Future VIROLOGY

Meta-Analysis

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Efficacy of chloroquine and hydroxychloroquine for the treatment of hospitalized COVID-19 patients: a meta-analysis

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Aims: To evaluate the efficacy and safety of hydroxychloroquine/chloroquine, with or without azithromycin, in treating hospitalized COVID-19 patients. **Materials & methods:** Data from randomized and observational studies were included in a random-effects meta-analysis. Primary outcomes included time to negative conversion of SARS-CoV-2 tests, length of stay, mortality, incidence of mechanical ventilation, time to normalization of body temperature, incidence of adverse events and incidence of QT prolongations. **Results:** Fifty-one studies (n = 61,221) were included. Hydroxychloroquine/chloroquine showed no efficacy in all primary efficacy outcomes, but was associated with increased odds of QT prolongations. **Conclusion:** Due to a lack of efficacy and increased odds of cardiac adverse events, hydroxychloroquine/chloroquine should not be used for treating hospitalized COVID-19 patients.

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Keywords: azithromycin • chloroquine • COVID-19 • hydroxychloroquine • length of stay • mechanical ventilation • mortality • QT prolongations • SARS-CoV-2

SARS-CoV-2, the etiological agent behind COVID-19, was first identified in Wuhan, China in December 2019 [1]. Shortly after in March 2020, the WHO designated COVID-19 as a global pandemic [2]. Due to the alarmingly high basic reproduction number [3], hospitalization rate [4] and mortality rate [5], numerous collaborative studies were conducted to identify the optimal treatment strategy for managing the disease. Currently, the most advisable option for combating the pandemic are vaccines, which can slow the transmission of SARS-CoV-2 [6] and prevent the onset of severe COVID-19 [7]. However, the widespread adoption of SARS-CoV-2 vaccines is hampered by vaccine hesitancy, as well as manufacturing and distribution obstacles, especially in underserved communities and low- to middle-income countries (LMICs) [8–11]. Evidently, there is still a need for effective COVID-19 treatment strategies despite clinical successes from the leading COVID-19 vaccine candidates.

Research efforts surrounding COVID-19 treatment during the early stages of the pandemic primarily focused on repurposing established antiviral and immunoregulatory drugs with known pharmacokinetic and pharmacodynamic profiles which dramatically reduced the cost and length of drug development [12]. The repurposing of chloroquine and its analog hydroxychloroquine, which are antimalarial drugs with anti-inflammatory properties commonly

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used to treat autoimmune conditions, was first proposed as an effective treatment strategy for COVID-19 based on strong *in vitro* activity against SARS-CoV and SARS-CoV-2 [13–15].

Chloroquine became widely used as an anti-inflammatory medication among patients with autoimmune diseases due to its ability to suppress toll-like receptor (TLR) signaling by increasing cellular endosomal pH. Hydroxychloroquine is a more commonly used analog of chloroquine with reduced toxicity [16]. It is speculated that the suppressive mechanism of chloroquine and hydroxychloroquine on TLR signaling is also involved in their *in vitro* activity against SARS-CoV-2, as the increased endosomal pH level may interfere with the function of host enzymes needed for the post-transcriptional modification of viral envelope glycoproteins [17]. This hypothesis is supported by a study conducted by Zhao *et al.*, which found that endosomal proteases, such as cathepsin L, play a critical role in the cleavage of SARS-CoV-2 spike proteins which are needed to facilitate viral entry [18]. In addition, chloroquine and hydroxychloroquine are also found to reduce sialic acid synthesis by inhibiting quinine reductase-2 [17]. As sialic acid is commonly used by airborne viruses as receptors for entry into the respiratory tract [19–21], a reduction in sialic acid synthesis may help protect the respiratory system against SARS-CoV-2 infections.

In addition to *in vitro* evidence, a multicenter, non-randomized clinical trial involving over 100 COVID-19 patients was conducted by Gao *et al.*, which found that chloroquine alleviated symptoms of pneumonia, improved radiographic findings and shortened the course of the disease [22]. However, Gao *et al.* did not publish any data supporting these claims [23]. Early observational evidence was quickly followed by a meta-analysis by Million *et al.* [24], which demonstrated more encouraging results. Regrettably, this meta-analysis was heavily criticized for its flawed methodologies [25]. The first randomized controlled trial (RCT) involving chloroquine compounds was purportedly conducted in China and disseminated to the preprint server medRxiv [26]. It reported efficacious results in terms of time to clinical recovery and radiological findings when comparing hydroxychloroquine to control, although the disseminated data were never peer-reviewed nor published in a medical journal. Despite various shortcomings associated with the quality of early clinical data, hydroxychloroquine was heavily endorsed by news media and government leaders [27], causing an increased demand for the drug and consequently leading to worldwide shortages for lupus and arthritis patients who rely on the medication for disease management [28,29].

Contrary to the optimistic findings from *in vitro* and early clinical studies, recent large-scale peer-reviewed RCTs, such as the RECOVERY [30] and TEACH trial [31], have shown that hydroxychloroquine is not effective in reducing mortality, incidence of severe outcomes, nor in improving patients' clinical scores. These conclusions are corroborated by subsequent meta-analyses of RCTs and observational studies [32,33]. However, these meta-analyses did not include all available studies in their analysis, nor did they study the correlation between patient outcomes and covariate factors such as cumulative chloroquine doses or time from symptom onset to drug administration/randomization. Additionally, these reviews did not evaluate incidences of QT prolongations, which is a commonly reported adverse event among COVID-19 patients being treated with chloroquine/hydroxychloroquine.

While the extensivity of the pandemic is coming under control in high-income countries with widely available vaccination programs, chloroquine and hydroxychloroquine may seem like an inexpensive and accessible treatment strategy for combating COVID-19 in LMICs or areas where vaccine shortages persist [34]. Considering that there have been multiple observational and randomized studies published since previous systematic reviews, we believe that an updated meta-analysis is needed to provide a more accurate summary of clinical evidence regarding the role of chloroquine and hydroxychloroquine in treating COVID-19 patients. This systematic review and meta-analysis assessed whether chloroquine and hydroxychloroquine, with or without azithromycin, is more beneficial compared with standard of care or adjuvant therapies alone with regards to time to negative conversion of SARS-CoV-2 tests, length of stay, mortality, incidence of mechanical ventilation, time to fever resolution and incidence of adverse events in hospitalized COVID-19 patients. We also assessed the impact of chloroquine/hydroxychloroquine treatment on the incidence of severe adverse events and incidence of QT prolongations.

Methods

We conducted this systematic review and meta-analysis following recommendations from the Cochrane Handbook for Systematic Reviews of Interventions [35] and in accordance with the latest PRISMA statements [36]. See online Supplementary Table 1 for the completed PRISMA 2020 checklist. This review was prospectively registered on PROSPERO (CRD42021233110), the international prospective register of systematic reviews [37].

Study identification

We searched the following databases from 1 January 2020 to 26 April 2021 using English search strategies consisting of relevant keywords such as 'hydroxychloroquine', 'chloroquine', and 'Plaquenil' in combination with database-specific COVID-19 search strings provided by the Rudolph Matas Library of the Health Sciences of Tulane University [38]: Medical Literature Analysis and Retrieval System Online (MEDLINE), Excerpta Medica Database (EMBASE) and PubMed. Additionally, we systematically searched the following Chinese databases from 1 January 2020 to 26 April 2021 using a custom Chinese search strategy: Wanfang Data, Wanfang Med Online, SinoMed, China National Knowledge Infrastructure (CNKI) and Chongqing VIP Information (CQVIP). The search strategies used for the database searches can be found in online Supplementary Tables 2–9.

Due to potential biased or problematic COVID-19 studies being published on preprint repositories [39–41], especially preprint articles concerning hydroxychloroquine, we limited our literature search to peer-reviewed sources only. We also hand-searched the reference sections of four previous systematic reviews [32,33,42,43] for relevant studies that were not identified by our database searches.

Eligibility criteria

We included both randomized and non-randomized comparative studies that met the following inclusion criteria in our review: compared hydroxychloroquine or chloroquine as a monotherapy or in combination with azithromycin with standard of care, or compared hydroxychloroquine or chloroquine with adjuvant therapies to adjuvant therapies alone, and included hospitalized COVID-19 patients. For studies involving hydroxychloroquine with adjuvant therapies, we only included studies that used the same adjunctive therapy for both its intervention and control arms to minimize the effect of adjuvant therapies on treatment outcomes, similar to the design of several other meta-analyses [44–46].

Outcome measures

Our primary efficacy outcomes included: time to negative conversion of SARS-CoV-2 tests, length of stay, mortality at the latest follow up, incidence of mechanical ventilation and time to normalization of body temperature. Our primary safety outcome was incidence of adverse events. Our secondary efficacy and safety outcomes included: rate of negative conversion of SARS-CoV-2 test at day 7 and day 14 and incidence of severe (or grade 3/4) adverse events. Due to multiple preliminary reports describing high incidences of QT prolongation in COVID-19 patients taking hydroxychloroquine [47–62], we added incidence of QT prolongation as a *post hoc* safety outcome as well.

Study selection

We performed independent and in-duplicate title and abstract screening using Rayyan (https://www.rayyan.ai/) [63] based on the aforementioned eligibility criteria. Abstract entries that were deemed eligible were then entered into an independent and in-duplicate full-text screening process. During study selection, disagreements on inclusion decisions were resolved by recruiting a senior author to attain consensus. Figure 1 shows the PRISMA flowchart [64] of our study selection process.

