REVIEW ARTICLE

Evidence for Chloroquine/Hydroxychloroquine in the Treatment of COVID-19

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Abstract

Introduction: Given the current lack of an approved and effective treatment or vaccine for severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), repositioning old drugs for use as an antiviral treatment is an interesting strategy because knowledge about these drugs' safety profile, posology, and drug interactions is already known. Chloroquine and hydroxychloroquine, widely used as antimalarial and autoimmune disease drugs, have recently been reported as a potential broad-spectrum antiviral drug.

Background: The *in vitro* antiviral activity of chloroquine has been identified since the late 1960s. However, antiviral mechanisms of chloroquine remain speculative. Several clinical trials have been conducted to test the efficacy and safety of chloroquine or hydroxychloroquine in the treatment of COVID-19-associated pneumonia. The quality of the studies and the outcomes are evaluated in this systematic review and meta-analysis.

Review results: Literature review revealed 23 clinical studies. Only 9 of 23 studies were randomized controlled trials. Of nine randomized controlled trials, only study by Skipper et al. was deemed to be at low risk of bias. All studies evaluated variedwith different outcomes. Mechanical ventilation and virological clearance were the only common outcomes evaluated in more than two studies. Virological clearance odds ratio (OR) was 1.25 (95% confidence interval [CI] of 0.57–2.73; $Chi^2 = 0.83$; $I^2 = 0\%$). GRADE quality of evidence was downgraded by three levels to very low due to concerns about the risk of bias, inconsistency, and imprecision. For mechanical ventilation, OR was 1.09 (95% CI 0.80–1.50; $Chi^2 = 0$; $I^2 = 0$). GRADE quality of evidence was downgraded by two levels to low due to concerns about the risk of bias and imprecision. There was no statistically significant difference between the groups for these two outcomes.

Conclusion: As per the available evidence, based on our review, we conclude that hydroxychloroquine/chloroquine has not shown to be beneficial when used for the treatment of patients with COVID-19 pneumonia.

Keywords: Acute hypoxemic respiratory failure (AHRF), Chloroquine, Coronavirus disease 2019, COVID-19 drug treatment, Hydroxychloroquine.

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Background

COVID-19

In December 2019, a new virus, severe acuterespiratory syndrome coronavirus 2 (SARS-CoV-2), emerged in China. Initial cases were reported from people working in the seafood wholesale market in Wuhan, capital city of Hubei province in Central China.[1] The researchers sequenced a novel beta-coronavirus, the genome with 86.9% identity to a previously published bat SARS-like CoV genome (bat-SL-CoVZC45, MG772933).[1] This virus was distinct from human SARS-CoV and Middle East respiratory syndrome coronavirus (MERS–CoV).[2] The World Health Organization (WHO) officially named the disease caused by this virus as coronavirus disease -2019 (COVID-19).[3] COVID-19 caused by SARS-CoV-2 ischaracterized byserious injuries to the lungs. The incubation period is about 14 days. Common presenting features are flu-like illnesses with lower respiratory tract symptoms.

WHO declared COVID-19 outbreak as global pandemic on March 11, 2020. Despite drastic containment measures, the virus is spreading extensively. As of January 3, 2021, the infection was reported from 222 countries globally, 8,25,79,768 patients have been confirmed to have COVID-19, and 18,18,849 of them have died.[4] The experts and researchers have been trying hard to find rapid diagnostic and therapeutic agents to counter the disease.[5]

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Chloroquine/Hydroxychloroquine

Repositioning old drugs for use as an antiviral treatment gained prominence in the beginning of the pandemic because the safety profile, side effects, posology, and drug interactions of these drugs are already known.[6,7] Many agents including Western medicines, natural products, and traditional Chinese medicines have shown potential efficacy against COVID-19.[8] Drugs, such as ribavirin, interferon, lopinavir–ritonavir, and corticosteroids,

have been used in patients with SARS or MERS.[9] Chloroquine is used in the treatment of malaria, rheumatoid arthritis, and lupus erythematosus. Chloroquine and its derivative, hydroxychloroquine, are inexpensive and safe drugs. They have been used for more than 70 years. The commonest side effect reported is eye damage after long-term use.[10] Both chloroquine and hydroxychloroquine have shown broad-spectrum antiviral effects.[11,12]

How does Chloroquine/Hydroxychloroquine Work?

In vitro studies have shown antiviral activity of chloroquine since the late 1960s.[13-15] Growth of many viruses can be inhibited in cell culture by both chloroquine and hydroxychloroquine, including the SARS-CoV.[16] Hydroxychloroquine sulfate was first synthesized in 1946 by introducing a hydroxyl group into chloroquine and this is much less (~40%) toxic than chloroquine in animals.[17] Previous studies have shown that chloroquine has therapeutic activity against many viruses,[18] including human coronavirus OC43 in animal model[19] and SARS-CoV in cell culture studies.[20] But antiviral mechanisms of chloroquine are not clearly confirmed.[21]

Both chloroquine and hydroxychloroquine are weak bases and elevate the pH of acidic intracellular organelles (endosomes/lysosomes) and inhibit pH-dependent viral fusion/replication[22] (Fig. 1). It also interferes with viral envelope glycoprotein and glycosylation of host cellular receptors of SARS-CoV.[12,18,20] In addition, chloroquineinhibits SARS-CoV entry by changing the glycosylation of angiotensin-converting enzyme 2 (ACE2) receptors and spikeproteins.[10] *In vitro* time-of-addition assay

