Title: Hydroxychloroquine and mortality risk of patients with COVID-19: a systematic review and meta-analysis of human comparative studies

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Abbreviations: HCQ: Hydroxychloroquine; AZ: Azithromycin; RR: Relative Risk; HR: Hazard Ratio, OR: Odds Ratio; US FDA: US Food and Drug Administration; EMA: European Medicine Agency, CI: Confidence Interval

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Abstract

Background: Global COVID-19 deaths reached at least 400,000 fatalities. Hydroxychloroquine is an antimalarial drug that elicit immunomodulatory effects and had shown in vitro antiviral effects against SRAS-CoV-2. This drug divided opinion worldwide in the medical community but also in the press, the general public and in public health policies. The aim of this systematic review and this meta-analysis was to bring a new overview on this controversial drug and to assess whether hydroxychloroquine could reduce COVID-19 mortality risk in hospitalized patients.

Methods and Findings: Pubmed, Web of Science, Cochrane Library, MedRxiv and grey literature were searched until 10 June 2020. Only studies of COVID-19 patients treated with hydroxychloroquine (with or without azithromycin) compared with a comparative standard care group and with full-text articles in English were included. Studies reporting effect sizes as Odds Ratios, Hazard Ratio and Relative Risk for mortality risk and the number of deaths per groups were included. This meta-analysis was conducted following PRISMA guidelines and registered on PROSPERO (Registration number: CRD42020190801). Independent extraction has been performed by two independent reviewers. Effect sizes were pooled using a random-effects model.

The initial search leaded to 112 articles, from which 16 articles met our inclusion criteria. 15 studies were retained for association between hydroxychloroquine and COVID-19 survival including 15,081 patients (8,072 patients in the hydroxychloroquine arm and 7,009 patients in the standard care arm with respectively, 1,578 deaths and 1,423 deaths). 6 studies were retained for hydroxychloroquine with azithromycin. Hydroxychloroquine was not significantly associated with mortality risk (pooled Relative Risk RR=0.82 (95% Confidence Interval: 0.62-1.07, I^2=82, $P_{heterogeneity}$<0.01, n=15)) within hospitalized patients, nor in association with azithromycin (pooled Relative Risk RR=1.33 (95% CI: 0.92-1.92, I^2=75%, $P_{heterogeneity}$<0.01, n=6)), nor in the numerous subgroup analysis by study design, median age population, published studies (vs unpublished articles), level of bias risk. However, stratified analysis by continents, we found a significant decreased risk of mortality associated with hydroxychlroquine alone but not with azithromycin among European (RR= 0.62 (95%CI: 0.41-0.93, n=7)) and Asian studies (RR=0.36 (95%CI:0.18-0.73, n=1)), with heterogeneity detected across continent ($P_{heterogeneity\ between}$=0.003). These finding should be interpreted with caution since several included studies had a low quality of evidence with a small sample size, a lack of adjustment on potential confounders or selection and intervention biases.

Conclusion: Our meta-analysis does not support the use of hydroxychloroquine with or without azithromycin to reduce COVID-19 mortality in hospitalized patients. It raises the question of the hydroxychloroquine use outside of clinical trial. Additional results from larger randomised controlled trials are needed

Introduction

On December 31, 2019, World Health Organization (WHO) identified in Wuhan (China) an unknown pneumonia caused by a new coronavirus, SARS-CoV-2. This new coronavirus rapidly spread around the world and on the 11th of March, the WHO declared it as a pandemic. By 17 June, 2020, WHO confirmed 8,006,427 cases and 436,899 deaths.

Recent publications identified the *in vitro* antiviral activity against SARS-CoV-2 of hydroxychloroquine (HCQ), an aminoquinoline like chloroquine. HCQ appeared as a potential treatment for COVID-19 patients at low costs(1). HCQ is also used as antimalarial drug, for rheumatoid arthritis and for lupus. This drug was widely advertised by international press and the United States President(2). Three *in vitro* studies tested HCQ on VeroE6 cells infected by SARS-CoV-2. This later suggested that HCQ decreased the viral replication with 50% inhibitory concentration (IC50) values of 2.2 µM (0.7 µg/mL) and 4.4 µM (1.4 µg/mL) in Maisonnasse *et al.* study, at 0.72 µM in *Yao* et al. study and between 4.51 – 12.96 µM for 50% maximal effective concentration (EC50) in Liu *et al.* study (1–3). Another study reported a synergistic effect of the HCQ with azithromycin (AZ) against SARS-CoV-2(6). The mechanism would be an acidification of the endosomes pH, and this pH modification would block the virus-endosome fusion (7).