Data extraction

We performed data extraction independently and in-duplicate using an extraction sheet developed *a priori*. Information relating to baseline demographics, descriptions of study methodology, treatment descriptions and outcome measures were extracted. The full list of extracted items can be found on our PROSPERO registration page. For observational studies that reported data from both matched and unmatched cohorts, we only extracted and analyzed data from the matched cohort.

Risk of bias assessment

We assessed the risk of bias of RCTs using the revised Cochrane risk of bias tool for randomized trials (RoB2) [65]. The risk of bias of non-randomized comparative studies was assessed using the risk of bias in non-randomized studies of interventions (ROBINS-I) tool [66]. All risk of bias assessments were conducted independently and in duplicate. Disagreements were resolved by consultation with a senior author to attain consensus.

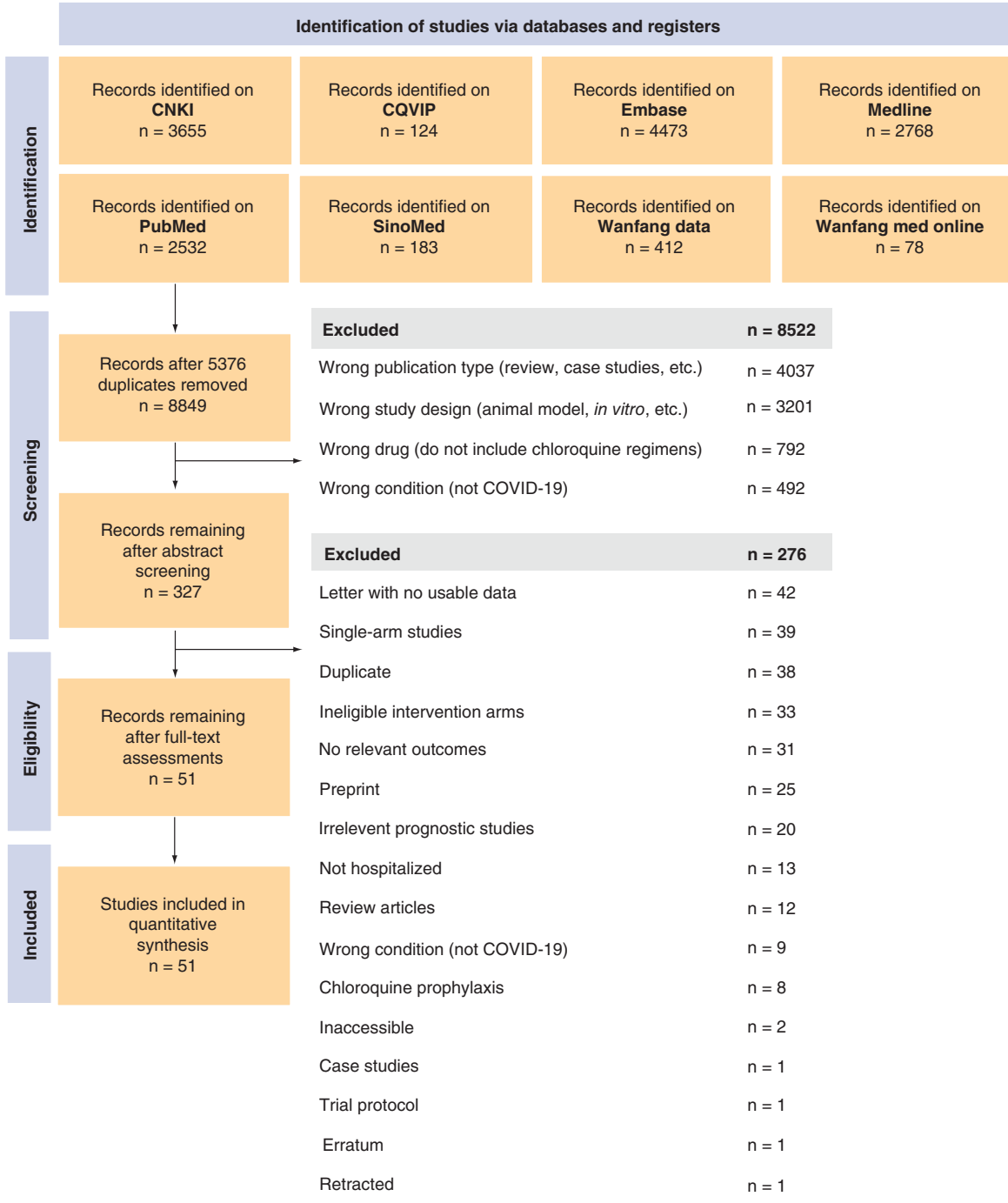

Figure 1. PRISMA flowchart for the identification and selection of studies.
CNKI: China National Knowledge Infrastructure; CQVIP: Chongqing VIP Information; EMBASE: Excerpta Medica Database; MEDLINE: Medical Literature Analysis and Retrieval System Online.

Quality of evidence

We assessed the quality of evidence for our primary outcomes using the Grading of Recommendations, Assessment, Development and Evaluations (GRADE) framework [67,68]. The GRADE approach evaluates the quality of evidence by assessing the following domains: study limitations (risk of bias), indirectness, inconsistency, imprecision and publication bias. The GRADE rating may also be upgraded due to magnitude of effects, dose-response gradients and plausible confounders. The overall quality of evidence for each outcome was rated as either high, moderate, low,

or very low, and the results of the primary outcomes and GRADE ratings were presented in a GRADE summary of findings table generated using GRADEpro (https://gradepro.org/).

Statistical analysis

We conducted all statistical analyses using R 3.6.3 (https://www.r-project.org/) [69], and we performed random-effects meta-analyses using the meta 4.18 library (https://cran.r-project.org/web/packages/meta/) [70]. We expressed and pooled the treatment effects of dichotomous outcomes as odds ratios (ORs) and 95% CIs. The treatment effect of continuous outcomes were expressed as mean differences (MDs) and 95% CIs. Results from individual studies and data synthesis were displayed in forest plots.

Missing data & rare events

For studies with missing data required for analysis, including the mean outcome value and measures of variance for continuous outcomes, attempts were made to contact the corresponding authors to obtain unpublished data. For studies that presented continuous outcomes in median and interquartile range (IQR), we detected the skewness of the data using methods proposed by Shi *et al.* [71], and used methods recommended by Luo *et al.* [72] and Wan *et al.* [73] to estimate the mean and standard deviation (SD) for data pooling if there was no significant skewness. We did not include skewed median and IQR values in the meta-analysis and only described these results narratively. We tested the impact of this assumption by conducting a subgroup analysis comparing the pooled results from studies with estimated mean and SD to studies that did not require estimation.

For studies reporting zero events in one or both of its treatment arms, we applied a continuity correction factor of 0.5 [74] to complete the meta-analysis.

Heterogeneity assessment

We examined the presence of heterogeneity using the Cochran's Q test [75] with a significance level of $p < 0.10$, as recommended by the Cochrane Handbook [35]. Heterogeneity was subsequently quantified using I^2 statistics [75,76]. We interpreted $30\% < I^2 < 75\%$ as moderate heterogeneity and $I^2 \geq 75\%$ as serious heterogeneity, following recommendations from the Cochrane Handbook [35].

Publication bias

We drew funnel plots [35] to identify small study effects within our meta-analyses as an indication for publication bias. Egger's regression test was used to quantitatively evaluate the presence of asymmetry within the funnel plots [77]. We did not perform Egger's test if fewer than ten studies were included in the analysis, as the test may lack power in these circumstances [78].

If the funnel plot revealed potential publication biases, we used the trim-and-fill method [79,80] to estimate the number of missing, unpublished studies and to observe the impact of unpublished studies on the pooled treatment effect.

Meta-regression & subgroup analysis

We performed meta-regression analyses on the proportion of patients with severe disease (defined as per individual study criteria), cumulative chloroquine QTbase dose (calculated based on conversion formula derived by Schlossberg & Samuel [81]), and follow-up duration (for the outcome of mortality only). As previous investigations have revealed that early administration of chloroquine compounds may yield better outcomes [82,83], we also performed a meta-regression analysis on the time from COVID-19 symptom onset to drug administration/randomization. We performed the following subgroup analyses based on factors defined *a priori*: study design (randomized design vs observational design), and risk of bias rating (low/moderate/some concerns rated studies vs high/serious/critical rated studies, similar to the subgroup analysis conducted in a previous study [84]). Additionally, we also performed subgroup analyses separating different treatment regimens into separate subgroups.