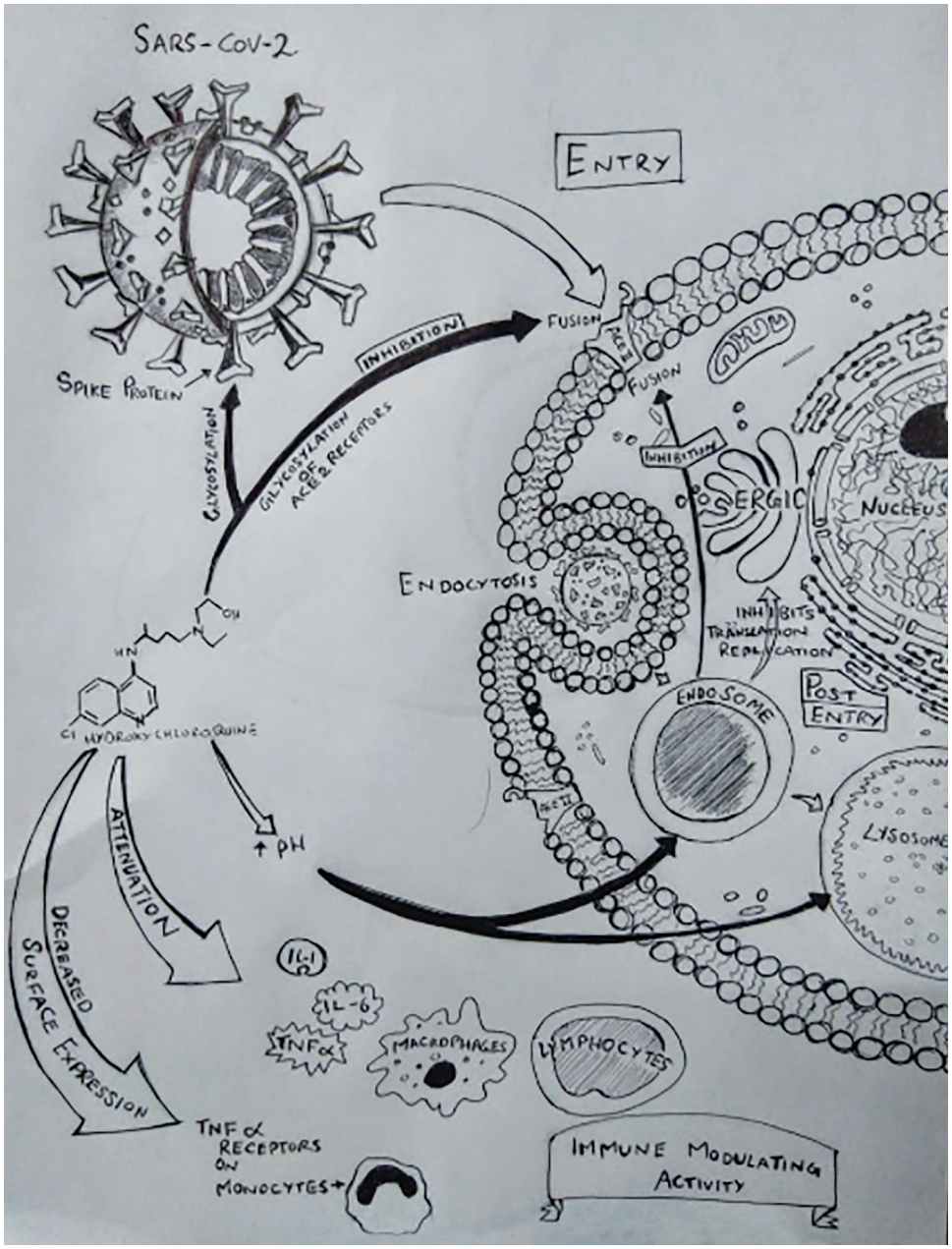


Fig. 1: Mechanism of action

demonstrated that chloroquine effectively inhibits both at entry and at postentry stages of the 2019-nCoV infection in Vero E6 cells.[23,24] In the *in vitro* studies, chloroquine blocked COVID-19 infection at a low-micromolar concentration, with a half-maximal effective concentration (EC50) of 1.13 μM and a half cytotoxic concentration (CC50) greater than 100 Mm and also showed a high selectivity index ([SI] >88.50).[18,25] Chloroquine also inhibits virion assembly in endoplasmic reticulum-Golgi intermediate compartment-like structures. It is possible that chloroquine exhibits host effects, independent of direct viral action, by attenuating the expression of proinflammatory factors and receptors,[18] which induces acute respiratory distress syndrome, the primary reason for coronavirus-associated mortality.[2] This immune-modulating activity of chloroquine possibly enhances its antiviral effect *in vivo* synergistically. After oral administration, chloroquine is widely distributed in the whole body. The EC90 value of chloroquine against the 2019-nCoV in VeroE6 cells was 6.90 μM. This is clinically achievable and demonstrated in the plasma of rheumatoid arthritis patients after receivingdoses of 500 mg/day.[26]

Why is it Important to do this Review?

