe Acute Respiratory Syndrome (SARS) and Middle Eastern Respiratory Syndrome (MERS).[1] Despite radical containment efforts, the SARS Coronavirus 2 (CoV 2) continues to spread globally. Currently, therapeutic tactics are only supportive as there are no proven pharmacological agents active against the virus.[2] International efforts are focussed on searching for effective therapies to counter the disease's effects. One strategy is to search for Nobel agents by repurposing older drugs with known antiviral activity that have been studied in the past.[3] In this regard, chloroquine (CQ) and hydroxychloroquine (HCQ) have garnered much attention as they were well studied during previous coronavirus epidemics with SARS and MERS.[4]

This study aims to discuss the general properties and antiviral history of CQ and HCQ, and to analyse the available evidence against COVID-19, either alone or in combination with other drugs.

Methods

A literature review was performed using PubMed and Google Scholar to identify all relevant English language scientific studies based on our study objectives. Non-specific combinations of the search strings included (Coronavirus OR Severe Acute Respiratory Syndrome OR SARS OR

Correspondence to:
Yogesh Acharya
Vascular and Endovascular Department, Western Vascular Institute, National University of Ireland, Galway, H91 TK33, Ireland
y.acharya1@nuigalway.ie
dryogeshach@gmail.com

Abida Sayed
Medicine Department, Avalon University School of Medicine, Willemstad, Curacao, Netherlands Antilles

Creative Commons Non Commercial CC BY-NC: This article is distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 License (https://creativecommons.org/licenses/by-nc/4.0/) which permits non-commercial use, reproduction and distribution of the work without further permission provided the original work is attributed as specified on the SAGE and Open Access pages (https://us.sagepub.com/en-us/nam/open-access-at-sage).

SARS-CoV OR Middle Eastern Respiratory Syndrome OR MERS OR MERS-CoV OR Severe Acute Respiratory Syndrome Coronavirus 2 OR SARS-CoV-2 OR 2019-nCoV OR Coronavirus disease OR COVID-19) AND (Quinine OR Chloroquine OR CQ OR Hydroxychloroquine OR HCQ), in association with related pharmacology and antiviral activity. For additional studies, we also searched medRxiv.org, the largest preprint repository of clinical medicine. Studies that analysed the efficacy and safety of CQ/HCQ in SARS, MERS, and COVID-19 both *in vitro* and *in vivo* models were included. Due to the scarcity of randomised control trials (RCT), other relevant studies, such as observational studies, case reports, case series, and review articles, were also incorporated. Studies conducted in the paediatric population and pregnant women were excluded. An independent analysis of the included studies was performed with a secondary reference search for the relevant supplementary studies.

Pharmacological properties

Chemically, CQ is made up of 7-chloro-4 (-4-dimethylamino-1-methyl butyl amino) quinoline. The CQ/HCQ belongs to the same family of weak bases, alkylated 4-aminoquinolines.[5] HCQ differs due to the presence of a hydroxyl group in its side chain with N-ethyl substitute being beta-hydroxylated. Both can easily cross cell membranes, with HCQ being more polar with a lower lipophilic effect.

CQ and HCQ share similar pharmacokinetics, with rapid absorption from the gastro-intestinal surfaces, and are renally and hepatically eliminated. As they are weak bases, they increase the pH of acidic vesicles.[6] Notably, HCQ increases intracellular pH levels by inhibiting lysosomal activity in immune cells to prevent downstream immune cell interaction, antigen processing, and cytokine responses. Furthermore, the increment in the pH interferes with viral entry into endosomes and blocks viral-endosome fusion.[6]

Although CQ and HCQ are relatively well-tolerated, both drugs are associated with systemic adverse effects, including QT syndrome, hypoglycemia, hepatitis, pancreatitis, neutropenia, retinopathy, anaphylaxis, and cardiac toxicities.[7] The United States Food and Drug Administration (FDA) has issued warnings on CQ/HCQ, particularly based on the associated cardiac side-effects.[8] The CQ/HCQ, either alone or in combination with other drugs like azithromycin, can cause possible cardiac complications, including conduction defects, such as bundle branch blocks, atrioventricular blocks, QT prolongation, torsades de point (TdP), and even dangerous ventricular arrhythmias. Although the CQ and HCQ use during pregnancy and in lactating mothers are believed to be safe,[9–11] they should be avoided in children due to narrow therapeutic and toxic windows. Children can suffer from apnea, seizures, and arrhythmias if they exceed the recommended therapeutic dose.[12]

Antiviral activity: SARS, MERS and others

Clark, in 1952, conducted studies depicting the potential anti-microbial activity of CQ by effective inhibition of deoxyribonucleic acid (DNA) synthesis on a protozoan parasite, *Plasmodium gallinaceum*.[13] This led to further investigations by Schellenberg and Coatney in 1960,[14] who demonstrated CQ-induced inhibition of the incorporation of radioactively labelled nucleic acid substituents in DNA and ribonucleic acid (RNA) of *Plasmodium gallinaceum.* These studies showed that CQ could inhibit the nucleic acid, and this nucleic-acid inhibitory potential could be utilised in viruses.

Mallucci conducted one of the first animal studies in 1966 to demonstrate the antiviral activity of CQ on lysosomes of hepatitis virus-infected mouse cells.[15] The mechanism of inhibition was unknown before the animal studies, and CQ was believed to lower virus yield, hypothesised by the prevention of new viral synthesis or viral uncoating. In 1972, Shimizu *et al.* investigated the antiviral effects of CQ on non-oncogenic viruses in animal cell culture.[16] They reported the antiviral effect of CQ on chick embryo cells infected with vesicular stomatitis virus with a subsequent reduction in the viral yield. The antiviral activities were attributed to the selective inhibition of vesicular stomatitis viral RNA without interfering with RNA synthesis in the host cells. In 1971, Lancz *et al.* showed CQ inhibiting herpes simplex virus replication in HeLa cell cultures,[17] and Banfield and Kisch subsequently confirmed these results in 1973.[18] However, they were unable to prove the inhibition *in vivo*. Other studies in the late 1970s and mid-1980s reported CQ-induced

inhibition of endocytosis and viral particle entry into cells, interfering with lysosomal enzyme activity, and preventing viral uncoating and multiplication and inhibition of DNA polymerase through intercalation.[19]

In 1994, Pazimo Yuhas and Tennant showed CQ/HCQ induced inhibition of retroviral infections in Moloney leukemia virus-cell cultures.[20] During the same period, Tsai *et al.* illustrated antiviral activity against human immunodeficiency virus (HIV) on the H9 cell line and avian reticuloendotheliosis virus on chick bone marrow cells resulting in the reduction in viral yield through the inhibition of viral protein glycosylation in the Golgi complex by CQ.[21] The antiviral activity of CQ was reinforced by further studies conducted by Sperber *et al.*, Chiang *et al.*, and Boelaert *et al.* by demonstrating the interference of HIV replication in T cells and monocytes.[22–24] These studies were the fundamental basis of drug repurposing of CQ/HCQ on subsequent viral epidemics. They opened the door for many further studies on CQ/HCQ, either alone or in combination with other antiviral drugs.