Results

Included studies

We identified and screened 8849 (after deduplication using Endnote 20 [https://endnote.com/]) potentially eligible titles and abstracts. A total of 327 full texts were subsequently retrieved and screened. Ultimately, 9 RCTs [30,31,85–91] and 42 non-randomized observational studies [92–134] were included in this systematic review and meta-analysis

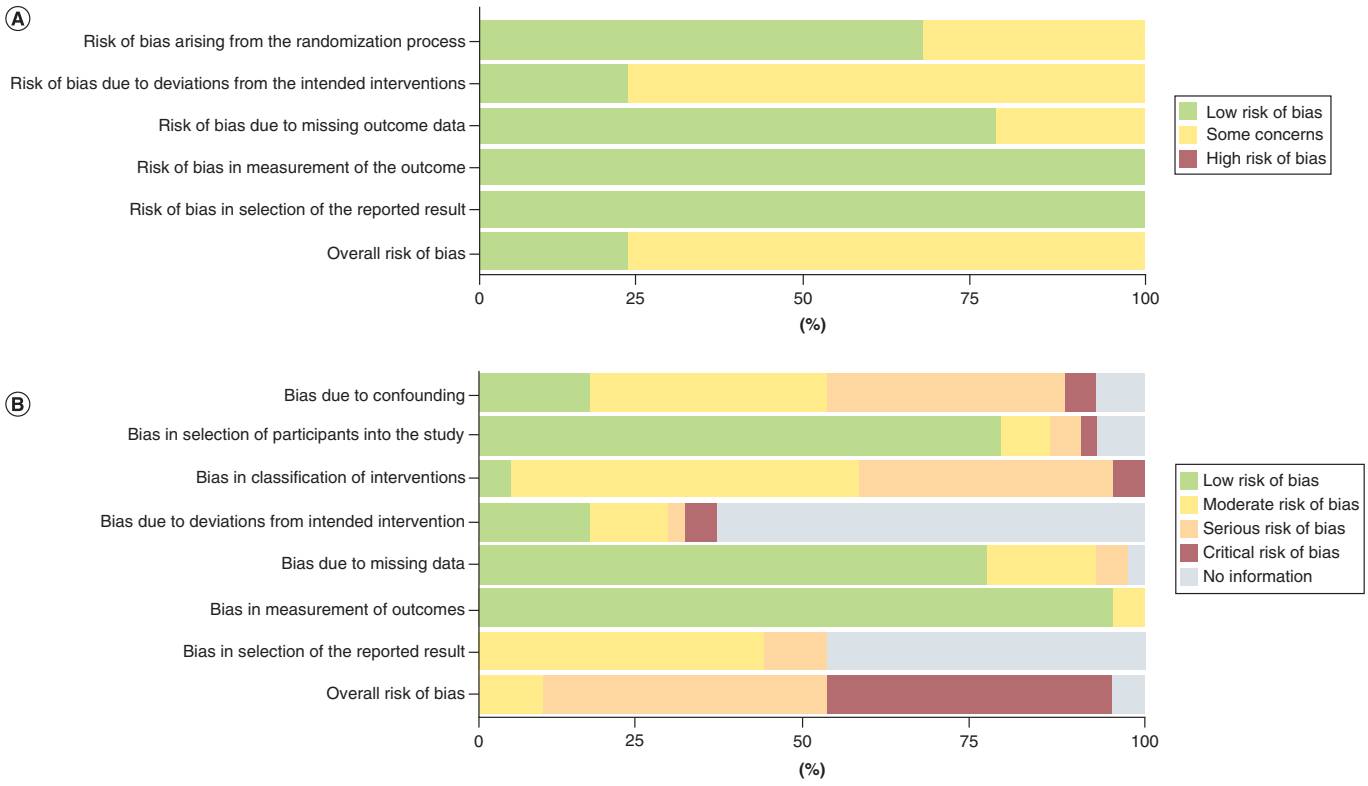

Figure 2. Risk of bias. (A) Percentage of studies with risk of bias ratings for randomized controlled trials using RoB2. **(B)** Percentage of studies with risk of bias ratings for observational studies using ROBINS-I.
RoB2: Revised Cochrane risk of bias tool for randomized trial; ROBINS-I: Risk of bias in non-randomized study of intervention.

(Figure 1) with a total of 61,221 hospitalized COVID-19 patients. Characteristics of the included studies are tabulated in Supplementary Table 10.

Risk of bias

The risk of bias in our included RCTs were evaluated using RoB2 (Figure 2A & Supplementary Table 11). Seven RCTs (78%) [30,85–89,91] were rated as having some concerns in regards to their risk of bias, primarily due to concerns regarding inadequate reporting of randomization and allocation concealment methodologies and the usage of an open-label study design. The remaining two RCTs (22%) [31,90] were rated as having a low risk of bias.

The risk of bias in observational studies was evaluated using ROBINS-I (Figure 2B & Supplementary Table 12). Eighteen studies (43%) [95,98,101,109,115,116,119,120,122,123,125–127,129–134] were rated as having a critical risk of bias, while another 18 studies (43%) [92–94,97,99,102–105,107,110–114,117,118,121] were rated as having a serious risk of bias. In general, studies were rated as having a serious or critical risk of bias due to confounding factors, poor descriptions of the regimen/interventions used, inadequate descriptions of treatment deviations and compliance and a lack of information in regards to *a priori* developed analysis plans and study protocols. Four studies (10%) [96,100,106,108] were rated as having a moderate risk of bias, and two studies (5%) [124,128] were rated as having no information. No observational study received a low risk of bias as evaluated by ROBINS-I.

Treatment outcomes

Time to negative conversion of SARS-CoV-2 tests

Six studies [85,87,88,95,118,121] with 581 hospitalized COVID-19 patients evaluated the effect of chloroquine and hydroxychloroquine on time to negative conversion of SARS-CoV-2 tests and were included in the meta-analysis. The overall pooled MD was -0.03 (95% CI: -4.69 to 4.62) with severe and significant heterogeneity (I^2 = 95%; P_Q <0.01). There were no significant differences between the pooled MD from randomized studies and non-randomized studies (p = 0.64, Supplementary Figure 1). As only randomized studies were rated as having a low or

moderate risk of bias for this outcome, we did not perform a subgroup analysis based on risk of bias ratings as it was redundant to the analysis by study design.

Six studies reported their continuous outcome measures in median with IQR. Data from two of these studies [87,121] were imputed and included in the meta-analysis following skewness testing. There were no significant subgroup differences between the pooled MD from studies using non-imputed data versus the pooled MD from studies using imputed data (p = 0.05, Supplementary Figure 2). Two of the remaining four studies (which included an observational study assessing chloroquine and a RCT assessing hydroxychloroquine) [91,105] did not find significant differences in time to negative test conversion between hydroxychloroquine and chloroquine against standard of care. An observational study by Huang *et al.* [93] found that chloroquine significantly reduced time to negative test conversion with a median difference of -6.0 (95% CI: -6.0 to -4.0). Last, an observational study by Karolyi *et al.* [94] reported that hydroxychloroquine conferred a moderate reduction in time to negative test conversion compared with standard of care (median 15 [IQR: 9 to 17] vs median 17.5 [IQR: 12.75 to 26.75]), however no hypothesis significance testing was conducted.

At 7 days after the commencement of chloroquine or hydroxychloroquine therapy, the OR of negative SARS-CoV-2 tests in the treatment group compared with the control group was 0.91 (95% CI: 0.35–2.38) with significant and moderate heterogeneity (I^2 = 73%; p_Q <0.01) based on 7 studies with 619 patients (Figure 3B) [31,87,91,107,117,118,133,134]. At 14 days, the OR of negative SARS-CoV-2 tests in the treatment group compared with the control group was 0.69 (95% CI: 0.14–3.44) with significant and severe heterogeneity (I^2 = 86%; P_Q <0.01) based on 6 studies with 649 patients (Figure 3C) [87,88,91,93,121,122]. There were no significant subgroup differences observed in any of the subgroup analyses (Supplementary Figures 3–6).

Length of stay

Nineteen studies [31,85,86,93–95,97,98,108,110,112,113,116,118,127,129,131–134] with 14,341 hospitalized COVID-19 patients assessed the effect of chloroquine and hydroxychloroquine on length of stay and were included in the meta-analysis. The overall pooled MD was 0.78 (95% CI: -0.34 to 1.89) with significant and severe heterogeneity (I^2 = 91%; p_Q <0.01). There were no significant differences between different regimen subgroups (p = 0.10, Figure 4). Additionally, there were no significant between-group differences among studies using a randomized versus a non-randomized design (p = 0.98, Supplementary Figure 7), nor any significant differences among studies with a high risk of bias versus studies with low/moderate risk of bias (p = 0.28, Supplementary Figure 8).

Nine studies reported the median length of stay. Data from four of the studies were imputed following skewness testing and included in the meta-analysis. There were no significant differences between the pooled MD from imputed studies versus non-imputed studies (p = 0.17, Supplementary Figure 9). Three observational studies [103,114,121] of the five remaining studies reported no significant differences between the median length of stay among patients taking hydroxychloroquine (with or without azithromycin) and patients receiving standard of care. The other two studies, specifically the RECOVERY trial [30] and a cohort study by Rosenberg *et al.* [126], reported higher median lengths of stay in the hydroxychloroquine group compared with their respective control groups.

Mortality

Forty-four studies [30,31,85–92,94,96,97,99–106,108,109,111–121,123,125–134] with 56,522 hospitalized COVID-19 patients assessed the effect of chloroquine and hydroxychloroquine on mortality and were included in the meta-analysis. The overall pooled OR was 0.89 (95% CI: 0.74–1.07) with significant and severe heterogeneity (I^2 = 85%; p_Q <0.01). There were no significant differences between regimen subgroups (p = 0.26, Figure 5). Additionally, there were no significant between-group differences among studies using a randomized versus a non-randomized design (p = 0.16, Supplementary Figure 10), nor any significant differences among studies with a high risk of bias versus studies with low/moderate risk of bias (p = 0.61, Supplementary Figure 11).

Time to fever resolution

Four studies [87,93,105,110] with 589 hospitalized COVID-19 patients assessed the effect of chloroquine and hydroxychloroquine on time to fever resolution and were included in the meta-analysis. The overall pooled MD was -0.44 (95% CI: -2.36 to 1.48) with significant and moderate heterogeneity (I^2 = 54%; p_Q = 0.09; Figure 6). There were no significant differences between the pooled MD of randomized and non-randomized studies (p = 0.93, Supplementary Figure 12) nor between imputed and non-imputed studies (p = 0.38, Supplementary Figure 13).