Several clinical trials have been conducted to test the efficacy and safety of chloroquine or hydroxychloroquine in the treatment of COVID-19-associated pneumonia.Studies consist of various methodologies, designs for control groups (none, different antivirals, placebo, etc.), and varied outcome measures. The final interpretation is therefore technically demanding, and it is difficult to reach any firm conclusion.[27] With this review, we aim to answer the question if there is any benefit of chloroquine/hydroxychloroquine in the treatment of patients with COVID-19.

Aim and Objective

Review of evidence for the benefit of chloroquine/hydroxychloroquine in the treatment of patients with COVID-19.

Materials and Methods

Search Methods for Identification of Studies

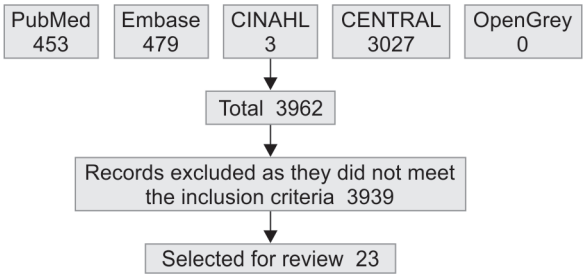
Electronic Searches

We searched the latest issue of the Cochrane Central Register of Controlled Trials (CENTRAL, Issue 1 of 12, January 2021), including Embase, CINAHL,and PubMed. We searched OpenGrey for information on Grey Literature. We used the search terms COVID, coronavirus, hydroxychloroquine, and chloroquine. We limited the time to last 1 year. We did not impose any language restrictions (Flowchart 1).

Searching Other Resources

We screened the reference lists of all eligible trials and relevant reviews.

Flowchart 1: Study flow diagram



Criteria for Considering Studies for this Review

Types of Studies

We included all studies comparing chloroquine/hydroxychloroquine with any other treatment protocols, which do not include chloroquine or hydroxychloroquine in hospitalized patients for the treatment of COVID-19, regardless of language and publication status.

Types of Participants

We included all studies conducted in COVID-19 patients as per author's criteria.

Types of Intervention

The intervention group comprised all participants who were treated with either chloroquine or hydroxychloroquine. The control group included all participants who were treated with any other medications, except chloroquine or hydroxychloroquine.

Types of Outcome Measures

We included all the outcomes reported by the authors as listed below:

- Reduction in all-cause hospital mortality
- Inhibiting the exacerbation of pneumonia
- Improving lung imaging findings
- Promoting virus-negative conversion
- Shortening the disease course
- Reduced need for escalation of respiratory support

Assessment of Risk of Bias in Included Studies

We assessed the risk of bias using the Cochrane "risk of bias" tool. We included only randomized controlled trials (RCTs) for risk of bias assessment. We assumed other methodologies at high risk of bias. Names of the study authors, institutions, journals, and results were not concealed. We judged the quality of studies on the basis of the risk of bias in the following domains:

- Selection bias
  - Random sequence generation
  - Allocation concealment
- Detection bias
  - Blinding of outcome assessors
  - Blinding of personnel
- Attrition bias
  - Incomplete outcome data
- Reporting bias
  - Selective reporting

We classified the studies as low risk, high risk, or unclear risk of bias for the above domains using information available from the studies. Studies were considered low risk of bias if all domains were assessed as adequate (low risk). Studieswereconsidered high risk of bias if one or more domains were assessed as inadequate (high or unclear risk), and as unclear risk if insufficient details of what happened in the study werereported.

We have presented a "risk of bias" table (Fig. 2) and a "risk of bias" graph (Fig. 3).

Measurement of Treatment Effect

We undertook analysis using RevMan 5.4.1 software.

For continuous outcomes, we presented the treatment effect as a mean difference (MD). Effect estimates along with 95% confidence intervals (CI) are presented.