Many pharmaceutical agents were tried empirically during the SARS epidemic in 2003. Savarino *et al.* were the first to hypothesise the possible use of CQ in SARS,[25] which led to further research by Keyaerts *et al.* in 2004 at the Belgian Catholic University of Leuven on the *in vitro* antiviral activity of CQ against SARS-CoV Frankfurt 1 strain.[26] This study was conducted using Vero E6 cell culture, and there were promising results on inhibition of viral replication. Soon CQ became the preferred choice for prophylaxis and treatment of SARS due to its easy accessibility, administration, and low cost.

In 2005, Vincent *et al.* replicated the results produced by Keyaerts *et al.*, demonstrating the inhibition of SARS virus in Vero E6 cells.[26,27] These inhibitory effects were noted with CQ treatment both before and after exposure to the SARS virus, suggesting its prophylactic as well as therapeutic utility. Vincent *et al.*[27] found that CQ interferes with the terminal glycosylation process of cellular receptors, particularly angiotensin-converting enzyme 2 (ACE2), with the potential to prevent virus-receptor interaction, and interferes with viral spreading by increasing vesicular pH levels.

In 2006, Savarino *et al.* pointed out the importance of the CQ as a broad-spectrum antiviral agent.[28] They stated, 'the broad-spectrum antiviral effects of chloroquine deserve particular attention in a time in which the world is threatened by the possibility of a new influenza pandemic, and the availability of effective drugs would be fundamental during evaluation of an effective vaccine.'

In 2009, Keyaerts *et al.* further investigated both the *in vitro* and *in vivo* antiviral activities of CQ against human CoV strain OC43 (HCoV-OC43) in newborn mice.[29] They concluded that CQ interferes with the *in vitro* replication of HCoV-OC43, and showed 100% survival in newborn mice treated pre-partum with CQ. Their result showed that CQ could be immensely useful against HCoV-OC43.

Although there were some promising results concerning CQ/HCQ in SARS/MERS, most of these credible pieces of evidence were based on *in vitro* studies. Only a few clinical studies were available, which were methodologically inferior with small sample size, high dropout rates, variable baseline viral loads compared with monotherapy, and combination therapy of CQ/HCQ and differences in toxicities, which impacted the quality and validity of the results obtained.[30] As the SARS/MERS outbreaks were limited within a particular region and lasted for a short time, there were no follow ups, more extensive observational studies, or controlled clinical trials to support this evidence.

Evidence: COVID-19

Since the beginning of the COVID-19 outbreak in December 2019, there have been reports of intensive investigations into the possible repurposing of antiviral agents. The World Health Organisation (WHO) global ethical guide on managing ethical issues in infectious disease outbreak regarding the use of the unproven experimental drug,[31] first published as an ethical guide after the Ebola epidemic in 2016, has allowed the 'off-label' use of the experimental intervention on a case-by-case basis if there are no proven effective treatments and the results of the controlled clinical studies are not available, or not expected to be available sooner. This ethical guide opened the door for re-testing of many drugs with possible antiviral properties during the ongoing COVID-19 pandemic.

The CQ and HCQ duo was one of the very first contenders in the race against COVID-19. Although there was no high-quality clinical evidence of CQ/HCQ against SARS and/or MERS, the *in vitro* and *in vivo* results of CQ/HCQ were promising. Therefore, as SARS-CoV 2 shared genetics and pathological similarities with SARS/MERS within the coronavirus families, CQ/HCQ were considered to be initial candidates for drug repurposing in COVID-19.[32–35] We have summarised these *in vitro* and *in vivo* studies in Table 1, and each of these studies is described in brief based on the sequential unfolding of the evidence.

On 4 February 2020, an editorial in Springer Nature® conducted by Wang *et al.* was one of the first to describe the effects of CQ in conjunction with other five antiviral drugs in Vero E6 cells infected with nCoV2019BetaCoV.[35] They performed a standard *in vitro* assay in 2019-nCoV clinical specimens to elucidate the antiviral activity in terms of cytotoxicity, virus yield, and infection rate. Notably, their time-of-addition assay showed that CQ blocked the virus at low concentrations during both entry and post-entry phases of cellular infection. They concluded that CQ has a high prospect in 2019-nCoV as it is a standard drug with known safety profile, is relatively cheap, achieves a wide volume of distribution after oral intake, and can easily attain the EC90 seen in Vero E6 (6.90 μM) with standard 500 mg dose.

On 19 February, a Chinese briefing reported CQ as a successful treatment regimen in greater than 100 patients infected with COVID-19.[53] The patients showed improvements in lung function demonstrated by radiological evidence, viral clearance, and slowing of disease progression. They recommended CQ to be listed in the standard Guidelines for management of COVID-19-associated pneumonia in the National Health Commission, China, based on the successful outcomes in terms of safety and efficacy within the country.

Another *in vitro* study conducted by Yao *et al.*[36] on 9 March 2020, showed that both CQ and HCQ have appropriate antiviral effects with HCQ being more potent than CQ against COVID-19. They tested the antiviral activity of CQ/HCQ in Vero cells infected with SARS-CoV2 through 'physiologically based pharmacokinetics' models with five different HQ dosing regimens simulated *in vitro*. Their results showed that a twice-daily HCQ for 4 days (loading dose: 400 mg and maintenance dose: 200 mg) achieved three times the potency of the standard 500 mg CQ given in advance for 5 days. Following these outcomes, many clinical trials were launched across different health centers in China to evaluate the efficacy of CQ and HCQ in COVID-19.

There is conflicting evidence on CQ/HCQ in COVID-19 regarding the available clinical studies to date. The European Medicines Agency (EMA) published a list of observational studies on CQ/HCQ in COVID-19 patients on 29 May 2020.[54] Although this is not an inclusive list of all the studies conducted so far, it provides an excellent reference to many current studies on COVID-19.

On 6 March 2020, Chen *et al.* reported a randomised control parallel arm equal group pilot study [ClinicalTrials.gov identifier: NCT042 61517] that treated 30 treatment-naive moderate COVID-19 infected patients with 400 mg HCQ once daily for 5 days against the conventional treatment at a public hospital in Shanghai, China.[37] More patients in the control group achieved a higher negative pharyngeal swab of viral nucleic acid at 7 days following the randomisation as the primary outcome (93.3%, $n = 14$ *versus* 86.7%, $n = 13$, $p > 0.05$). While only one patient in the HCQ intervention group progressed to develop a severe infection, the authors suggested probable discrepancies in outcomes to relatively small sample sizes. They recommended more extensive studies to evaluate the conclusive effect of the drug.