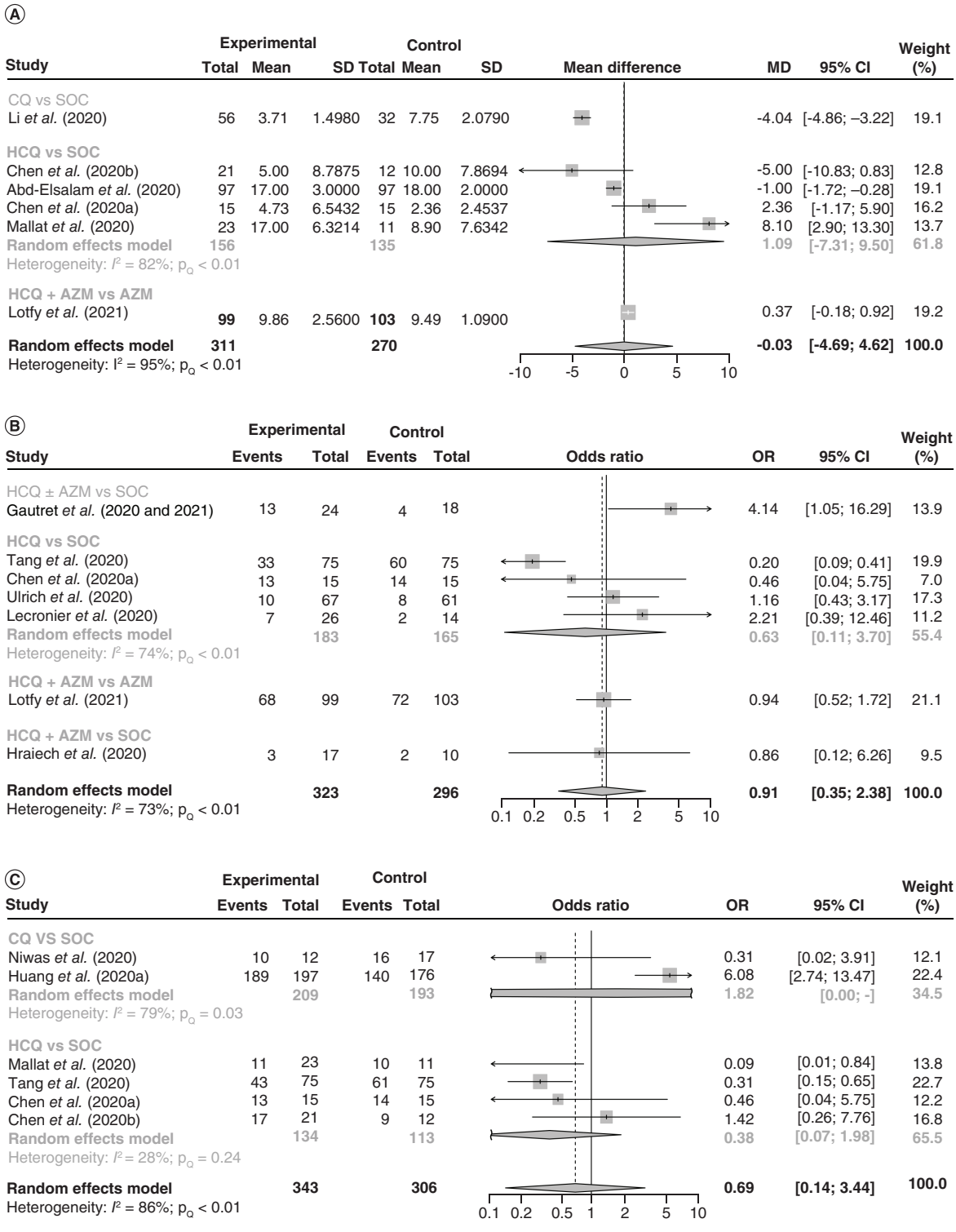

Figure 3. Forest plots for the pooling of mean differences for the outcome of time to negative conversion of SARS-CoV-2 test and for the pooling of odds ratios for secondary efficacy outcomes. The use of hydroxychloroquine/chloroquine regimens was compared with control groups using standard of care or adjuvant therapies without hydroxychloroquine/chloroquine. Heterogeneity was quantified using I^2 statistics. **(A)** Forest plot for the pooling of MDs for the outcome of time to negative conversion of SARS-CoV-2 test. MD <0 indicates beneficial treatment effects of hydroxychloroquine/chloroquine regimens compared with the control groups. **(B)** Forest plot for the pooling of ORs for incidences of negative SARS-CoV-2 tests at day 7. **(C)** Forest plot for the pooling of ORs for incidences of negative SARS-CoV-2 tests at day 14; there were no significant differences between regimen subgroups (p = 0.31). OR >1 indicates beneficial treatment effects of hydroxychloroquine/chloroquine regimens compared with the control groups for all secondary efficacy outcomes.
AZM: Azithromycin; CQ: Chloroquine; HCQ: Hydroxychloroquine; MD: Mean difference; OR: Odds ratio; SOC: Standard of care.

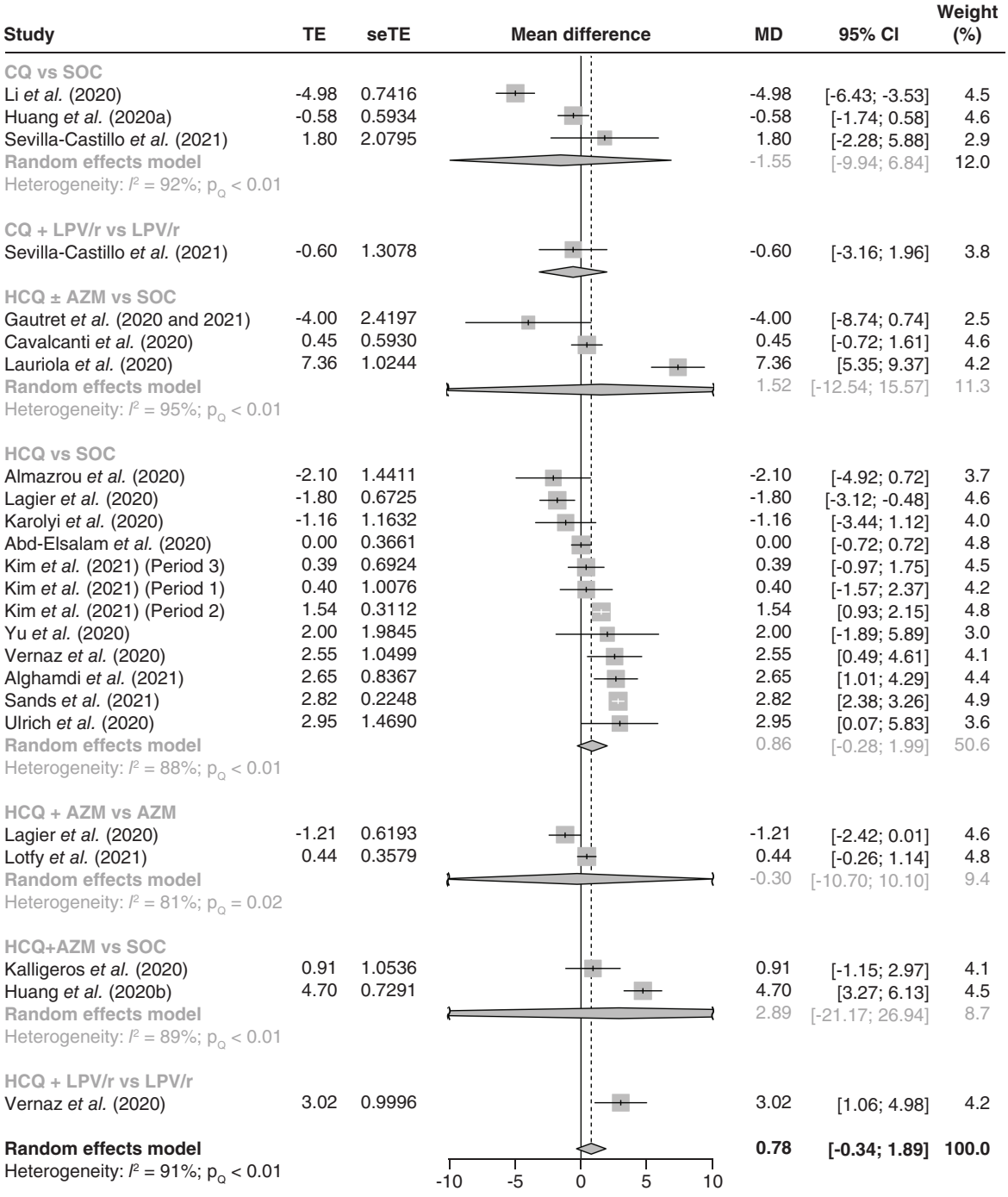

Figure 4. Forest plot for the pooling of mean differences for length of stay. The use of hydroxychloroquine/chloroquine regimens was compared with control groups using standard of care or adjuvant therapies without hydroxychloroquine/chloroquine. Heterogeneity was quantified using I^2 statistics. MD <0 indicates beneficial treatment effects of hydroxychloroquine/chloroquine compared with the control groups. There were no significant differences between regimen subgroups (p = 0.10).
AZM: Azithromycin; CQ: Chloroquine; HCQ: Hydroxychloroquine; LPV/r: Lopinavir-Ritonavir combination therapy; MD: Mean difference; seTE: Standard error of the treatment effect; SOC: Standard of care; TE: Treatment effect (mean difference).