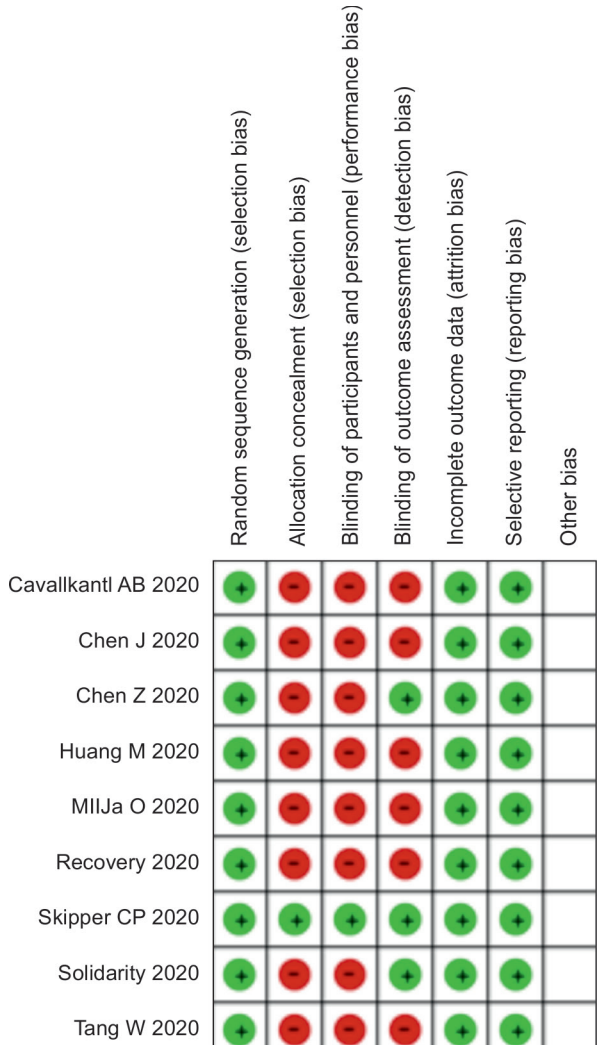


Fig. 2: Risk of bias table

Assessment of Heterogeneity

We had planned not to perform a meta-analysis if we suspected an important clinical heterogeneity on examination of the included studies. We used the chi-squarestatistic to test statistical heterogeneity between studies and considered a $p \leq 0.10$ indicating significant heterogeneity; we used the I^2 statistic to assess the magnitude of heterogeneity.[28] We considered that an I^2 >50% would indicate problematic heterogeneity between studies and, in such cases, we would carefully consider the value of any pooled analyses. To determine the best estimate of the intervention effect, we used a fixed-effect model. We prepared forest plots, summarizing findings from the included studies.

Assessment of Reporting Biases

Comprehensive electronic search was carried out to minimize the effects of publication bias. As we had very few eligible studies, funnel plots of effect estimate against their standard errors (on a reverse scale) to differentiate asymmetry due to publication bias were not created as per the guideline.

Data Synthesis

We used the Cochrane's statistical software (RevMan 5.4.1) for analysis. We expressed risk ratios for proportionsand pooled estimates of MD for continuous variables.

Results are presented in the form of forest plots (Figs 4 and 5).

"Summary of Findings" Table and GRADE

"Summary of findings" table (Table 1) includes a list of all important outcomes, the number of participants and studies addressing each outcome, and a grade for the overall quality of the body of evidence for each outcome. GRADE system is used to assess the quality of body of evidence associated with specific outcomes (virological clearance and mechanical ventilation). Evaluation considerswithin-study risk of bias, directness of the evidence, heterogeneity of data, precision of effect estimates, and risk of publication bias.[29]

Discussions

Summary of Main Results

Literature review revealed 23 clinical studies (Flowchart 1). All the 23 studies are briefly described in Table 2. Only 9 of 23 studies were RCTs. All studies evaluated varied withdifferent outcomes. The outcomes reported in these studies are described in Table 3. Even when the same outcomeswereevaluated, the tool used for evaluating the outcomes wasdifferent (e.g., all-cause 28-day mortality, in-hospital mortality, and survival benefit). Mechanical ventilation and virological clearance were the only common outcomes evaluated in more than two studies. Forest plots of these two outcomes are included in Figures 4 and 5. For virological