On 10 March 2020, Coregiani *et al.* reported a systematic review of CQ's safety and efficacy in COVID-19.[55] After going through six different studies, they could only identify promising preclinical studies but non-conclusive clinical evidence. They recommended that the clinical use of CQ/HCQ must adhere to the Monitored Emergency Use of Unregistered Interventions (MEURI) framework, or the WHO ethical guidelines for the clinical trials, and expressed the urgent need for high-quality clinical trials to provide conclusive evidence.

An open-label non-randomised French trial by Gautret *et al.* on 20 March 2020, consisting of 36

Table 1. Summary of *in vitro* and *in vivo* studies of the use of CQ/HCQ in COVID-19.

Study	Study type	Objective	Outcome	Result/s	Conclusion
Wang *et al.*[35]	*In vitro*	To evaluate antiviral efficiency of ribavirin, penciclovir, nitazoxanide, nafamostat, chloroquine, remdesivir and favipiravir against *in vitro* SARS-CoV 2	Drug efficacy was quantified *via* viral numbers in cell supernatant (qRT-PCR) and viral nucleoprotein expression (immunofluorescence microscopy)	CQ blocked the virus at low concentrations during both entry and post-entry phases of cellular infection	CQ has potential for clinical use against the SARS-CoV 2 due to potent blocking viral infection demonstrating its antiviral effects, however, *in vivo* studies are warranted
Yao *et al.*[36]	*In vitro*	To test CQ/HCQ *in vitro* activity against SARS-CoV 2 infected Vero cells	Drug efficacy was quantified using the detection of viral RNA *via* RT-PCR	A twice-daily HCQ (loading dose of 400 mg and maintenance dose of 200 mg) for four days achieved three times the potency of the standard 500 mg CQ given in advance for five days	HCQ has higher potency than CQ in inhibiting SARS-CoV 2 *in vitro*
Chen *et al.*[37]	Pilot	To evaluate HCQ against COVID-19	Negative SARS-CoV 2 nucleic acid conversion rate *via* respiratory pharyngeal swab	More patients in the control group achieved a higher negative pharyngeal swab of viral nucleic acid at 7 days (93.3%, $n = 14$ *versus* 86.7%, $n = 13$, $p > 0.05$).	Although HCQ showed good prognosis in moderate COVID-19 infection, further investigation is needed with larger sample sizes and better endpoints
Gautret *et al.*[38]	Open-label non-randomised clinical trial	To evaluate HCQ against COVID-19 *via* respiratory viral load	Post-inclusion virus presence and/or absence	Higher viral clearance in the HCQ group (200 mg thrice-daily) compared with the control (70% *versus* 12.5%, $p = 0.001$). HCQ + azithromycin showed 100% viral clearance at the same time, while HCQ only had 57.1% and control group 12.5% ($p < 0.001$)	HCQ resulted in reduced or absent viral load
Molina *et al.*[39]	Prospective study	To assess HCQ + azithromycin antiviral properties against COVID.	Viral detection *via* nasopharyngeal swabs *via* PCR assay	Positive SARS-CoV 2 nucleic acids in 8 of the 10 patients, while one patient died.	No substantial evidence on HCQ + azithromycin in severe COVID-19 patients
Chen *et al.*[40]	Randomised clinical trial	To evaluate HCQ efficacy against COVID-19	Time to clinical recovery, clinical characteristics, radiological results occurring 5 days following study enrollment, or severe adverse effects	Shortened time to clinical recovery in the HCQ group and a higher degree of improvement in pneumonia than the control (80.6% *versus* 54.8%)	HCQ showed significant shortening of time to clinical recovery and promotion of pneumonia absorption

(Continued)

Table 1. (Continued)

Study	Study type	Objective	Outcome	Result/s	Conclusion
Mahévas *et al.*[41]	Clinical trial	To assess the clinical efficacy of oral HCQ (600 mg)	ICU transfer and death from any cause	In total, 20.2% of patients receiving HCQ ended up in the ICU or had died within 1–7 days compared with 22.1% without HCQ (16 *versus* 21 adverse events; RR 0.91; 95% CI: 0.47–1.80)	Results do not support HCQ use in COVID-19 patients as no significant reduction in ICU admission, death, or ARDS occurred
Borba *et al.*[42]	Randomised clinical trial (preliminary Results)	A comprehensive evaluation of CQ safety and efficacy against COVID-9	Mortality by day-28	High fatality rates (27%, 95% CI: 17.9–38.2%) with high dose CQ (600 mg twice daily or 12 g total) than the low dose (450 mg daily or 2.7 g total). More QTc and higher lethality in the high CQ dose (18.9% and 39%)	High CQ dose is not advised for use in COVID-19 due to safety risks
Magagnoli *et al.*[43]	Retrospective Study	To assess HCQ, either alone or in combination with azithromycin, against COVID-19	Hospitalisation (discharge/death), requirement of ventilation, patient status following ventilation in hospitalised patients	Death rate in HCQ, HCQ + azithromycin, and non-HCQ groups were 27.8%, 22.1%, 11.4%, respectively. Significantly high risk of death in HCQ groups (adjusted HR 2.61, 95% CI 1.10–6.17, $p = 0.03$); but it was non-significant HCQ + azithromycin groups (adjusted HR 1.14, 95% CI 0.56–2.32; $p = 0.72$)	No evidence that HCQ (+azithromycin) decreased the risk of mechanical ventilation in COVID-19 hospitalised patients
Saleh *et al.*[44]	Prospective observational safety study	To evaluate CQ, HCQ, and azithromycin in association with QT interval, Torsade de Pointes risk and sudden cardiac death in COVID-19	QT prolongation leading to Torsade de Pointes	No TdP or sudden death due to arrhythmia was noted. These medications did prolong the QT interval but did not lead to the discontinuation of therapy	Although there were no TdP or sudden death due to arrhythmia, more studies are needed for the conclusive results
van den Broek *et al.*[45]	Retrospective Observational cohort safety study	To evaluate CQ-induced CTc prolongation among COVID-19 patients	QTc prolongation *via* ECG recording	Mean QTc prolongation of CQ: 35 ms (95% CI 28–43 ms) with computerised and 34 ms (95% CI 25–43 ms) with manual interpretation, respectively	CQ significantly prolonged QTc interval emphasizing the need for patient monitoring and caution with its use
Mercuro *et al.*[46]	Observational cohort study	To identify risk/degree of QT prolongation by HCQ +/−azithromycin in COVID-19 patients	QT interval change	HCQ + azithromycin had a greater median change in QT interval than HCQ alone (23 msec *versus* 5.5 msec $p = 0.03$)	HCQ use both alone or with azithromycin was associated with an elevated risk of QTc prolongation

(Continued)

Table 1. (Continued)