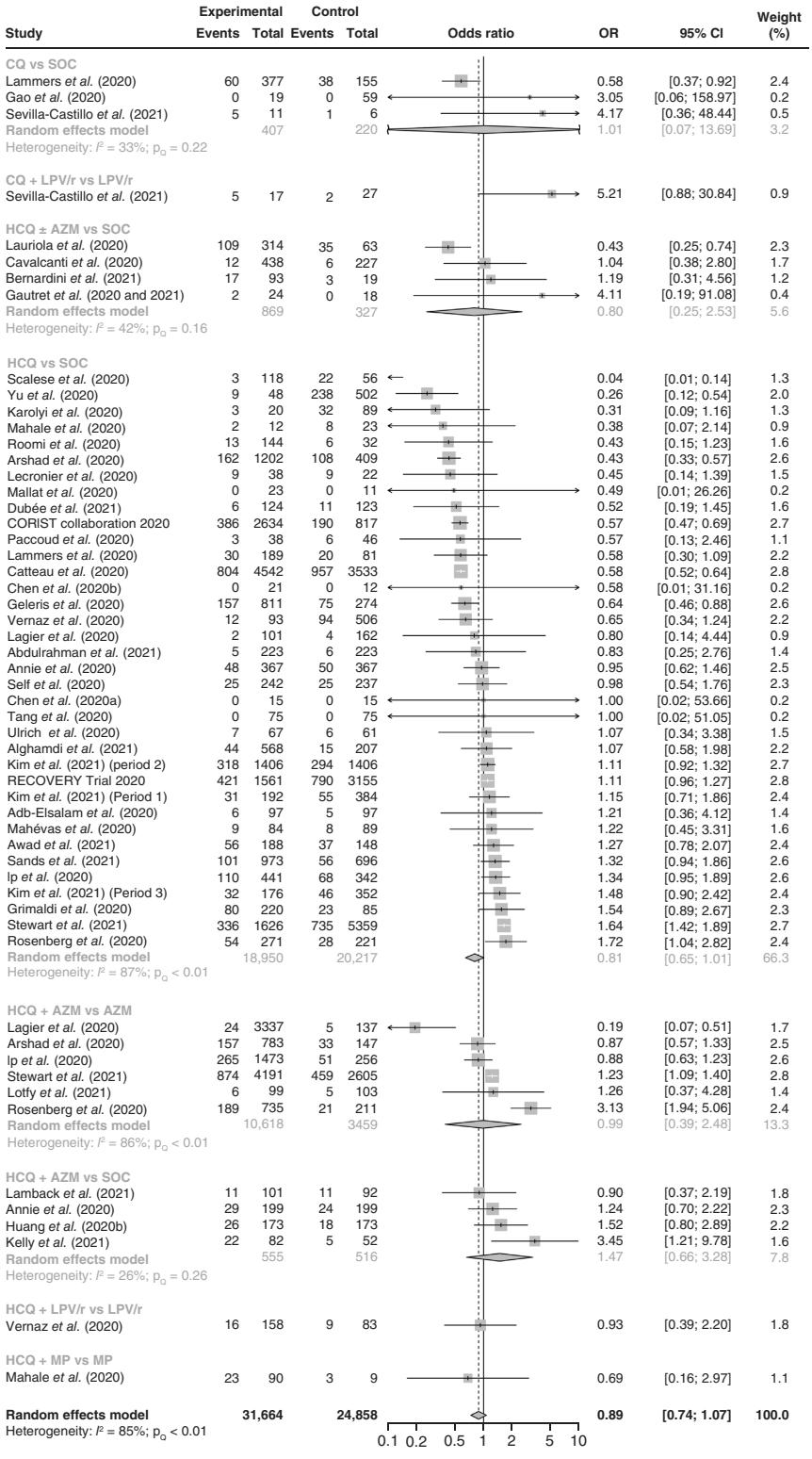

Figure 5. Forest plot for the pooling of odds ratios for mortality. The use of hydroxychloroquine/chloroquine was compared with control groups using standard of care or adjuvant therapies without hydroxychloroquine/chloroquine. Heterogeneity was quantified using I^2 statistics. OR <1 indicates beneficial treatment effects of hydroxychloroquine/chloroquine compared with the control groups. There were no significant differences between regimen subgroups (p = 0.26).
AZM: Azithromycin; CQ: Chloroquine; HCQ: Hydroxychloroquine; LPV/r: Lopinavir-ritonavir combination therapy; MP: Methylprednisolone; OR: Odds ratio; SOC: Standard of care.

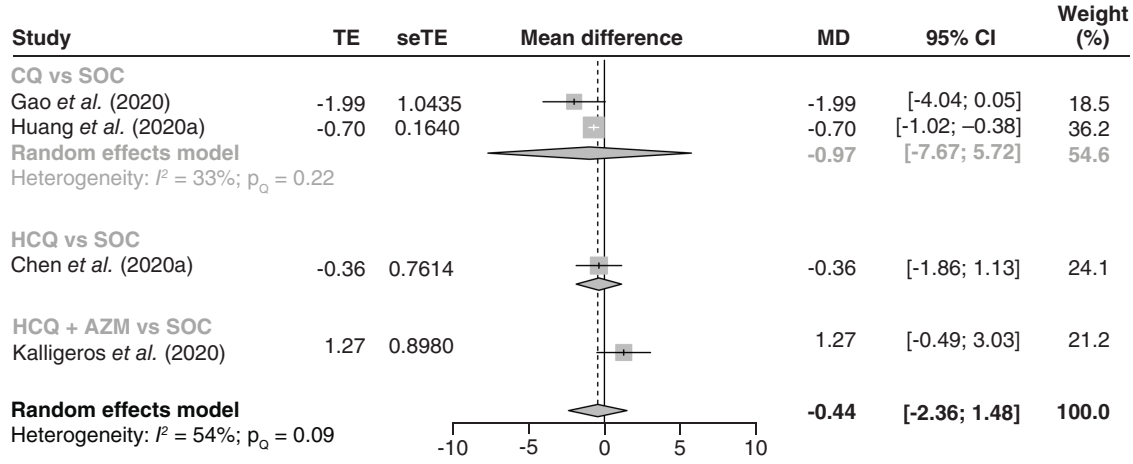

Figure 6. Forest plot for the pooling of mean differences for time to fever resolution. The use of hydroxychloroquine/chloroquine was compared with control groups using standard of care or adjuvant therapies without hydroxychloroquine/chloroquine. Heterogeneity was quantified using I^2 statistics. MD <0 indicates beneficial treatment effects of hydroxychloroquine/chloroquine compared with the control groups. There were no significant differences between regimen subgroups (p = 0.10).
AZM: Azithromycin; CQ: Chloroquine; HCQ: Hydroxychloroquine; MD: Mean difference; seTE: Standard error of the treatment effect; SOC: Standard of care; TE: Treatment effect (mean difference).

We did not conduct a subgroup analysis by risk of bias rating, as only randomized studies received a low/moderate risk of bias rating for this outcome.

Incidence of mechanical ventilation

Twenty-one studies [30,31,86,89,90,92,94,97,98,101,102,106,112,114,117–119,121,125,126,130] with 25,343 hospitalized COVID-19 patients assessed the effect of chloroquine and hydroxychloroquine on incidences of mechanical ventilation. The overall pooled OR was 1.26 (95% CI: 0.85–1.87) with significant and severe heterogeneity (I^2 = 87%; p_Q <0.01). There were no significant differences between different regimen subgroups (p = 0.34, Figure 7). In addition, there were no significant subgroup differences between randomized and non-randomized studies (p = 0.39, Supplementary Figure 14), nor were there any differences between studies with a high risk of bias and studies with a low/moderate risk of bias (p = 0.77, Supplementary Figure 15).

Adverse events

Twelve studies [31,86,87,89–91,93,96,108,111,113,114] with 6875 hospitalized COVID-19 patients assessed the effect of chloroquine and hydroxychloroquine on incidences of adverse events. The overall pooled OR was 1.49 (95% CI: 0.91–2.42) with significant and moderate heterogeneity (I^2 = 64%; p_Q <0.01). There were no significant differences between different regimen subgroups (p = 0.05, Figure 8A). There were no significant subgroup differences between randomized and non-randomized studies (p = 0.88, Supplementary Figure 16), nor were there any differences between studies with a high risk of bias and studies with a low/moderate risk of bias (p = 0.74, Supplementary Figure 17). However, the subgroup of studies with low/moderate risk of bias ratings yielded a significant treatment effect (OR: 1.67; 95% CI: 1.11–2.52) contrary to the overall pooled OR, with no heterogeneity (I^2 = 35%; p_Q = 0.15).

In terms of severe adverse events, the pooled OR was 0.96 (95% CI: 0.32 to 2.84) with no heterogeneity (I^2 = 0%; P_Q = 0.80) based on 3 RCTs [86,88,89] with 892 patients and moderate risks of biases (Figure 8B). We did not perform any other planned subgroup analyses due to insufficient data.