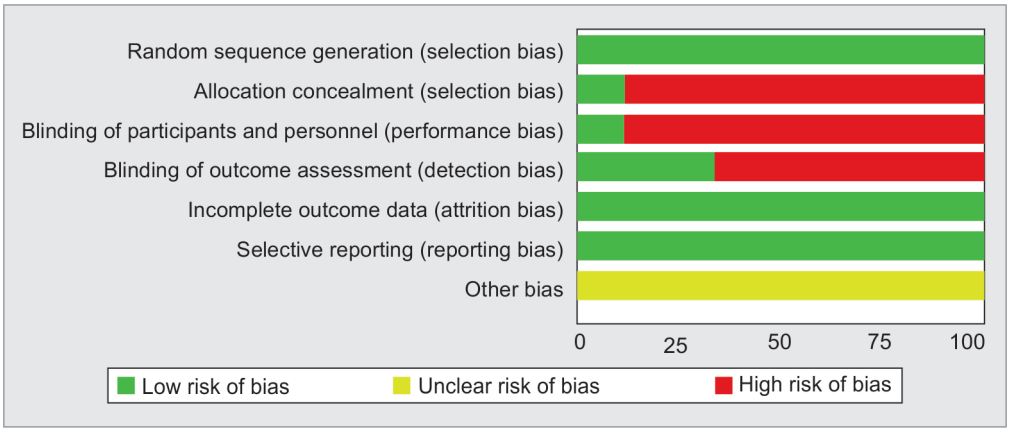


Fig. 3: Risk of bias graph

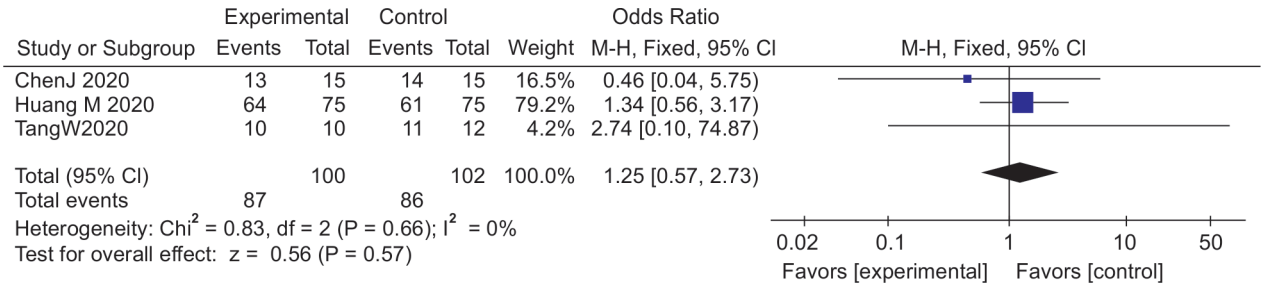


Fig. 4: Virological clearance

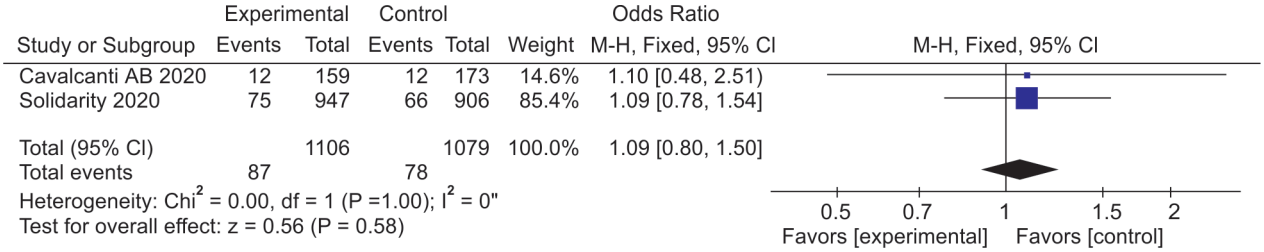


Fig. 5: Forest plot mechanical ventilation

Table 1: GRADE summary of findings

Hydroxychloroquine/chloroquine compared to usual care in patients with COVID-19						
Patient or population: Patients with COVID-19 **Setting**: **Intervention**: Hydroxychloroquine/chloroquine **Comparison**: Usual care						
	Anticipated absolute effects (95% CI)*					
Outcomes	*Risk with usual care*	*Risk with hydroxychloroquine/ chloroquine*	*Relative effect (95% CI)*	*No of participants (studies)*	*Certainty of the evidence (GRADE)*	*Comments*
Virological clearance	843 per 1,000	**870 per 1,000** (754–936)	**OR 1.25** (0.57–2.73)	202 (3 RCTs)	⊕OOO VERY LOW[a,b,c]	
Mechanical ventilation	72 per 1,000	**78 per 1,000** (59–105)	**OR 1.09** (0.80–1.50)	2,185 (2 RCTs)	⊕OOO VERY LOW[d,e]	

*The risk in the intervention group (and its 95% CI) is based on the assumed risk in the comparison group and the relative effect of the intervention (and its 95% CI); CI, confidence interval; OR: odds ratio

GRADE working group grades of evidence
High certainty: We are very confident that the true effect lies close to that of estimate of effect
Moderate certainty: We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of effect, but there is a possibility that it is substantially different
Low certainty: Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of effect
Very low certainty: We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect

Explanations: a. Allocation concealment, blinding of participants, and blinding of outcomes are assessed inadequately in all the three studies; b. Huang M and Tang studies showed benefits, whereas Chen J study showed no benefit; c. Large CI; d. Allocation concealment and blinding of participants are assessed inadequately in both the studies; e. Large CI

clearance, odds ratio (OR) was 1.25 (95% CI of 0.57–2.73; $Chi^2 = 0.83$; $I^2 = 0\%$). GRADE quality of evidence was downgraded to very low due to concerns about the risk of bias, inconsistency, and imprecision. For mechanical ventilation, OR was 1.09 (95% CI 0.80–1.50; $Chi^2 = 0$; $I^2 = 0$). GRADE quality of evidence was downgraded to low due to concerns about the risk of bias and imprecision. There was no statistically significant difference between the groups for these two outcomes.

Quality of Evidence

Of nine RCTs, only study by Skipper et al. was deemed to be at low risk of bias (Figs 2 and 3). However, in the Skipper et al. study, the

Table 2: List of clinical trials on the use of chloroquine or hydroxychloroquine in the treatment of COVID-19

Author	*Country*	*Published*	*Full text available*	*RCT*	*Peer reviewed*	*Number of patients*	*Study arm*	*Control arm*	*Outcome*	*Weakness*
Authors name unknown[6]	China	No	No	No details available	No	>100	Chloroquine	No information available about the care given in the control arm	Chloroquine phosphate is superior to the control treatment in inhibiting the exacerbation of pneumonia, improving lung imaging findings, promoting a virus-negative conversion, and shortening the disease course	Study never published,hence no data available
Chen et al.[30]	China	No	Yes	Yes	No	62	Hydroxy-chloroquine and standard treatment	Standard treatment only	Body temperature recovery time and cough-remission time were significantly shortened and a larger proportion of patients with improved pneumonia in the hydroxychloroquine group	No information is available about how many patients received antivirals and immunoglobulins as part of standard treatment. Clinically, useful outcomes, such as the number of patients requiring ventilation, intensive care unit (ICU) days, ventilator days, and mortality not measured
Chen et al.[3]	China	Yes	Yes	Yes	Yes	30	Hydroxy-chloroquine and conventional treatment	Conventional treatment only	No significant improvement in viral clearance, clinical outcomes, or radiological picture with hydroxychloroquine	Not powered to detect differences in outcomes
Gautret et al.[7]	France	Yes	Yes	No	Yes	36	Hydroxy-chloroquine ± azithromycin	No information available about the care given in the control arm	Viral load reduction/disappearance with hydroxychloroquine and its effect reinforced by azithromycin	Underpowered as per author's calculation. Controls were those patients who met exclusion criteria for inclusion in the study arm or patients from other centers. Six patients with poor outcomes were excluded from the hydroxychloroquine group
Million et al.[31]	France	Yes	Yes	No	Yes	1,061	Hydroxy-chloroquine and azithromycin	No information available about the care given in the control arm	Good clinical outcome, virological cure and lower mortality in hydroxychloroquine group	Observational study. No control arm. Almost all patients had mild disease where the risk of mortality is very low
Molina et al.[32]	France	Yes	Yes	No	Yes	11	Hydroxy-chloroquine and azithromycin	No control arm	No evidence of strongantiviral activity or clinical benefit with thecombination of hydroxychloroquine and azithromycin.	Itis a letter to the editor, complete data not published. Small patient numbers.No control arm and observational nature of the study with its inherent risk of bias