Study	Study type	Objective	Outcome	Result/s	Conclusion
Jain *et al.*[47]	Safety study	To improve ECG monitoring of COVID-19 patients	QT prolongation *via* ECG monitoring and SBAR tool (situation background assessment recommendation)	SBAR efficiently identified QT prolongation, with 95.1% being related to QT-prolonging medications.	A high percentage of QT prolongation was identified using SBAR leading to earlier intervention
Geleris *et al.*[48]	Observational study	To examine the association between HCQ and risk of intubation/death in COVID-19 patients.	Intubation, dead, or discharge within 24 hrs of ER presentation.	No significant association of HCQ with lowering the risk of intubation or death (HR 1.04; 95% CI 0.82–1.32)	HCQ did not show lowered/increased risk of intubation or death. Further studies are needed on HCQ *via* RCT
Tang *et al.*[49]	Open-label randomised controlled trial	To assess HCQ efficacy and safety profile in COVID-19 patients.	Negative virus conversion	The probability of negative viral conversion was similar in the HCQ (85.4%, 95% CI: 71.8–93.8) and control (81.3%, 95% CI: 71.2–89.6). But, adverse events were higher in HCQ (30% *versus* 8.8%)	HCQ did not increase negative viral conversion; however, higher rates of adverse events were noted with HCQ
Rosenberg *et al.*[50]	Retrospective multicentre cohort study	To describe the association between HCQ, either alone or in combination with azithromucin, and clinical outcome among hospitalised COVID-19 patients	In-hospital mortality	No significant association between HCQ and a reduction or increased mortality rate due to COVID-19 complications (HR 1.08; 95% CI: 0.63–1.85)	The majority of the observed fatalities are attributed to coexisting clinical comorbidities, like hypertension, obesity, diabetes mellitus, liver, or kidney dysfunction
Boulware *et al.*[51]	Double-blind randomised controlled trial	To assess post-exposure prophylaxis with HCQ after SARS-CoV 2 exposure.	Laboratory-confirmed COVID-19, or COVID-19 like illness within 14 days	No significant difference between the HCQ group and placebo (11.8% *versus* 14.3%, absolute difference -2.4 percentage points; 95% CI: –7.0 to 2.2; $p = 0.35$), although a higher side effect profile was observed with HCQ than placebo (40.2% *versus* 16.8%)	HCQ did not prevent COVID-19 or illness compatible with COVID-19 following risk exposure. HCQ is not recommended to be used as a post-exposure prophylaxis in COVID-19.
Randomised Evaluation of COVID-19 therapy (RECOVERY) trial[52]	Open-labeled clinical trial	To evaluate whether treatment with either Lopinavir-Ritonavir, HCQ, Corticosteroids, Azithromycin, Convalescent plasma or Tocilizumab prevents death in COVID-19 patients	All-cause mortality Time - within 28 days following randomisation	No significant difference was observed in the primary outcome of all-cause mortality within 28 days (25.7% *versus* 23.5%, HR 1.11, 95% CI: 0.98–1.26; $p = 0.10$)	No mortality benefit with HCQ in COVID-19 patients, leading to the stoppage of any further participants' enrollment in the HCQ arm.

ARDS, acute respiratory distress syndrome; COVID-19, coronavirus disease 2019; CQ, chloroquine; HCQ, hydroxychloroquine; HR, hazard ratio; ICU, intensive care unit; RR, relative risk; RT-PCR, reverse transcriptase polymerase chain reaction; SARS-CoV 2, severe acute respiratory syndrome coronavirus 2.

patients (26 HCQ groups *versus* 16 control groups), was conducted to investigate HCQ efficacy against COVID-19.[38] Their primary endpoint was a virological clearance, as demonstrated by the negative nasopharyngeal polymerase chain reaction (PCR) results on the sixth day of inclusion. A significantly higher proportion of patients on the HCQ group (200 mg thrice-daily) achieved viral clearance as the primary outcome compared with the control (70% *versus* 12.5%, $p = 0.001$). Similarly, patients treated with HCQ and azithromycin showed 100% viral clearance at the same time as 57.1% in HCQ only group and 12.5% in the control group ($p < 0.001$). The investigators concluded that HCQ, in conjunction with azithromycin, effectively reduces viral load in COVID-19. Although this study showed a promising clinical prospect of HCQ in COVID-19, either alone or in combination with azithromycin, their conclusion is severely limited due to the relatively small sample size.

A prospective study by Molina *et al.* assessed antiviral clearance properties of HCQ and azithromycin in COVID-19 patients following the French trial by Gautret *et al.*[38,39] Their study involved administration of HCQ (600 mg/day for 10 days) and azithromycin (500 mg on day 1 and 250 mg on days 2–5). PCR after the drug regimen showed positive SARS-CoV 2 nucleic acids in 8 of the 10 patients, while 1 patient died. Although this was a relatively smaller study, the results showed a lack of clinical benefit of high dose HCQ plus azithromycin. They contradicted the results observed by Gautret *et al.*, casting suspicion to HCQ's strong antiviral properties in COVID-19.

On 10 April 2020, Chen *et al.* published a parallel-group RCT that evaluated HCQ against COVID-19.[40] Their study incorporated 62 COVID-19 patients, of whom 31 were administered an additional 400 mg/day HCQ for 5 days. They reported a shortened time to clinical recovery in regard to temperature and cough remission in the HCQ group. Also, a greater degree of improvement in pneumonia was noted compared with the control (80.6% *versus* 54.8%). They concluded that HCQ could significantly decrease the clinical recovery time and enhance pneumonia absorption in COVID-19.

On 13 April 2020, a systematic review by Shah *et al.* analyzed the prophylactic importance of CQ/HCQ in COVID-19.[56] They screened 45 articles, of which 5 were chosen, including *in vitro* preclinical research as well as clinical opinion papers. Preclinical studies and clinical opinions demonstrated the role of CQ/HCQ as a prophylactic measure for COVID-19. Meanwhile, there was no original clinical research to show the prophylactic effects of the CQ/HCQ. The authors concluded that the prophylactic use of these pharmaceutical agents still needs to be evaluated carefully in clinical studies due to the lack of clear evidence on prophylaxis and the specific safety concerns.

Furthermore, on 14 April 2020, a target French trial by Mahévas *et al.* comprising 4 French hospitals to evaluate the effectiveness of HCQ in COVID-19 patients requiring oxygen was reported to be prematurely terminated following the unanticipated adverse drug events with high dose HCQ (600 mg daily), in particular, severe pulmonary and cardiogenic side effects.[41] The study recruited 181 patients with COVID-19 induced pneumonia, of which 84 received HCQ while 97 did not. In total, 20.2% of patients receiving HCQ ended up in the ICU or had died within 1–7 days (primary endpoint) compared with 22.1% amongst those not taking HCQ [16 *versus* 21 adverse events; relative risk (RR) 0.91; 95% confidence interval (CI) 0.47–1.80]. The trial concluded that the use of HCQ against COVID-19 with hypoxic pneumonia should be discouraged.