Hydroxychloroquine was also tested in a study where macaques were infected by SARS-CoV-2 and received either a high dose of hydroxychloroquine (90 mg/kg on day 1 then 45 mg/kg) either a low HCQ dose (30 mg/kg on day 1 then 15 mg/kg) (3). Hydroxychloroquine did not improve the time to viral clearance. Another study in preprint also reported that there is no evidence of efficacy for the drug hydroxychloroquine (6.5 mg/kg) against infection with SARS-CoV-2 in hamsters or macaque models(8).

By June 17, about 132 trials have been referenced to test hydroxychloroquine for COVID-19 on ClinicalTrials.gov (9). Until today, most of the published studies on hydroxychloroquine with a comparative group (standard care) were observational and non-randomized with inconsistent results (10–16). This study is the first meta-analysis to pool adjusted relative risk and to include 16 studies. Previous meta-analysis on COVID-19 included a very limited number of studies and used unadjusted risk ratio (17–19). Thus, the aim of this meta-analysis was to provide a systematically quantitative assessment of the association between HCQ treatment (vs standard care) and COVID-19 survival risk among human trials and observational studies.

Material and methods

Data sources, search strategy

Research question was: does hydroxychloroquine treatment (vs standard care) have an effect (positive or negative) on survival of patients with COVID-19? A search was performed via PubMed and Web of Science and Cochrane Review until 10 June 2020 with this string search: (COVID-19 OR SRAS-CoV-2) AND (MORTALITY OR DEATH) AND (HYDROXYCHLOROQUINE OR HCQ) (Supplementary text S1). Given that the number of articles about hydroxychloroquine and COVID-19 is rapidly growing, we also manually searched additional reference on MedRxiv preprint server and on google scholar. The language was limited to English. This meta-analysis was conducted following PRISMA statements in Supplementary text S2. This study has been recorded on the international database of prospectively registered systematic reviews, PROSPERO (Registration number: CRD42020190801).

Criteria for study selection:

Inclusion criteria were 1) reports must contain original data with available risk estimates (Hazard Ration, Odds Ratios, Relative Risk and/or with data on the number of death in HCQ and control groups 2) all publication dates will be considered 3) publications in English language 4) comparative studies with a control group without hydroxychloroquine and 5) COVID-19 confirmed cases by RT-PCR. Reviews and meta-analysis, commentaries, *in vitro* and *in vivo* studies were excluded.

Data extraction

Data extraction was performed by two investigators (Mr. T. Fiolet and Mr. Y. Mahamat-Saleh) who screened the titles and abstracts. Discrepancies were resolved by a third investigator (Dr. Anthony Guihur).

The following data were extracted from each study: study design, publication date, location, number of participants (total, in treatment and control groups, doses when available, effect size (Hazard Ratio, Odds Ratio or Relative Risk) and 95% confidence intervals for reported risk estimates. Hazard Ratio (HR) refers to the ratio of hazards in the intervention group divided by those occurring in the control group. Hazard represents the instantaneous event rate, which means the probability that an individual would experience an event (e.g. death) at a particular given point in time after the intervention, assuming that this individual has survived to that particular point of time without experiencing any event. In contrast, Relative Risk (RR) and Odds Ratio (OR) does not take account of the timing of each event. RR and OR are similar when the event (death) is rare. The most adjusted effect size reflecting the greatest control of potential confounders was extracted.

Three included studies did not report effect size for mortality risk (15,20,21). Thus we used the number of death per groups to calculate an unadjusted relative risk using *metabin* function in *meta* package in R Software (22). RR calculation is based on Cochrane Handbook for Systematic Reviews of Interventions formula $RR = \frac{\frac{\text{number of deaths in treatment group}}{\text{number of participants in treatment group}}}{\frac{\text{number of deaths in control group}}{\text{number of participants in control group}}}$ (23)

For all the other studies, reported adjusted OR, RR or HR were used. The quality of each study was assessed with ROBIN-I tool following Cochrane guidelines for non-randomized studies and with Rob2 for randomized studies (24,25).