Incidence of QT prolongation

Twelve studies [31,86,88,90,96,102,108,111,113,117,124,126] with 7,580 hospitalized COVID-19 patients assessed the effects of chloroquine and hydroxychloroquine on incidences of QT prolongation. Four studies [31,90,96,102] defined QT prolongation as a corrected QT interval >500 ms. Lagier *et al.* [113] defined QT prolongation as a change in corrected QT interval >60 ms. Huang *et al.* [108] defined QT prolongation as either a corrected QT interval

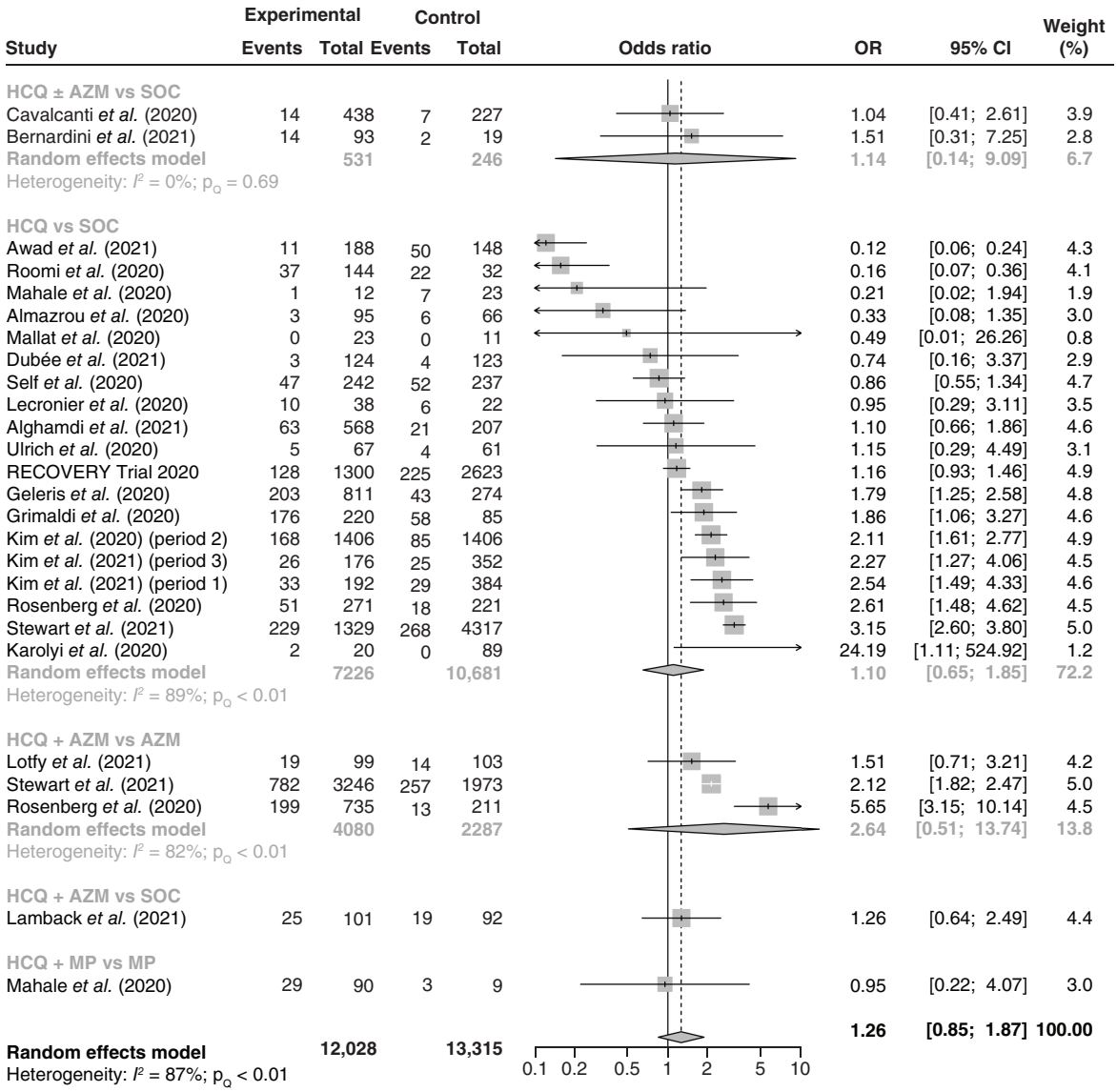

Figure 7. Forest plot for the pooling of odds ratios for incidence of mechanical ventilation. The use of hydroxychloroquine/chloroquine was compared with control groups using standard of care or adjuvant therapies without hydroxychloroquine/chloroquine. Heterogeneity was quantified using I^2 statistics. OR <1 indicates beneficial treatment effects of hydroxychloroquine/chloroquine compared with the control groups. There were no significant differences between regimen subgroups (p = 0.34).
AZM: Azithromycin; HCQ: Hydroxychloroquine; MP: Methylprednisolone; OR: Odds ratio; SOC: Standard of care.

>500 ms or a change in corrected QT interval >60 ms. Lecronier *et al.* [117] defined QT prolongation as a corrected QT interval >470 ms for male patients and a corrected QT interval >450 ms for female patients. Last, Cavalcanti *et al.* [86] defined QT prolongation as a corrected QT interval >480 ms. The remaining four studies [88,111,124,126] did not define QT prolongation.

Overall, the pooled OR was 2.82 (95% CI: 1.45–5.50) with significant and moderate heterogeneity (I^2 = 51%; p_Q = 0.02). There were significant differences between regimen subgroups (p < 0.01, Figure 8C); notably, the subgroup of studies comparing hydroxychloroquine (with a subset of patients also taking azithromycin) against standard of care yielded a substantially higher pooled OR compared with the other regimen subgroups (OR: 13.27; 95% CI: 3.00–58.68) with no heterogeneity (I^2 = 0%; p_Q = 0.71). There were no significant differences between randomized and non-randomized studies (p = 0.89, Supplementary Figure 18), nor between studies with a high risk of bias and studies with a low/moderate risk of bias (p = 0.79, Supplementary Figure 19). Nevertheless, it is

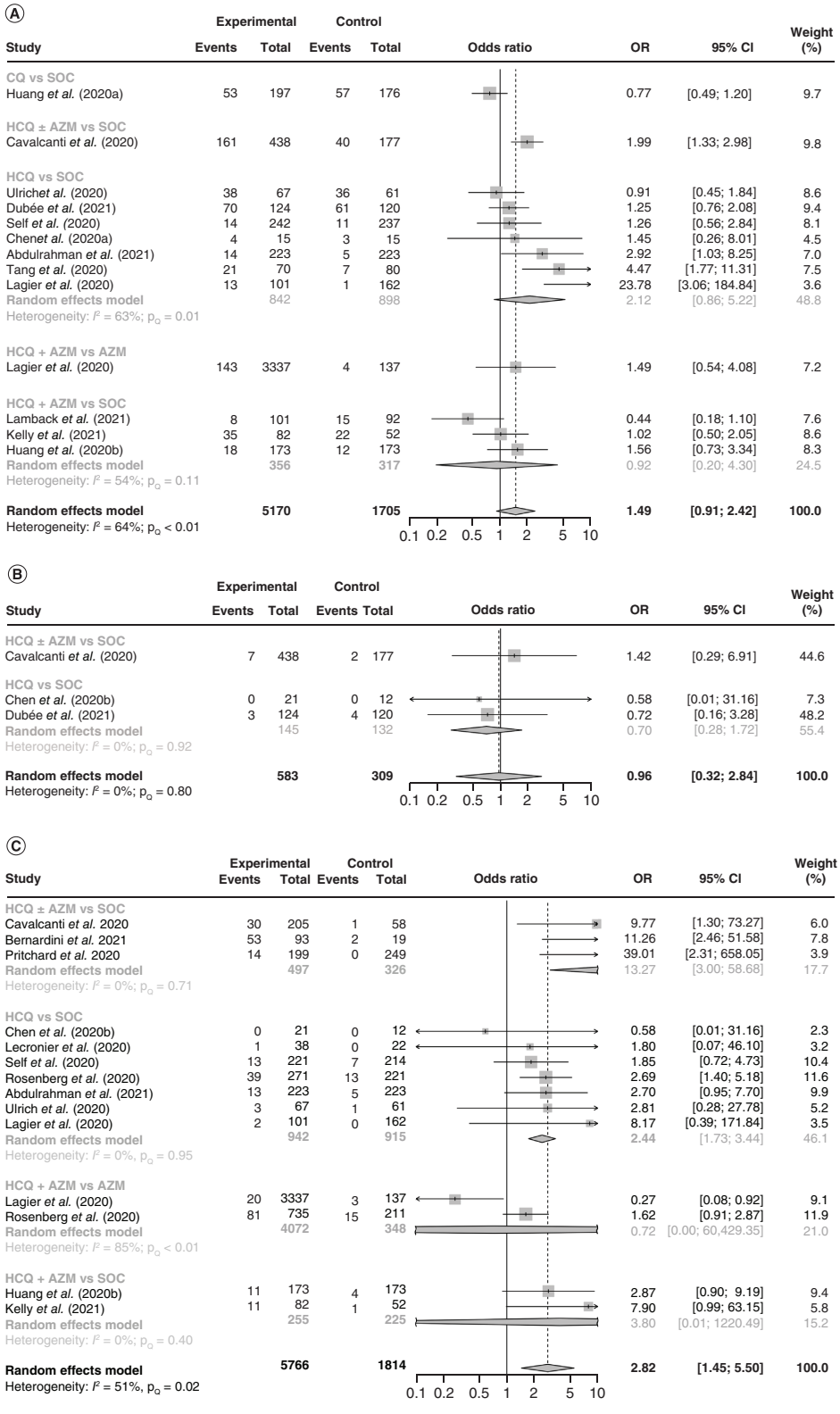

Figure 8. Forest plot for the pooling of odds ratios for primary and secondary safety outcomes. The use of hydroxychloroquine/chloroquine was compared with control groups using standard of care or adjuvant therapies without hydroxychloroquine/chloroquine. Heterogeneity was quantified using I^2 statistics. OR <1 indicates better safety outcomes of hydroxychloroquine/chloroquine compared with the control groups. **(A)** Forest plot for the pooling of ORs for incidence of adverse events. There were no significant differences between regimen subgroups ($p = 0.05$). **(B)** Forest plot for the pooling of ORs for incidence of severe adverse events. There were no significant differences between regimen subgroups ($p = 0.38$). **(C)** Forest plot for the pooling of ORs for incidence of QT prolongations. There were significant differences between regimen subgroups ($p < 0.01$).
AZM: Azithromycin; CQ: Chloroquine; HCQ: Hydroxychloroquine; OR: Odds ratio; SOC: Standard of care.

notable that the RCT subgroup did not yield a significant treatment effect (OR: 2.69; 95% CI: 0.62–11.58) with no heterogeneity (I^2 = 0%; P_Q = 0.74). Similarly, the subgroup of studies with a high risk of bias also did not achieve a significant treatment effect (OR: 3.13; 95% CI: 0.89–10.93) with moderate heterogeneity (I^2 = 70%; p_Q <0.01).