On 16 April 2020, Borba *et al.* reported a study on preliminary safety results on two CQ doses in severe COVID-19 patients.[42] This randomised double-blinded phase IIb trial showed high fatality rates (27%, 95% CI: 17.9–38.2%) with high dose CQ (600 mg twice daily or 12 g total) when compared with the low dose (450 mg daily or 2.7 g total). Furthermore, QTc and higher lethality were observed more in the high CQ dose (18.9% and 39%, respectively). They concluded that higher CQ doses should not be recommended for COVID-19 treatment because of potential safety risks. These adverse events caused the premature halting of further patient enlistment.

A systematic review and meta-analysis published by Sarma *et al.* evaluated the safety and efficacy of HCQ in COVID-19.[57] Seven studies were incorporated into this systematic review, with a total of

1358 participants. Compared with the control groups, HCQ showed reduced radiological progression in lung injury [odds ratio (OR) 0.31, 95% CI 0.11–0.9], while no significant difference was seen in virological cure (OR 2.37, 95% CI 0.13–44.53), death or symptomatic worsening of illness (OR 1.37, 95% CI 1.37–21.97) and safety (OR 2.19, 95% CI 0.59–8.18). They stressed the need for more convincing study and comprehensive data to reach a definitive consensus, despite having promising early results of HCQ and azithromycin in COVID-19.

On 23 April 2020, a retrospective study by Magognoli *et al.* evaluated HCQ, either alone or in combination with azithromycin in COVID-19.[43] The study evaluated 368 veterans in three groups; 97 HCQ, 113 'HCQ plus azithromycin', and 158 without HCQ. The death rate was seen to be 27.8%, 22.1%, 11.4% in the HCQ, 'HCQ plus azithromycin', and non-HCQ group, respectively. The risk of death was observed to be significantly higher in HCQ groups [adjusted hazard ratio (HR) 2.61, 95% CI 1.10–6.17, $p=0.03$]; however, this was not significant in 'HCQ plus azithromycin' groups (adjusted HR 1.14, 95% CI 0.56–2.32; $p=0.72$). They emphasised to exercise caution and patience on awaiting further evidence on the efficacy of the HCQ before the extensive adoption of these therapies in the clinical settings.

On 29 April 2020, Saleh *et al.* conducted a prospective observational safety study to evaluate the effect of CQ, HCQ, and azithromycin in association with QT interval and risk of TdP and sudden cardiac death in COVID-19 patients.[44] In this extensive cohort study to date with CQ/HCQ/azithromycin, no TdP or sudden death due to arrhythmia was noted. These medications did prolong the QT interval but did not lead to the discontinuation of therapy. They concluded that further research is needed to establish drug safety before a definitive recommendation can be made. In addition, a retrospective observational cohort safety study conducted by van den Broek *et al.* on 29 April 2020, investigated CQ-induced QT prolongation in COVID-19 patients.[45] They concluded that CQ leads to QT prolongation and recommended ECG monitoring of all patients taking CQ.

Similarly, on 1 May 2020, Mercuro *et al.* in Boston, Massachusetts, investigated the risk of QT prolongation with the use of HCQ with or without azithromycin used in combination in COVID-19 confirmed-patients.[46] They observed that patients receiving HCQ as a treatment for COVID-19 pneumonia were at significant risk for QT prolongation and that parallel treatment with azithromycin further elevated the risk. They emphasised extreme caution and careful monitoring of the patients with a careful weighing of both risk and benefits before initiation of the treatment with HCQ/azithromycin.

On 2 May 2020, a rapid systematic review consisting of clinical trials carried out by Chowdhury, Rathod and Gernsheimer showed inadequate evidence to encourage the use of CQ/HCQ in COVID-19.[58] They concluded that healthcare professionals should dissuade from the clinical use of CQ/HCQ until the ongoing studies provide more evidence on efficacy and safety profiles. Similarly, on 5 May 2020, Jain *et al.* conducted a safety study to establish an improved ECG monitor system of COVID-19 patients undergoing pharmaceutical treatment associated with a risk of QT prolongation.[47] They created a tool called Situation Background Assessment Recommendation (SBAR) that identifies patients requiring ECG monitoring and tags QT prolongation within the ECGs. They showed that SBAR efficiently identified QT prolongation, with 95.1% being related to QT-prolonging medications.

Furthermore, on 7 May 2020, Geleris *et al.* conducted an observational study to determine an association between HCQ and the need for intubation or risk of death.[48] Based on the observations of 1376 consecutive patients with COVID-19 who received HCQ (600 mg twice daily on initial admission followed by 400 mg daily for 5 days), they conclude that there was no significant association of HCQ with lowering the risk of intubation or death (HR 1.04; 95% CI 0.82–1.32). Geleris *et al.*[48] concluded that HCQ should only be used within clinical trial settings unless its efficacy can be thoroughly tested and established. This study had many potential limitations, including missing data and inaccurate health record-keeping, incomplete documentation on smoking, and comorbidity status on patients as well as a single-centre design, limiting the generalisability of the study data.

An open-labeled randomised controlled trial was published on 7 May 2020, by Tang *et al.*, to assess

the HCQ's efficacy and safety against COVID-19.[49] The trial involved 150 patients; 75 received HCQ along with standard care groups, while other 75 received standard care only. The probability of negative viral conversion was almost similar in the HCQ (85.4%, 95% CI: 71.8–93.8) and the standard of care group (81.3%, 95% CI: 71.2–89.6). But, adverse events were higher with HCQ than with standard of care (30% *versus* 8.8%).

On 11 May 2020, Rosenberg *et al.* reported a retrospective multicentre cohort study involving 1438 patients to evaluate an association between HCQ and COVID-19-induced mortality.[50] They concluded that there was no significant association between HCQ use and a reduction or increased mortality rate due to COVID-19 complications (HR 1.08; 95% CI, 0.63–1.85). The majority of the observed fatalities are attributed to coexisting clinical comorbidities, like hypertension, obesity, diabetes mellitus, liver, or kidney dysfunction.

A systematic review and meta-analysis was published on 12 March 2020, by Singh *et al.* to summarise available evidence of HCQ in COVID-19.[59] Efficacy was measured through quantification by viral clearance *via* reverse transcriptase-polymerase chain reaction (RT-PCR) as well as all-cause mortality. The meta-analysis regarding viral clearance involved three studies with a total of 120 patients. It showed no benefit by HCQ on viral clearance (RR 1.05, 95% CI: 0.79–1.38; $p = 0.74$ and $I2 = 61.7\%$, $p = 0.007$). Similarly, meta-analysis regarding all-cause death also involved three studies, but with a total of 474 patients. It revealed significantly higher rates of death with HCQ than with the control (RR 2.17, 95% CI: 1.32–3.57, $p = 0.002$, $I2 = 0.0\%$, $p = 0.43$). Based on the given results, the authors recommended great caution in HCQ utility until further evidence from clinical trials is available.