Outcome

The outcome is COVID-19 mortality.

Statistical analysis

Effect of HCQ alone and HCQ + AZ

A primary meta-analysis was performed to assess the association between hydroxychloroquine alone (vs standard care) and risk of death. In a second time, the relationship between hydroxychloroquine associated with azithromycin and mortality was assessed. HRs, ORs and RRs were treated as equivalent measures of mortality risk. Pooled RRs were determined by using a random effect model with inverse variance weighting (DerSimonian-Laird method) (26). Significance was checked by Z-test ($p<0.05$ was considered as significant).

Heterogeneity was assessed by the Chi-square test and I^2 test. $30\%<I^2<60\%$ was interpreted as moderate heterogeneity and $I^2>60$ as high heterogeneity. Funnel plot was constructed to assess the publication bias. Begg's and Egger's test were conducted to assess the publication bias (7,27). RR or HR and their 95% confidence interval were used to assessed mortality risk.

Subgroup analysis

Subgroup analyses were further conducted according to the quality assessment to explore the source of heterogeneity among observational studies. We performed stratified analyses by continents, the type of article (peer-reviewed vs unpublished), the use of an adjustment on confounding factors (studies with $RR_{unadjusted}$ vs $RR_{adjusted}$), the mean daily dose of hydroxychloroquine (continuous), the median population age across the studies (median age>63 years) and the level of bias risk identified with ROBIN-I (moderate/serious/critical) (24), the exclusion of studies with cancer and dialysis patients. Mean daily dose of hydroxychloroquine is a daily average between the loading dose and the maintenance doses. Additionally, influence analysis was conducted by omitting each study to find potential outliers (28). It is used to detect studies which influence the overall estimate of our meta-analysis the most, omitting one study at a time (leave-one-out method).

A two-sided p-value <0.05 was considered statistically significant. All analysis were conducted using R version 3.6.1 with *meta* package and *robvis* package (29).

Results

Literature Search

After searching Pubmed and Web of Science, 105 results were identified. 7 articles from Medrxiv/Google Scholar were added. After screening the title and the abstract, only 9 articles about hydroxychloroquine and COVID-19 were included. 144 articles were excluded for not meeting the inclusion criteria. 16 articles were included for further consideration including 14 observational studies and one non-randomized trial and one unpublished randomized controlled trial (RCT): 15 articles for HCQ (10–17,20,21,30–34) and 6 articles for HCQ+AZ (10,16,30,31,35,36). Flow chart is presented in Figure 1.