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Author	*Country*	*Published*	*Full text available*	*RCT*	*Peer reviewed*	*Number of patients*	*Study arm*	*Control arm*	*Outcome*	*Weakness*
Gautret et al., second study[33]	France	Yes	Yes	No	Yes	80	Hydroxy-chloroquine and azithromycin	No control arm	Majority (81.3%) of patients had a favorable outcome with hydroxychloroquine and azithromycin and were discharged from the unit with low NEWS scores (93.8%). A rapid fall of nasopharyngeal viral load tested by quantitative polymerase chain reaction (qPCR) was noted, with 83% negative at day 7, and 93% at day 8. The number of patients presumably contagious (with a PCR cycle threshold value <34) steadily decreased overtime and reached zero on day 12	No control arm and observational nature of the study with its inherent risk of bias
Geleris et al.[34]	USA	Yes	Yes	No	Yes	1,376	Hydroxy-chloroquine 600 mg bd for 1 day and 400 mg daily for the next 4 days	No hydroxy-chloroquine	No significant association was found between hydroxychloroquine use and intubation or death	Observational study. Patients in the hydroxychloroquine group were sicker with lower PaO_2: FiO_2 ratio at baseline. Some patients in both groups received remdesivir
Rosenber et al.[35]	USA	Yes	Yes	No	Yes	1,438	Hydroxy-chloroquine, azithromycin, or both	Neither	There was no significant difference in mortality between those receiving hydroxychloroquine, azithromycin or both compared with neither treatment. The risk of cardiac arrest was significantly more in patients receiving hydroxychloroquine and azithromycin together	Observational study. Patients receiving hydroxychloroquine, azithromycin, or both were sicker with higher incidences of diabetes, respiratory rate >22/min, abnormal chest x-ray, oxygen saturation <90%, and aspartate transaminase >410 units/L
Tang et al.[36]	China	Yes	Yes	Yes	Yes	150	Hydroxy-chloroquine at a dose of 1200 mg/day for 3 days followed by 800 mg/day for 2–3 weeks for mild-to-moderate cases, respectively	No hydroxy-chloroquine	Administration of hydroxychloroquine did not result in a significantly higher probability of negative conversion compared to standard care. Adverse events were higher in the hydroxychloroquine group	Unblinded study. No allocation concealment as sequential envelops used. Only two patients with severe disease were included and most patients enrolled late in the disease course (median 15 days)

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Author	*Country*	*Published*	*Full text available*	*RCT*	*Peer reviewed*	*Number of patients*	*Study arm*	*Control arm*	*Outcome*	*Weakness*
Mahevas et al.[37]	France	Yes	Yes	No	Yes	181	Hydroxy-chloroquine	No hydroxy-chloroquine	Administration of hydroxychloroquine was not associated with the reduction of admission to intensive care or death 21 days after hospital admission. Patients (10%) had electrocardiogram changes requiring discontinuation of medicine	Observational study. Antibiotics were given unequally between groups
Magagnoli et al.[38]	USA	No	No	No	No	807	Hydroxy-chloroquine alone and hydroxy-chloroquine + azithromycin	No hydroxy-chloroquine	Hydroxychloroquine with or without azithromycin did not improve mortality or the need for mechanical ventilation	Observational study. Not peer review
Arshad et al.[39]	USA	Yes	Yes	No	Yes	2,541	Hydroxy-chloroquine 400 mg bd for 1 day and 200 mg bd for the next 4 days	No hydroxy-chloroquine	Hydroxychloroquine alone decreased the mortality–hazard ratio by 66% and hydroxychloroquine and azithromycin combination by 71%	Observational study. Corticosteroids and tocilizumab were given to a various number of patients in both groups
Huang et al.	China	Yes	Yes	Yes	Yes	22	Chloroquine	Lopinavir/ ritonavir	All became negative by day 13. Better computed tomography (CT) clearance. All discharged by day 10	Eligibility criteria not defined. A large number of patients excluded. Small patient numbers. No blinding. All patients in the control arm received medication later in the disease course (6.5 days vs 2.5 days), indicating possibly sicker patient group
Mitja et al.	Spain	Yes	Yes	Yes	Yes	293	Hydroxy-chloroquine	Usual care	No difference in reduction of viral load, risk of hospitalization, time to complete resolution	No blinding. Included mainly healthcare workers. Concerns about generalizability to non-healthcare population
Skipper et al.	USA and Canada	Yes	Yes	Yes	Yes	423	Hydroxy-chloroquine	Placebo	No difference in change in symptom severity. More adverse effects	Population was relatively young with most aged 50 years or less, with a few comorbid conditions. Blacks and African-Americans underrepresented. After the commencement of the study, the primary endpoint was changed from the rate of hospitalization and death to the change in overall symptom severity on a 10-point visual analog scale as authors found the rate of hospitalization and death was much lower than expected