On 3 June 2020, Boulware *et al.* reported on an RCT that evaluated HCQ's effect on preventing symptomatic illness following SARS-CoV 2 exposure.[51] In total, 821 positive COVID-19 patients were enrolled. Following 4 days of exposure, participants were allocated randomly to either a placebo group or a treatment group. Participants in the treatment group were given the initial dose of 800 mg HCQ, followed by 600 mg in 6–8 h, and 600 mg daily for 4 days. Primary outcomes were set as confirmed COVID-19 infection or COVID-19-like illness occurring during 14 days post exposure. Post-exposure prophylaxis was not observed with HCQ as there was no significant difference between the HCQ group and placebo (11.8% *versus* 14.3%, absolute difference −2.4 percentage points; 95% CI: −7.0 to 2.2; $p = 0.35$), although a higher side effect profile was observed with HCQ than placebo (40.2% *versus* 16.8%). Boulware *et al.* concluded that HCQ is not effective at preventing COVID-19 infection and does not show the benefits of post-exposure prophylaxis.[51]

Furthermore, on 4 June 2020, Randomised Evaluation of COVID-19 therapy (RECOVERY) Trial,[52] the most significant ongoing open-labeled clinical trial to date in COVID-19 covering 175 hospitals throughout the United Kingdom (UK), published their result of the HCQ arm after a request from the UK Medicines and Healthcare Products Regulatory Agency (MHRA) to the independent Data Monitoring Committee to review the trial. The trial results were published after randomisation of the 4674 patients; 1542 in the HCQ group and 3132 in the control group with usual care. No significant difference was observed in the primary outcome of all-cause mortality within 28 days (25.7% *versus* 23.5%, HR 1.11, 95% CI: 0.98–1.26; $p = 0.10$). The RECOVERY trial concluded no mortality benefit with HCQ in COVID-19 patients, leading to the stoppage of any further participants' enrollment in the HCQ arm.

Safety warning on CQ/HCQ

Apart from a general warning, the FDA has cautioned against the indiscriminate use of CQ/HCQ, either alone or in combination with azithromycin, in COVID-19 patients due to the potential linkage to cardiac toxicities, including severe complications like rhythm disturbances.[60] While the FDA continues to explore these adverse events and will communicate their findings with the public once more information becomes available, the latest COVID-19 NIH Treatment Guidelines Panel recommends 'against using high-dose chloroquine (600 mg twice daily for 10 days) for the treatment of COVID-19 (AI), because the high dose carries a higher risk of toxicities than the lower dose'.[61] Similarly, the EMA has advised the close monitoring of the COVID-19 patients receiving CQ/HCQ.[54]

Recommendation

Current clinical recommendations on CQ/HCQ in COVID-19 are not backed by substantial evidence, and most of the studies are methodologically inferior. In this unique circumstance, we are made to decide between providing medical care or producing reliable and scientifically valid data. This dilemma may lead to compromising the generation of evidence-based and clinically reliable results. In many cases, drugs like CQ and HCQ are being given compassionately due to the severe nature of the disease, despite the discrepancies and safety warnings. As there is insufficient clinical evidence to either refute or accept the use of CQ/HCQ in COVID-19, we would like to advise physicians across the globe to avoid high dose and exercise extreme caution in the compassionate use of CQ/HCQ, particularly in patients with cardiac comorbidities.

Quality of the current evidence

Understandably, there is an overwhelming need to identify plausible treatment options against a devastating and deadly disease like COVID-19. In their quality analysis of the existing studies of CQ/HCQ in COVID-19, Alexander *et al.*, reported that the majority of the existing studies on COVID-19 are general, biased, and methodologically non-rigorous.[62] They concluded that the available clinical studies have many limitations, including sample size, unclear reporting of study methodology, no blinding and/or randomisation, missing clinical data, inconsistencies in treatment *versus* control groups such as groups taken from different healthcare centers, lack of control groups, lack of matching and stratification, inconsistent and low event rates, and an unadjusted analysis. Observational studies can lead to biases, like selection, collider, and confounding bias, that can significantly influence studies outcomes, making them challenging to interpret and/or replicate.[63,64] This is mostly due to the high demand for evidence on ongoing pandemics and uncertainty regarding the virus.

Although we did not perform the quality assessment of the available studies, the majority of the available studies were case-based small studies that are often not controlled with randomisation and/or standardisation. There is no denial that high-quality research, such as RCTs or extensive registry-based observational studies, are still warranted to have conclusive evidence before reaching any consensus. Despite these methodological challenges, it is still up to the research community and clinicians to accurately appraise studies and prioritise the publication of credible evidence.[65]

Conclusion

Given the promising early *in vitro* results of CQ/HCQ in SARS-CoV 2 and existing therapeutic dilemmas, the compassionate use of CQ/HCQ can be an option in the ongoing crisis despite the absence of convincing clinical evidence in COVID-19. However, physicians should avoid high doses of CQ/HCQ and exercise caution, particularly in patients with existing cardiovascular disease. We suggest that clinicians and researchers regularly update and adhere to the available credible evidence and findings of the ongoing clinical trials.

Highlights

- Given the promising early *in vitro* results and therapeutic dilemma, the compassionate use of CQ/HCQ in COVID-19 can be an option.
- However, there is no convincing clinical evidence to support the use of CQ and HCQ, either alone or in combination with azithromycin, in COVID-19.
- Misuse of CQ/HCQ or use beyond the prescription can result in serious health problems, including cardiac toxicity and even death.
- It is recommended to avoid high dose CQ/HCQ and exercise extreme caution while using CQ/HCQ even in hospitalised patients.
- Clinicians and researchers should regularly update and adhere to the available credible evidence and findings of the ongoing clinical trials.

Author Contributions

YA and AS made substantial contributions to the conception and study design, data acquisition and interpretation. All authors were involved in drafting the manuscript as well as the revision process. All authors have approved the final version of the manuscript.

Conflict of interest statement

The authors declare that there is no conflict of interest.

Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

ORCID iD

Yogesh Acharya https://orcid.org/0000-0003-1829-5911

References

1. Liu Y, Gayle AA, Wilder-Smith A, *et al*. The reproductive number of COVID-19 is higher compared to SARS coronavirus. *J Travel Med* 2020; 27: taaa021.
2. Cascella M, Rajnik M, Cuomo A, *et al*. Features, evaluation and treatment coronavirus (COVID-19). In *StatPearls*, Treasure Island, FL: StatPearls Publishing, 2020.
3. Ciliberto G, Mancini R and Paggi MG. Drug repurposing against COVID-19: focus on anticancer agents. *J Exp Clin Cancer Res* 2020; 39: 86.
4. Cortegiani A, Ippolito M, Ingoglia G, *et al*. Chloroquine for COVID-19: rationale, facts, hopes. *Crit Care* 2020; 24: 210.
5. Browning DJ. Pharmacology of chloroquine and hydroxychloroquine. In: *Hydroxychloroquine Chloroquine Retinopathy*. Springer, New York, 2014, pp. 35–63.
6. Zhou D, Dai SM and Tong Q. COVID-19: a recommendation to examine the effect of hydroxychloroquine in preventing infection and progression. *J Antimicrob Chemother* 2020; 75: 1667–1670.
7. Kalil AC. Treating COVID-19-off-label drug use, compassionate use, and randomized clinical trials during pandemics. *JAMA*. Epub ahead of print 24 March 2020. DOI: 10.1001/jama.2020.4742.
8. The Food and Drug Administration. Aralen® chloroquine phosphate, USP: description [Internet], https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/006002s044lbl.pdf (2020, accessed 5 May 2020).
9. Lexicomp Inc. Chloroquine [Internet], http://online.lexi.com (2016, accessed 7 May 2020).
10. Lexicomp Inc. Hydroxyhloroquine [Internet], http://online.lexi.com (2016, accessed 7 May 2020).
11. Law I, Ilett KF, Hackett LP, *et al*. Transfer of chloroquine and desethylchloroquine across the placenta and into milk in Melanesian mothers. *Br J Clin Pharmacol* 2008; 65: 674–679.
12. Kelly JC, Wasserman GS, Bernard WD, *et al*. Chloroquine poisoning in a child. *Ann Emerg Med* 1990; 19: 47–50.
13. Clarke DH. The use of phosphorus 32 in studies on Plasmodium gallinaceum. II. Studies on conditions affecting parasite growth in intact cells and in lysates. *J Exp Med* 1952; 96: 451–463.
14. Schellenberg KA and Coatney GR. The influence of antimalarial drugs on nucleic acid synthesis in Plasmodium gallinaceum and Plasmodium berghei. *Biochem Pharmacol* 1961; 6: 143–152.
15. Mallucci L. Effect of chloroquine on lysosomes and on growth of mouse hepatitis virus (MHV-3). *Virology* 1966; 28: 355–362.
16. Shimizu Y, Yayiamoto S, Homma M, *et al*. Effect of chloroquine on the growth of animal viruses. *Arch Gesamte Virusforsch* 1972; 36: 93–104.
17. Lancz GJ, McLaren LC, James CG, *et al*. Chloroquine mediated alterations in mammalian cell metabolism and viral replication. *Proc Soc Exp Biol Med* 1971; 136: 1289–1293.
18. Banfield WJ and Kisch AL. The effect of chloroquine on herpesvirus infection in vitro and in vivo. *Proc Soc Esp Biol Med* 1973; 142: 1018–1022.
19. Chandramohan M, Vivekananthan SC, Sivakumar D, *et al*. Preliminary report of anti-hepatitis C virus activity of chloroquine and hydroxychloroquine in huh-5-2 cell line. *Indian J Pharm Sci* 2006; 68: 538–540.
20. Pazmiño NH, Yuhas JM and Tennant RW. Inhibition of murine RNA tumor virus replication and oncogenesis by chloroquine. *Int J Cancer* 1974; 14: 379–385.
21. Tsai WP, Nara PL, Kung HF, *et al*. Inhibition of human immunodeficiency virus infectivity by chloroquine. *AIDS Res Hum Retroviruses* 1990; 6: 481–489.
22. Sperber K, Kalb TH, Stecher VJ, *et al*. Inhibition of human immunodeficiency virus type 1 replication by hydroxychloroquine in T cells and monocytes. *AIDS Res Hum Retroviruses* 1993; 9: 91–98.
23. Chiang G, Sassaroli M, Louie M, *et al*. Inhibition of HIV-1 replication by hydroxychloroquine: mechanism of action and comparison with zidovudine. *Clin Ther* 1996; 18: 1080–1092.
24. Boelaert JR, Sperber K and Piette J. Chloroquine exerts an additive in vitro anti-HIV type 1

effect when associated with didanosine and hydroxyurea. *AIDS Res Hum Retroviruses* 1999; 15: 1241–1247.

25. Savarino A, Boelaert JR, Cassone A, *et al*. Effects of chloroquine on viral infections: an old drug against today's diseases? *Lancet Infect Dis* 2003; 3: 722–727.
26. Keyaerts E, Vijgen L, Maes P, *et al*. In vitro inhibition of severe acute respiratory syndrome coronavirus by chloroquine. *Biochem Biophys Res Commun* 2004; 323: 264–268.
27. Vincent MJ, Bergeron E, Benjannet S, *et al*. Chloroquine is a potent inhibitor of SARS coronavirus infection and spread. *Virol J* 2005; 2: 69.
28. Savarino A, Di Trani L, Donatelli I, *et al*. New insights into the antiviral effects of chloroquine. *Lancet Infect Dis* 2006; 6: 67–69.
29. Keyaerts E, Li S, Vijgen L, *et al*. Antiviral activity of chloroquine against human coronavirus OC43 infection in newborn mice. *Antimicrob Agents Chemother* 2009; 53: 3416–3421.
30. Sanders JM, Monogue ML, Jodlowski TZ, *et al*. Pharmacologic treatments for coronavirus disease 2019 (COVID-19): a review. *JAMA*. Epub ahead of print 13 April 2020. DOI: 10.1001/jama.2020.6019.
31. The World Health Organization [Internet], https://www.who.int/ (2020, accessed 27 May 2020).
32. Zhu N, Zhang D, Wang W, *et al*.; China Novel Coronavirus Investigating and Research Team. A novel coronavirus from patients with pneumonia in China, 2019. *N Engl J Med* 2020; 382: 727–733.
33. Liu J, Zheng X, Tong Q, *et al*. Overlapping and discrete aspects of the pathology and pathogenesis of the emerging human pathogenic coronaviruses SARS-CoV, MERS-CoV, and 2019-nCoV. *J Med Virol* 2020; 92: 491–494.
34. Wang H, Li X, Li T, *et al*. The genetic sequence, origin, and diagnosis of SARS-CoV-2. *Eur J Clin Microbiol Infect Dis*. Epub ahead of print 24 April 2020. DOI: 10.1007/s10096-020-03899-4.
35. Wang M, Cao R, Zhang L, *et al*. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. *Cell Res* 2020; 30: 269–271.
36. Yao X, Ye F, Zhang M, *et al*. In vitro antiviral activity and projection of optimized dosing design of hydroxychloroquine for the treatment of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). *Clin Infect Dis*. Epub ahead of print 9 March 2020. DOI: 10.1093/cid/ciaa237.
37. Chen J, Liu D, Liu L, *et al*. A pilot study of hydroxychloroquine in treatment of patients with moderate COVID-19. *J Zhejiang Univ (Med Sci)* 2020; 49: 215–219.
38. Gautret P, Lagier JC, Parola P, *et al*. Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial. *Int J Antimicrob Agents*. Epub ahead of print 20 March 2020. DOI: 10.1016/j.ijantimicag.2020.105949.
39. Molina JM, Delaugerre C, Le Goff J, *et al*. No evidence of rapid antiviral clearance or clinical benefit with the combination of hydroxychloroquine and azithromycin in patients with severe COVID-19 infection. *Med Mal Infect* 2020; 50: 384.
40. Chen Z, Hu J and Zhang Z. Efficacy of hydroxychloroquine in patients with COVID-19: results of a randomized clinical trial. *medRxiv* 2020.03.22.20040758. DOI: 10.1101/2020.03.22.20040758.
41. Mahevas M, Tran VT, Roumier M, *et al*. No evidence of clinical efficacy of hydroxychloroquine in patients hospitalized for COVID-19 infection with oxygen requirement: results of a study using routinely collected data to emulate a target trial. *medRxiv* 2020.04.10.20060699. DOI: 10.1101/2020.04.10.20060699.
42. Borba M, de Almeida Val F, Sampaio VS, *et al*. Chloroquine diphosphate in two different dosages as adjunctive therapy of hospitalized patients with severe respiratory syndrome in the context of coronavirus (SARS-CoV-2) infection: preliminary safety results of a randomized, double-blinded, phase IIb clinical trial (CloroCovid-19 study). *medRxiv* 2020.04.07.20056424. DOI: 10.1101/2020.04.07.20056424.
43. Magagnoli J, Narendran S, Pereira F, *et al*. Outcomes of hydroxychloroquine usage in United States veterans hospitalized with Covid-19. *medRxiv* 2020.04.16.20065920. DOI: 10.1101/2020.04.16.20065920.
44. Saleh M, Gabriels J, Chang D, *et al*. Effect of chloroquine, hydroxychloroquine, and azithromycin on the corrected QT interval in patients with SARS-CoV-2 infection. *Circ Arrhythm Electrophysiol* 2020; 13: e008662.
45. van den Broek MPH, Möhlmann JE, Abeln BGS, *et al*. Chloroquine-induced QTc prolongation in COVID-19 patients. *Neth Heart J*. Epub ahead of