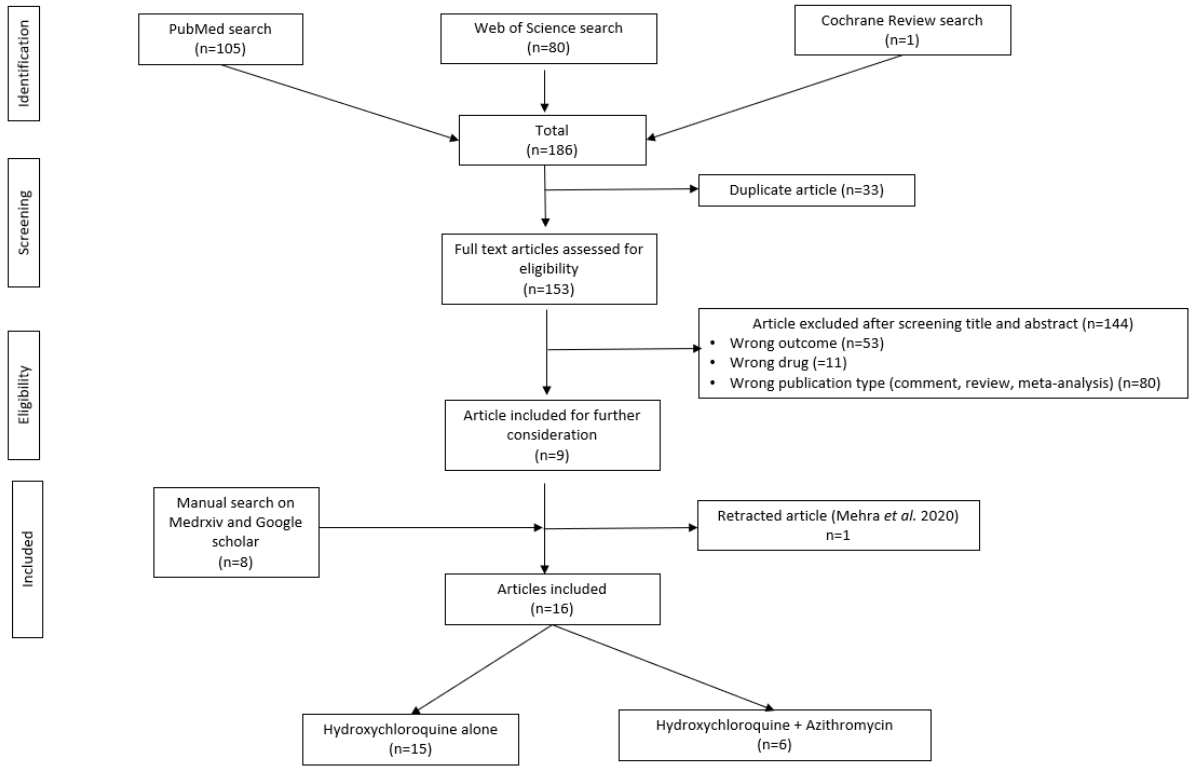

Figure 1: Flow diagram of study selection process

Study characteristics

This meta-analysis includes 8,072 patients in the hydroxychloroquine group and 7,009 patients in the standard care group with respectively 1,578 deaths and 1,423 deaths. Individual studies are described in Table 1. It appears that all the included studies were carried on hospitalized patients. No study meeting our inclusion criteria addressed the effect of HCQ on asymptomatic forms of COVID-19. Mean and median age of participants ranged from 53 to 72 across the studies. Studies were conducted in the USA (n=6) (13,16,20,30,31,36), in Spain (n=4) (14,15,33,35), in France (n=2) (11,21), in the UK (n=1)(37), in Italy (n=1) (32), in China (n=1) (12) and in 3 countries (USA, Canada and Spain)(10). 9 articles were published, and 4 articles were preprints. RECOVERY Trial data were reported by a press communication (34,37). Mean daily dose of hydroxychloroquine ranged from 333 mg/j to 945 mg/j.

First Author	Journal	Type of study	Country	Number of deaths		Number of participants		Treatment	Mean HCQ dose per day	Age[a] (years)	Patients	Study quality
				Control	HCQ	Control	HCQ					
Alberici et al(32), 2020	Kidney International	Observational, cohort	Italy	Not reported	Not reported	22	72	Not specified	NA	72 (median) IQ=62-79	Hospitalized patients with haemodialysis	Critical
Ayerbe et al(15), 2020	Journal of Thrombosis and Thrombolysis	Observational, cohort	Spain	49	237	162	1857	Not specified	NA	67,57 (mean)	Hospitalized patients	Serious
Barbosa Joshua et al(20), 2020	Unpublished	Observational, cohort	USA	1	2	21	17	800 mg for 2 days then 200-400mg for 3-4 days	600	62.7 (mean) SD=15.1	Hospitalized patients (mild/moderate symptoms)	Critical
Geleris et al(13), 2020	NEJM	Observational, cohort	USA	75	157	565	811	1200mg at day 1 then 400mg for 4 days	560	From <40 to >80	Hospitalized patients (moderate/severe symptoms)	Moderate
Ip et al(31), 2020	PrePrint	Observational, cohort	USA	115	432	598	1914	800mg at day 1 then 400mg on day 2-5 (80%)	400	64 (median) IQ=52-76	Hospitalized patients (44% moderate/severe symptoms)	Moderate
Kuderer et al(10), 2020	The Lancet	Observational, cohort	USA Canada Spain	41	11	486	89	Not specified	NA	66 (median) IQ=57-76	Hospitalized patients with who have a current or past diagnosis of cancer	Moderate
Magagnoli et al(30), 2020	Clinical Advances	Observational, cohort	USA	37	38	395	198	Median HCQ dose: 400mg/day Median HCQ+AZ dose: 422.2 mg/day	400	70 (median) IQ=60-75	Hospitalized patients	Serious
Mahevas et al(11), 2020	BMJ	Observational, cohort	France	8	9	89	84	600mg/day	600	60 (median) IQ=52-68	Hospitalized patients with covid-19	Moderate