Meta-regressions

Through the proposed meta-regression analyses, we identified a significant correlation between the incidence of adverse events and cumulative chloroquine base dose ($p < 0.05$, Supplementary Figure 20), where the odds of adverse events increased according to increases in cumulative dose (β = 0.0002; 95% CI: 0–0.0004). This correlation accounted for 27.2% of heterogeneity in the analysis (R^2 = 27.2%). The remaining analyses were either non-significant or were not conducted due to insufficient data (Supplementary Table 13).

Publication bias

Using visual observation of funnel plots and Egger's regression analysis, we found significant publication bias for the outcome of incidence of mechanical ventilation (p_{Egger} <0.05, Supplementary Figure 21). Using the trim-and-fill method, we estimate that there were 8 unpublished studies that would increase the pooled treatment effect (Supplementary Figure 22). The estimated treatment effect, including the unpublished studies, was OR 1.98 (95% CI: 1.24–3.16). The remaining analyses were either non-significant or were not conducted if less than 10 studies were included in the meta-analyses (Supplementary Figures 23–32).

Quality of evidence

The summary of findings for primary outcomes is tabulated in Table 1.

Discussion

Main findings

Our systematic review and meta-analysis included 9 RCTs and 42 observational studies involving 61,221 hospitalized COVID-19 patients. The use of hydroxychloroquine/chloroquine, with or without azithromycin, was not significantly associated with a reduction in time to negative conversion of SARS-CoV-2 tests, length of stay, mortality, time to fever resolution or incidence of mechanical ventilation compared with control groups based on very low quality of evidence. Additionally, the use of hydroxychloroquine/chloroquine, with or without azithromycin, was not significantly associated with reductions in odds of negative SARS-CoV-2 test conversion at day 7 or day 14.

In terms of safety, the use of hydroxychloroquine/chloroquine, with or without azithromycin, was not significantly associated with an increased incidence of adverse events or severe adverse events. However, the odds of adverse events was significantly correlated with the cumulative chloroquine base dose, indicating a potential dose-response gradient with regards to incidences of adverse events in patients taking hydroxychloroquine/chloroquine. Additionally, there was a difference in outcomes between studies with a high risk of bias versus studies with a low/moderate risk of bias, as the latter subgroup found a significant, 67% increase in the odds of adverse events for patients taking hydroxychloroquine/chloroquine with no heterogeneity. Both of the aforementioned observations suggest that the use of hydroxychloroquine/chloroquine may be associated with increased adverse events in hospitalized COVID-19 patients. Therefore, the safety of these drugs in the context of the ongoing pandemic requires further elaboration and investigation.

One particular category of adverse events that were commonly reported in COVID-19 patients taking hydroxychloroquine/chloroquine was cardiac adverse events, namely QT prolongation. In this systematic review, we found that patients taking hydroxychloroquine/chloroquine, with or without azithromycin, had a 182% increased odds of QT prolongation compared with the control groups, based on a low quality of evidence. In particular, we found that studies that gave a subset of patients azithromycin reported a significantly higher incidence of QT prolongations compared with studies that only administered hydroxychloroquine monotherapy. This observation was not replicated in studies that gave all patients both hydroxychloroquine and azithromycin as the pooled treatment effect did not reach significance in that subgroup. However, we suspect that imprecision due to a small sample size may have contributed to this disagreement in findings.

Table 1. Summary of findings, hydroxychloroquine/chloroquine regimens compared with standard of care/adjuvant therapies for the management of hospitalized COVID-19 patients.

Primary outcomes	Relative effect (95% CI)	Anticipated absolute effects (95% CI)†			Patients (n) Studies (n)	Quality of evidence (GRADE)
		Risk without CQ/HCQ	Risk with CQ/HCQ	Risk difference (95% CI)		
Time to negative conversion of SARS-CoV-2 tests	-	Mean time in the control group was 12 days	-	MD 0.03 fewer days (4.69 fewer to 4.62 more)	581 (3 RCTs, 3 OSs)	⊕◯◯◯ very low‡,§,¶
Length of stay	-	Mean length of stay in the control group was 11 days	-	MD 0.78 more days (0.34 fewer to 1.89 more)	14,341 (3 RCTs, 16 OSs)	⊕◯◯◯ very low‡,§,¶,#
Mortality	OR 0.89 (0.74 to 1.07)	191 per 1000	174 per 1000 (149 to 202)	17 fewer per 1000 (42 fewer to 11 more)	56,522 (9 RCTs, 35 OSs)	⊕◯◯◯ very low‡,§,¶,#
Time to fever resolution	-	Mean time in the control group was 3 days	-	MD 0.44 fewer days (2.36 fewer to 1.48 more)	589 (1 RCT, 3 OSs)	⊕◯◯◯ very low‡,¶,#
Incidence of mechanical ventilation	OR 1.26 (0.85 to 1.87)	93 per 1000	114 per 1000 (80 to 161)	21 more per 1000 (13 fewer to 68 more)	25,343 (5 RCTs, 16 OSs)	⊕◯◯◯ very low‡,§,¶,#,††
Incidence of adverse events	OR 1.49 (0.91 to 2.42)	161 per 1000	222 per 1000 (149 to 317)	61 more per 1000 (12 fewer to 156 more)	6,875 (6 RCTs, 6 OSs)	⊕⊕⊕◯ moderate¶,‡‡
Incidence of QT prolongation	OR 2.82 (1.45 to 5.50)	29 per 1000	77 per 1000 (41 to 140)	48 more per 1000 (12 more to 111 more)	7,580 (4 RCTs, 8 OSs)	⊕⊕◯◯ low ‡,#,§§

†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).
GRADE Working Group quality of evidence rating [67,68].
High quality: We are very confident that the true effect lies close to that of the estimate of the effect.
Moderate quality: We are moderately confident in the effect estimate; the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
Low quality: Our confidence in the effect estimate is limited; the true effect may be substantially different from the estimate of the effect.
Very low quality: We have very little confidence in the effect estimate; the true effect is likely to be substantially different from the estimate of effect.
‡Downgraded due to study limitations; a majority of included studies were rated as having serious or critical risk of bias according to ROBINS-I and/or RoB2.
§Downgraded due to inconsistency; significant and severe heterogeneity was observed in the analysis.
¶Downgraded due to imprecision; confidence intervals could not rule out the possibility of no effect (crosses null).
#Quality of study was rated as low prior to downgrading or upgrading as a majority of the included studies were observational studies.
††Downgraded due to publication bias; visual inspection of the funnel plot and/or Egger's regression test indicated the presence of funnel plot asymmetry.
‡‡Upgraded due to the potential presence of a dose-response gradient.
§§Upgraded due to a large magnitude of effect.
CQ: Chloroquine; GRADE: Grading of recommendation, assessment, development and evaluation; HCQ: Hydroxychloroquine; MD: Mean difference; OR: Odds ratio; OS: Observational study; RCT: Randomized controlled trial.

Comparison with other studies

The lack of efficacy of chloroquine compounds in treating COVID-19 is well documented in previous meta-analyses with smaller sample sizes. A previous systematic review by Kashour *et al.* [32] found that hydroxychloroquine, with or without azithromycin, was not effective in reducing short-term mortality in hospitalized COVID-19 patients. Ghazy *et al.* [42] found that hydroxychloroquine/chloroquine did not improve the incidence of negative test conversion on day 14, nor reduce the need for mechanical ventilation or the time to negative test conversion. A collaborative meta-analysis involving both published and unpublished RCTs with over 10,000 patients even concluded that hydroxychloroquine may be associated with increased mortality in COVID-19 patients [33], although we could not replicate this finding in our analyses. Nevertheless, these earlier studies lend credibility to our findings that hydroxychloroquine/chloroquine, with or without azithromycin, were not efficacious in improving any of our outcomes of interest.

The therapeutic use of chloroquine and hydroxychloroquine in viral diseases has been theorized since their introduction more than half a century ago [135,136]. Since then, there has been a plethora of *in vitro* studies showing that chloroquine compounds can exhibit broad-spectrum antiviral effects. Some of the most notable viruses that were shown to be inhibited by chloroquine *in vitro* include SARS-CoV [137,138], Middle East respiratory syndrome–related coronavirus (MERS-CoV) [139], human immunodeficiency viruses-1 (HIV-1) [140,141], dengue virus-2 (DENV-2) [142,143], Zika virus [144,145], Chikungunya virus [146,147] and Ebola virus [148,149], among others [150]. However, these *in vitro* findings often cannot be translated to clinical benefits. For instance, a previous RCT

investigating the effect of hydroxychloroquine on HIV found that hydroxychloroquine actually increased HIV viral load *in vivo* [151], contrary to *in vitro* observations. Similarly, RCTs involving the use of chloroquine for treating other diseases such DENV [152] and Chikungunya virus [153] yielded minimal to no efficacy.