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Author	*Country*	*Published*	*Full text available*	*RCT*	*Peer reviewed*	*Number of patients*	*Study arm*	*Control arm*	*Outcome*	*Weakness*
Mercuro et al.	USA	Yes	Yes	No	Yes	90	Hydroxy-chloroquine + azithromycin vs hydroxy-chloroquine alone	No control	Hydroxychloroquine and azithromycin were associated with a greater change in QTc compared to hydroxychloroquine alone	Observational study
Membrillo et al.	Spain	Yes	Yes	No	Yes	164	Hydroxy-chloroquine	No control	Patients (48.8%) not treated with hydroxychloroquine died compared to 22% of patients treated with hydroxychloroquine ($p = 0.002$)	Observational study. Elderly, sicker patients with comorbidities were underrepresented in the intervention group, which may have impacted the outcome
Ip et al.	USA	Yes	Yes	No	Yes	2,512	Hydroxy-chloroquine ± azithromycin	No hydroxy-chloroquine	Hydroxychloroquine not associated with survival benefit	Preprint, not peer reviewed. It is an Observational study. Dosing and timing of hydroxychloroquine varied between hospitals
Paccoud et al.	France	Yes	Yes	No	Yes	84	Standard of care + hydroxy-chloroquine	Standard of care alone	Hydroxychloroquine not associated with a significantly reduced risk of unfavorable outcomes or overall survival	Observational study. Small sample size
Cavalcanti et al.	Brazil	Yes	Yes	Yes	Yes	504	Standard of care + hydroxy-chloroquine/ standard of care + hydroxy-chloroquine + Azithromycin	Standard of care	Prolongation of QTc and elevation of liver enzymes	Some patients may have been exposed to the medications before randomization and some of the patients may have been included relatively later in the course of the disease (up to 14 days after the beginning of the symptoms)
Recovery trial[40]	UK	Yes	Yes	Yes	Yes	4,716	Hydroxy-chloroquine	No hydroxy-chloroquine	No significant difference in 28-day mortality or duration of hospital stay	Study stopped abruptly after interim analysis revealed no benefit
Solidarity trial	Multi-national	Yes	Yes	Yes	Yes	1,853	Standard care + hydroxy-chloroquine	Standard care alone	Hydroxychloroquine produced no reduction in in-hospital mortality. There were no differences in initiation of ventilation or death due to cardiac causes in both groups	Study was stopped after interim analysis. Nonblinded

Table 3: Reported outcomes in various studies

Author	*Type of study*	*Outcome*	*Results*
Recovery	RCT	All-cause 28-day mortality	27% hydroxychloroquine group vs 25% usual care; rate ratio 1.09, 95% CI 0.97–1.23, $p = 0.15$
Solidarity	RCT	In-hospital mortality	Death rate 1.19, 0.89–1.59, $p = 0.23$
Million	Observational study	Overall case fatality rate	Overall case fatality rate was 0.9%
Geleris	Observational study	Composite of intubation and death	Hazard ratio (HR) 1.04, 95% CI 0.82–1.32
Rosenberg	Observational study	In-hospital mortality	The probability of death for patients receiving hydroxychloroquine + azithromycin was 25.7%, 95% CI 22.3–28.9%; hydroxychloroquine alone 19.9%, 95% CI 15.2–24.7%; and neither drug 12.7%, 95% CI 8.3–17.1%
Mahvesa	Observational study	Death at 21 days	Overall survival at day 21 was 89% in the treatment group and 91% in the control group (HR 1.2, 0.4–3.3)
Arshad	Observational study	Mortality–hazard ratio	Crude mortality was 13.5% with hydroxychloroquine alone, 20% with hydroxychloroquine and azithromycin together, and 26.45% with neither of the medications (p <0.001)
Magagnoli	Observational study	Mortality	Hydroxychloroquine with or without azithromycin compared to neither did not improve mortality (19.2, 22.2 and 9.4%, p <0.001)
Membrillo	Observational study	Death	Patients (48.8%)not treated with hydroxychloroquine died compared to 22% of patients treated with hydroxychloroquine ($p = 0.002$)
Ip A	Observational study	Survival benefit	Use of hydroxychloroquine with or without cotreatment with azithromycin was not associated with a reduction in mortality (adjusted HR, 0.99, 95% CI 0.80–1.22. Unadjusted 30-day mortality for patients receiving hydroxychloroquine alone, azithromycin alone, the combination, or neither drug was 25, 20, 18, and 20%, respectively
Paccoud	Observational study	Overall survival	Overall survival was not significantly different between the two groups (HR 0.89 [0.23–3.47], $p = 1$)
Unknown authors	Unknown	Pneumonia resolution	No data available
Chen	RCT	Pneumonia resolution	A larger proportion of patients with improved pneumonia in the hydroxychloroquine group (80.6%, 25 of 31) compared to control group (54.8%, 17 of 31)
Unknown authors	Unknown	Radiological clearance	No data available
Chen	RCT	Radiological clearance	Radiological progression was shown on CT images in five cases (33.3%) of the hydroxychloroquine group and seven cases (46.7%) of the control group
Huang	RCT	Radiological clearance	By day 9, 60% of patients in the chloroquine group reached lung clearance, compared to 25% from the lopinavir/ritonavir group. By day 14, the incidence rate of lung improvement based on CT imaging from the chloroquine group was more than doubled to that of the lopinavir/ritonavir group (rate ratio 2.21, 95% CI 0.81–6.62)
Unknown authors	Unknown	Viral clearance	No data available
Chen J	RCT	Viral clearance	COVID-19 nucleic acid of throat swabs was negative on day 7 in 86.7% of cases in the hydroxychloroquine group and 93.3% of the control group (p >0.05)
Gautret	Observational study	Viral clearance	At day 6, 70% of hydroxychloroquine-treated patients was virologically cured, compared to 12.5% in the control group ($p = 0.001$). Patients (100%) treated with hydroxychloroquine and azithromycin combination were virologically cured, compared to 57.1% of patients treated with only hydroxychloroquine (p <0.001)
Million	Observational study	Viral clearance	A good clinical outcome and virological cure was obtained in 973 patients within 10 days (91.7%)
Gautret (second study)	Observational study	Viral clearance	A rapid fall of nasopharyngeal viral load tested by PCR was noted, with 83% negative at day 7 and 93% at day 8
Tang	RCT	Viral clearance	Probability of negative conversion by 28 days in the standard of care plus hydroxychloroquine group was 85.4% (95% CI 73.8–93.8%), similar to that of in the standard-of-care group (81.3%, 71.2–89.6%)
Huang	RCT	Viral clearance	All patients on chloroquine became negative on day 13, compared to lopinavir/ritonavir group, where 11 of 12 turned negative at day 14