											pneumonia who require oxygen:	
Membrillo et al(14), 2020	PrePrint	Observational, cohort	Spain	21	27	43	123	Loading dose of 800 mg + 400 mg in following days (ten days for moderate cases)	440	HCQ: 61.5 No HCQ: 68.7 (mean)	Hospitalized patients	Serious
Philippe Gautret et al(21), 2020	International Journal of Antimicrobial Agents	Non-randomised controlled trial	France	0	1	16	26	A maintenance dose of 600 mg/day	600	45,1 (mean) SD=22	Hospitalized patients (mild symptoms)	Critical
RECOVERY TRIAL	Unpublished	Randomized controlled trial	UK	736	396	3132	1542	A loading dose of 2400mg at day 1, then 800mg/day for 10 days	945	Not specified	Hospitalized patients	Not applicable
Rogado et al(35), 2020	Clinical and Translational Oncology	Observational, cohort	Spain	Not reported	Not reported	8	18	Not specified	NA	71 (median) Range:34-90	Hospitalized patients (64% severe cases)	Critical
Rosenberg et al(16), 2020	JAMA	Observational, cohort	USA	28	54	221	271	400mg then 200-400mg at 2nd prescription then 200-400mg at 3rd	333	63 (median)	Hospitalized patients	Moderate
Sanchez-Alvarez et al(33), 2020	Nefrología	Observational, cohort	Spain	32	166	53	322	Not specified	NA	71 SD=15	(85%) required hospital admission, 8% in intensive care units, with haemodialysis	Serious
Singh et al(36), 2020	PrePrint	Observational, cohort	USA	104	109	910	910	Not specified	NA	62 SD=17	Hospitalized patients	Serious
Yu et al(12),	Science	Observational,	China	238	9	502	48	400mg during	NA	68 (median)	Critically ill	Serious

2020	China Life Sciences	cohort						7-10 days		IQ: 59-77	patients	

Table 1 (continued): Characteristics of studies included in the meta-analysis for COVID-19 mortality
IQ=Interquartile range, SD=Standard Deviation, HCQ=Hydroxychloroquine, AZ=Azithromycine, NA=Not available

First Author	Effect size reported in each study[b]	Adjustments	Treatment	Control
Alberici et al(32), 2020	OR=0,44 [0,16-1,24]	Not adjusted	HCQ alone	Other antiviral and antibiotic were administered
Ayerbe et al(15), 2020	$RR_{calculated}$=0,422 [0,325-0,546]	Not adjusted	HCQ alone	Other antiviral and antibiotic were administered
Barbosa Joshua et al(20), 2020	$RR_{calculated}$=2,47 [0,24-24,98]	Not adjusted	HCQ alone	Supportive care
Geleris et al(13), 2020	HR=1,04 [0,82-1,32]	inverse probability weighting from a propensity-score	HCQ alone	Standard care not specified
Ip et al(31), 2020	HR=0.99 [0.8-1.22] HR=0.98 [0.75-1.28]	Cox model adjusted on the propensity-score variable: gender, coronary disease, stroke, heart failure, arrhythmia, African American, COPD, , renal failure, rheumatologic disorder, inflammatory bowel disease, advanced liver disease, age, diabetes mellitus, insulin use prior to hospitalization, asthma, HIV/hepatitis, any cancer, and log ferritin	HCQ alone HCQ+AZ	Group without drug
Kuderer et al(10), 2020[c]	OR=1,06 [0,51,2,2] OR=2.93 [1.79-4.79]	Adjusted for age, sex, smoking status, and obesity	HCQ alone HCQ+AZ	Treatment without AZ
Magagnoli et al(30), 2020	HR=1.83 [1.16-2.89] HR=1.31 [0.80-2.15]	Propensity score adjustment. All baseline covariates were included in the propensity score models (age, race, BMI, SpO2, breaths per minute, heart rate, T°, systolic blood pressure, ALT, AST, serum albumin, Total bilirubin, Creatinine, Erythrocytes, Haematocrit, Leukocytes, Lymphocytes, Platelets, Blood urea nitrogen, C-reactive protein	HCQ alone HCQ+AZ	Standard care
Mahevas et al(11), 2020	HR=1,2 [0,4,3,3]	Inverse probability of treatment weighting in Cox model. age, sex, comorbidities (presence of chronic respiratory insufficiency during oxygen treatment, or asthma, cystic fibrosis, or any chronic respiratory disease likely to result in decompensation during a viral infection; heart failure (New York Heart Association class III or IV); chronic kidney disease; liver cirrhosis with Child-Pugh class B or more; personal history of cardiovascular disease (hypertension, stroke, coronary artery disease, or cardiac surgery); insulin dependent diabetes mellitus, or	HCQ alone	Standard care