The reasoning behind the lack of clinical efficacy associated with chloroquine in the context of viral illnesses is likely due to its mechanism of action. As mentioned previously, it was speculated that chloroquine and hydroxychloroquine achieves their antiviral effects by increasing pH in endosomal compartments, preventing post-translational modifications of viral glycoproteins [17]. Paradoxically, this mechanism is also involved in producing the anti-inflammatory and immunomodulatory effects associated with chloroquine [154], which is not desirable when it comes to viral clearance (except for in the case of patients with severe disease due to hyperinflammatory reactions [155]). The specific immune receptors inhibited by this mechanism, namingly TLR7 and TLR9, are crucial for detecting viral RNA and are needed to mount an effective antiviral immune response [156]. Additionally, chloroquine has been found to impair interferon-induced antiviral response [157], which is often one of the first lines of host defence against viral infections.

Apart from inhibiting antiviral host defences, previous reviews also highlighted insufficient tissue and serum drug concentration as a potential factor behind the lack of efficacy [32]. A study by Balevic *et al.* compiled all available data on the pharmacokinetics of hydroxychloroquine in patients with rheumatic diseases and found that the average serum hydroxychloroquine concentration was lower than the lowest estimated concentration needed for treating SARS-CoV-2 at typical dosages [158]. It was speculated that using high dose hydroxychloroquine may achieve clinically significant benefits in COVID-19 patients; however, this approach was associated with increased incidences of adverse events according to the results of a RCT comparing high-dose versus low-dose hydroxychloroquine [159]. This finding was replicated in our own analyses as we identified a potential correlation between the odds of adverse events and cumulative chloroquine base dose.

A common type of adverse events associated with the use of chloroquine is cardiac adverse events [160], such as long QT syndrome and Torsades de Pointes [161]. This is primarily caused by the blockage of the human ether-à-go-go related gene (hERG) potassium channel, which is a voltage-gated ion channel that allows a delayed, sustained K^+ efflux following cardiac depolarization. The hERG channel mediates the delayed rectifier potassium current, which is essential for cardiac repolarization. Thus, the blockage of the hERG channel by chloroquine lengthens vascular repolarization, resulting in QT prolongations [162]. In some circumstances, this blockage can also lead to early afterdepolarizations, resulting in Torsades de Pointes and cardiac arrhythmias [163]. In our meta-analysis, we found that there was a significant increase in the odds of QT prolongations associated with hydroxychloroquine/chloroquine use. This finding corresponds with observations from numerous single-armed cohort studies, which found that the use of chloroquine or hydroxychloroquine generally increased corrected QT intervals, often to a greater extent compared with studies conducted in healthy volunteers [47–62,164].

This adverse effect may be compounded by the use of adjunctive azithromycin, which is a macrolide antibiotic commonly used to prevent bacterial co-infections in COVID-19 patients [165]. Arrhythmias via blockage of the hERG potassium channel is a well documented side effect of macrolide antibiotics [166], although azithromycin is one of the safest macrolides available in terms of cardiac toxicity [165]. Nevertheless, we found that studies where a subset of patients was administered azithromycin had a higher odds of QT prolongations compared with hydroxychloroquine alone. This mirrors the findings from a RCT conducted by Pfizer Labs in 2013 [167], which found a correlation between azithromycin use and QT prolongations in healthy volunteers receiving chloroquine. While studies administering all patients with adjunctive azithromycin did not yield a significant increase in the odds of QT prolongations compared with control due to low sample sizes and imprecision, the proarrhythmic potential of the azithromycin and chloroquine combination should not be ignored. Chloroquine should not be administered to patients with known risk factors for QT prolongations, such as patients with electrolyte imbalance, left ventricular hypertrophy, or left ventricular ejection fraction, and concurrent use of chloroquine with other QT-prolonging drugs, such as azithromycin, should be avoided whenever possible during the treatment of COVID-19.

Strengths & limitations

This systematic review and meta-analysis has several strengths. First of all, we performed several subgroup analyses to examine the impact of our study methodologies on the treatment effect, such as comparing the pooled effect from randomized versus non-randomized studies, studies with low/moderate risks of bias versus studies with high risks of bias, and studies that required imputations versus studies that did not require imputations. Secondly, we examined potential correlations between time from symptom onset to administration/randomization, cumulative

chloroquine base dose, and proportion of patients with severe diseases and the treatment effect using meta-regression analyses, which were not done in previous reviews. Lastly, our meta-analyses included more studies involving a larger sample size of patients, which helped to improve the power and precision of our analyses.

Our study also has several limitations. A majority of our included studies are observational studies, which are prone to biases due to sources of confounding and risk modification. In fact, we found that most of the included observational studies had a high risk of bias. Inclusion of observational studies in meta-analyses may result in large treatment effects [168,169] and overly precise estimates [170,171]; although in the case of our efficacy outcomes, this would mean that the direction of bias would likely favor hydroxychloroquine/chloroquine. In addition, we found evidence of publication bias in the outcome of mechanical ventilation incidence. However, the trim-and-fill analysis revealed that the direction of bias is likely to favor hydroxychloroquine/chloroquine.

Conclusion

This systematic review and meta-analysis showed that the use of hydroxychloroquine/chloroquine, with or without azithromycin, is not significantly associated with reductions in time to negative conversion of SARS-CoV-2 tests, length of stay, mortality, time to fever resolution, or incidence of mechanical ventilation compared with control groups based on very low quality of evidence. In addition, the use of hydroxychloroquine/chloroquine may not be associated with increased odds of adverse events, although this finding should be interpreted with caution as we observed a correlation between cumulative chloroquine base dose and the odds of adverse events, and the use of hydroxychloroquine/chloroquine was significantly associated with increased odds of adverse events when only studies with a low/moderate risk of bias was included in the analysis. Lastly, the use of hydroxychloroquine/chloroquine may be associated with increased odds of QT prolongations. Given that the use of hydroxychloroquine/chloroquine is not associated with improved efficacy outcome measures, and their use may result in cardiac adverse events, we do not recommend the use of hydroxychloroquine/chloroquine for the treatment of hospitalized COVID-19 patients based on the available evidence.

Summary points

- Chloroquine is an antimalarial, antirheumatic agent that was speculated to have antiviral applications; this led to its adoption during the early stages of the pandemic for treating SARS-CoV-2 infections following encouraging results from *in vitro* and observational studies.
- In this systematic review and meta-analysis, data from 9 randomized controlled trials and 42 observational studies (n = 61,221) were analyzed to assess the efficacy and safety of using chloroquine and its analog, hydroxychloroquine, for treating hospitalized COVID-19 patients.
- A majority of RCTs included in the analysis were rated as having some concerns in terms of risk of bias according to RoB2, while a majority of the included observational studies were rated as having serious or critical risk of bias according to ROBINS-I.
- The use of hydroxychloroquine/chloroquine, with or without azithromycin, was not associated with any significant reductions in mortality, length of stay, time to fever resolution, incidence of mechanical ventilation, and time for negative SARS-CoV-2 test conversions.
- While the use of hydroxychloroquine/chloroquine was not associated with a significant increase in the odds of adverse events, there was a positive correlation between cumulative chloroquine base dose and odds of adverse events according to meta-regression analyses.
- The use of hydroxychloroquine/chloroquine was significantly associated with increased odds of QT prolongations, and the use of adjunctive azithromycin may exacerbate this effect; however, the exact impact of azithromycin on the risk of QT prolongations need to be further investigated.
- All primary efficacy outcomes were based on very low quality of evidence, while the outcome of adverse event incidence was based on moderate quality of evidence, and the outcome of QT prolongation incidence was based on low quality of evidence, according to the GRADE framework.
- Due to the lack of efficacy and increased odds of cardiac adverse events, hydroxychloroquine/chloroquine should not be used for treating hospitalized COVID-19 patients.

Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at: www.futuremedicine.com/doi/suppl/10.2217/fvl-2021-0119

Author contributions

J Deng was responsible for conception and design of the work, as well as for search strategy development, conducting database searches, performing data analyses (including meta-analyses, meta-regressions, subgroup analyses and GRADE ratings) and drafting of the final manuscript. F Zhou was responsible for conception and design of the work, as well as for supervising and contributing to all parts of the data acquisition process (including article screening, full-text retrieval, data extraction and risk of bias analyses) and revised the final manuscript critically for important intellectual content. K Heybati was responsible for supervising and contributing to all parts of the data acquisition process (including article screening, full-text retrieval, data extraction, and risk of bias analyses) and revised the final manuscript critically for important intellectual content. S Ali, Q Zuo, W Hou, T Dhivagaran, HB Ramaraju, O Chang, CY Wong and Z Silver contributed to all parts of the data acquisition process (including article screening, full-text retrieval, data extraction, and risk of bias analyses) and revised the final manuscript critically for important intellectual content. All authors have given final approval for the final version of this manuscript to be submitted for publication and agree to be held accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Ethical conduct of research

There are no relevant ethical disclosures as only published, aggregate patient data was used for this study. Aggregate patient data extracted by study authors are available upon reasonable request.

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