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Author	*Type of study*	*Outcome*	*Results*
Mitja	RCT	Viral clearance	No significant differences were found in the mean reduction of viral load at day 3 (−1.41 vs −1.41 Log10 copies/mL in the control and intervention arm, respectively; difference of 0.01 [95% CI −0.28;0.29]) or at day 7 (−3.37 vs −3.44; d −0.07 [−0.44;0.29])
Molina	Observational study	Viral clearance	Nasopharyngeal swabs using a qualitative PCR assay were still positive for SARS-CoV-2 RNA in 8 of 10 patients (80%, 95% CI: 49–94) at days 5–6 after treatment initiation
Solidarity	RCT	Mechanical ventilation	No differences in initiation of ventilation (75 vs 66)
Magagnoli	Observational study	Mechanical ventilation	Risk of ventilation was similar in the hydroxychloroquine group (adjusted HR 1.43, 95% CI 0.53–3.79, $p = 0.48$) and in the hydroxychloroquine and azithromycin group (adjusted HR 0.43, 95% CI 0.16–1.12, $p = 0.09$) compared to the no hydroxychloroquine group
Geleris	Observational study	Composite of intubation and death	HR 1.04, 95% CI 0.82–1.32
Cavalcanti	RCT	Mechanical ventilation	Patients (11%) in the hydroxychloroquine + azithromycin group, 7.5% in hydroxychloroquine alone group, and 6.9% in the control group received mechanical ventilation during the first 15 days. Effect estimate with 95% CI was 1.77 (0.81–3.87) for hydroxychloroquine + azithromycin vs control and 1.15 (0.49–2.70) for hydroxychloroquine vs control
Mercuro	Observational study	QTc prolongation	19% of patients who received hydroxychloroquine monotherapy developed prolonged QTc of 500 ms or more, and 3% of patients had a change in QTc of 60 ms or more. Of those who received concomitant azithromycin, 21% had prolonged QTc of 500 ms or more and 13% had a change in QTc of 60 ms or more
Cavalcanti	RCT	QTc prolongation	QTc duration more than 480 ms was seen in 14.7% of patients in the hydroxychloroquine + azithromycin group, 14.6% of patients in the hydroxychloroquine group, and 1.7% of patients who received neither of the medications
Solidarity	RCT	Death during 14 days with any cardiac cause	No difference (4 vs 2)
Ip A	Observational study	Death due to cardiac causes	No difference (21 vs 16%)

primary outcome was changed from the rate of hospitalization and death to the change in overall symptom severity on a 10-point visual analog scale. In this study, hydroxychloroquine did not significantly reduce symptom severity in early, mild COVID-19. This outcome was not analyzed in any other studies, making it difficult to compare the outcomes. Recovery and Solidarity trials are the two biggest studies. Both Recovery and Solidarity studies were stopped after interim analysis and outcome assessment wereunblinded.

Conclusion

Our analysis found 23 studies. Only nine were RCTs. Only one study was deemed as low risk of bias. Mechanical ventilationand virological clearance were the only common outcomes evaluated by more than two RCTs. There was no statistically significant difference between those who received hydroxychloroquine/chloroquine and who did not for these outcomes. It is not possible to comment on other outcomes and adverse effects of hydroxychloroquine/chloroquine as they are not reported uniformly. As per the available evidence, based on our review, we conclude that hydroxychloroquine/chloroquine has not shown to be beneficial when used for the treatment of patients with COVID-19 pneumonia.

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