		diabetic microangiopathy or macroangiopathy; treatment with immunosuppressive drugs, including anticancer chemotherapy; uncontrolled HIV infection or HIV infection with CD4 cell counts <200/µL; or a haematological malignancy); body mass index (≥30 or not); third trimester of pregnancy; treatment by angiotensin converting enzyme inhibitors or angiotensin receptor blockers13; time since symptom onset; and severity of condition at admission (percentage of lung affected: ≥50% or not; presence of confusion; respiratory frequency; oxygen saturation without oxygen; oxygen flow; systolic blood pressure; and C reactive protein level).		
Membrillo et al(14), 2020	OR=0,07 [0,012,0,402]	Adjusted on variables with p<0,25in univariate analysis	HCQ alone	Standard care + other antivirals, immunomodulators, anti-inflammatory drugs
Philippe Gautret et al(21), 2020	$RR_{calculated}$ =3,41 [0,1505,77,45]	Not adjusted	HCQ alone	Group without HCQ
RECOVERY TRIAL	HR=1,1 [0,98,1,26]	Adjustment not precised	HCQ alone	Standard care
Rogado et al(35), 2020	OR=0,02 [0,01,0,73]	Adjusted by median age, histology, staging, cancer treatment received and hypertension	HCQ+AZ	Group without HCQ
Rosenberg et al(16), 2020	HR=1,08 [0,63,1,85] HR=1.35 [0.76-2.4]	Multiple adjustments on potential confounders (age>65, sex, hospital, comorbidities, respiratory capacities	HCQ alone HCQ+AZ	Group without drug
Sanchez-Alvarez et al(33), 2020	OR=0,471 [0,28,0,792]	No information about adjustments in logistic regression	HCQ alone	Standard care + other antivirals
Singh et al(36), 2020	HR=0,95 [0,74,1,23] HR=1.19 [0.89-1.60]	Creation of groups based on propensity score matching for age, gender, race, confounding comorbidities	HCQ alone HCQ+AZ	Group without HCQ
Yu et al(12), 2020	HR=0,36 [0,18,0,75]	Adjustment: respiratory rate, shortness of breath, alanine aminotransferase (when p<0,01 in univariate Cox model)	HCQ alone	Standard care

Table 1 (continued): Characteristics of studies included in the meta-analysis for COVID-19 mortality

IQ=Interquartile range, SD=Standard Deviation, HCQ=Hydroxychloroquine, AZ=Azithromycine, NA=Not available

[a]Some studies did not report mean or median age

[b]HR and OR are the most adjusted effect size reported in each study. Some studies did not report effect size. $RR_{calculated}$ were calculated using the number of death in the treatment and the control groups

Study quality

Risk of bias was assessed with ROBIN-I for non-randomised studies (n=14) and Rob2 was not applicable for RECOVERY RCT because data were not available (Figure S1). Details on the assessment of studies quality are provided in Fig S2. Among the non-randomized studies, the majority of these observational studies had a high or critical risk of bias (10 out of 16) (12,14,15,20,21,30,32,33,35,36). Five articles had a moderate risk of bias(10,11,13,16,31). Some studies did not report adjusted effect sizes to control confusion and selection bias (15,20,21,32,33,35). Studies quality was lowered by the lack of information about the assignment of treatment, the time between start of follow-up and start of intervention), some unbalanced co-intervention with other antiviral and antibiotic drugs.