print 29 Apr 2020. DOI: 10.1007/s12471-020-01429-7.

46. Mercuro NJ, Yen CF, Shim DJ, *et al*. Risk of QT interval prolongation associated with use of hydroxychloroquine with or without concomitant azithromycin among hospitalized patients testing positive for coronavirus disease 2019 (COVID-19). *JAMA Cardiol*. Epub ahead of print 1 May 2020. DOI: 10.1001/jamacardio.2020.1834.
47. Jain S, Workman V, Ganeshan R, *et al*. Enhanced electrocardiographic monitoring of patients with coronavirus disease 2019. *Heart Rhythm*. Epub ahead of print 6 May 2020. DOI: 10.1016/j.hrthm.2020.04.047.
48. Geleris J, Sun Y, Platt J, *et al*. Observational study of hydroxychloroquine in hospitalized patients with Covid-19. *N Engl J Med* 2020; 382: 2411–2418.
49. Tang W, Cao Z, Han M, *et al*. Hydroxychloroquine in patients with COVID-19: an open-label, randomized, controlled trial. *medRxiv* 2020.04.10.20060558. DOI: 10.1101/2020.04.10.20060558.
50. Rosenberg ES, Dufort EM, Udo T, *et al*. Association of treatment with hydroxychloroquine or azithromycin with in-hospital mortality in patients with COVID-19 in New York state. *JAMA*. Epub ahead of print 11 May 2020. DOI: 10.1001/jama.2020.8630.
51. Boulware DR, Pullen MF, Bangdiwala AS, *et al*. A randomized trial of hydroxychloroquine as postexposure prophylaxis for Covid-19. *N Engl J Med*. Epub ahead of print 3 June 2020. DOI: 10.1056/NEJMoa2016638.
52. RECOVERY trial [Internet], Recoverytrial.net. https://www.recoverytrial.net/ (2020, accessed 30 May 2020).
53. Gao J, Tian Z and Yang X. Breakthrough: chloroquine phosphate has shown apparent efficacy in treatment of COVID-19 associated pneumonia in clinical studies. *Biosci Trends* 2020; 14: 72–73.
54. European Medicines Agency [Internet], https://www.ema.europa.eu/en (2020, accessed 30 May 2020).
55. Cortegiani A, Ingoglia G, Ippolito M, *et al*. A systematic review on the efficacy and safety of chloroquine for the treatment of COVID-19. *J Crit Care*. Epub ahead of print 10 March 2020. DOI: 10.1016/j.jcrc.2020.03.005.
56. Shah S, Das S, Jain A, *et al*. A systematic review of the prophylactic role of chloroquine and hydroxychloroquine in coronavirus disease-19 (COVID-19). *Int J Rheum Dis* 2020; 23: 613–619.
57. Sarma P, Kaur H, Kumar H, *et al*. Virological and clinical cure in COVID-19 patients treated with hydroxychloroquine: a systematic review and meta-analysis. *J Med Virol* 2020; 92: 776–785.
58. Chowdhury MS, Rathod J and Gernsheimer J. A rapid systematic review of clinical trials utilizing chloroquine and hydroxychloroquine as a treatment for COVID-19. *Acad Emerg Med* 2020; 27: 493–504.
59. Singh AK, Singh A, Singh R, *et al*. Hydroxychloroquine in patients with COVID-19: a systematic review and meta-analysis. *Diabetes Metab Syndr* 2020; 14: 589–596.
60. Food and Drug Administration. FDA cautions use of hydroxychloroquine/chloroquine for COVID-19 [Internet], https://www.fda.gov/drugs/drug-safety-and-availability/fda-cautions-against-use-hydroxychloroquine-or-chloroquine-covid-19-outside-hospital-setting-or (2020, accessed 30 May 2020).
61. National Institute of Health. Coronavirus disease 2019 (COVID-19) treatment guidelines [Internet], https://www.covid19treatmentguidelines.nih.gov/ (2020, accessed 30 May 2020).
62. Alexander PE, Debono VB, Mammen MJ, *et al*. COVID-19 coronavirus research has overall low methodological quality thus far: case in point for chloroquine/hydroxychloroquine. *J Clin Epidemiol* 2020; 123: 120–126.
63. Griffith G, Morris TT, Tudball M, *et al*. Collider bias undermines our understanding of COVID-19 disease risk and severity. *medRxiv* 2020.05.04.20090506. DOI: 10.1101/2020.05.04.20090506.
64. Sterne JAC, Hernán MA, Reeves BC, *et al*. ROBINS-I: a tool for assessing risk of bias in non-randomised studies of interventions. *BMJ* 2016; 355: i4919.
65. Knottnerus JA and Tugwell P. Methodological challenges in studying the COVID-19 pandemic crisis. *J Clin Epidemiol* 2020; 121: A5–A7.

Visit SAGE journals online journals.sagepub.com/home/tai

SAGE journals