Hydroxychloroquine and mortality

The pooled RR for COVID-19 mortality was 0.82 (95% CI: 0.62-1.07, I^2=82, $P_{heterogeneity}$<0.01, n=15) (Figure 2) indicating no significant association between hydroxychloroquine and COVID-19 survival or increased mortality. There was significant high heterogeneity across the included studies (I^2 =83%, p<0.01). Egger's test (p= 0.42) and Begg's test (P=0.88) were not significant for asymmetry of the funnel plot indicating that there is not a major publication bias (Figure S3). In our separated analysis by study design, we found a positive but not significant association between hydroxychloroquine alone and mortality among interventional studies (RR: 1.10, 95%CI: 0.97-1.25, I^2=0%, $P_{heterogeneity\ within}$=0.5, n=2); however an inverse but not significant association was found among observational studies (RR: 0.78, 95%CI: 0.58-1.05, I^2=82%, $P_{heterogeneity\ within}$ <0.01), with heterogeneity observed across the study design ($P_{heterogeneity\ between}$ = 0.03).

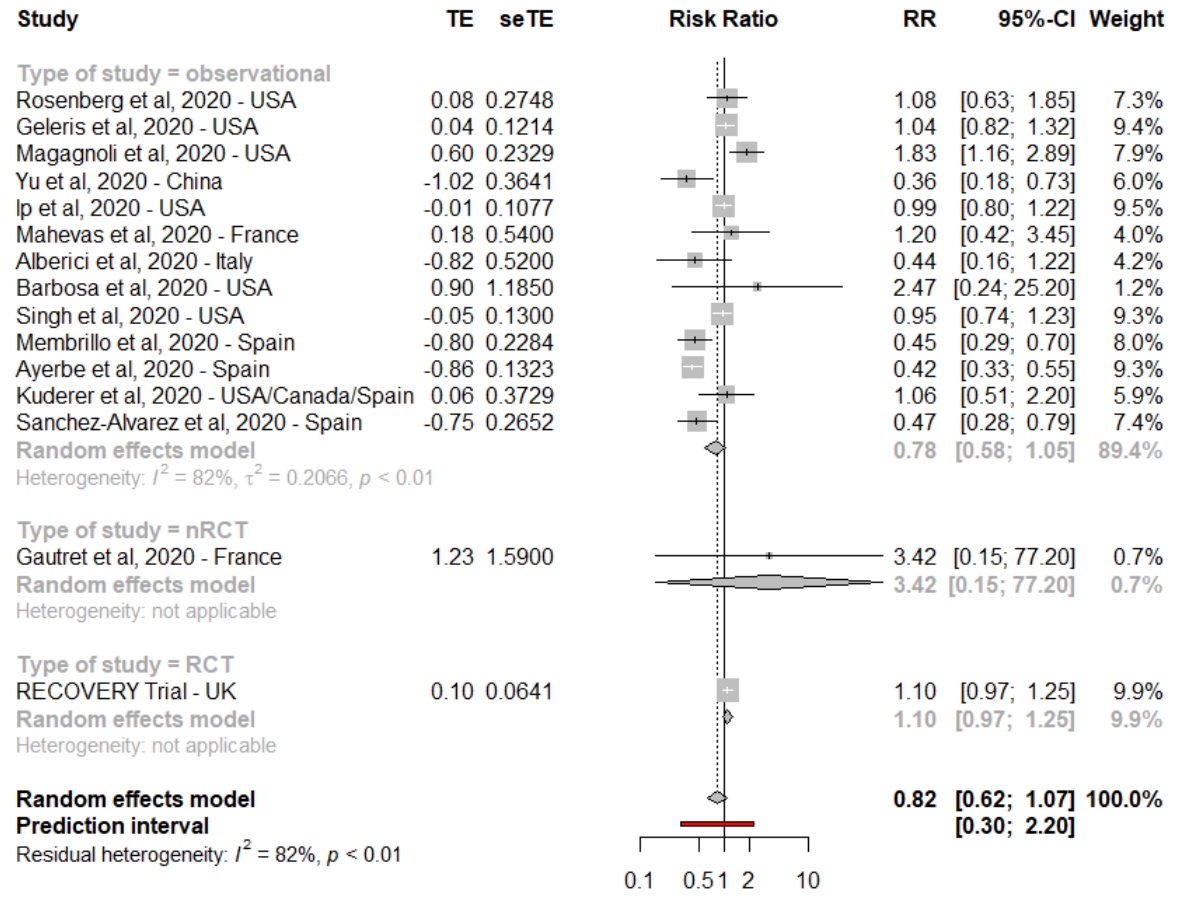

Figure 2: Meta-analysis showing association between hydroxychloroquine alone and COVID-19 mortality. RCT=Randomised Controled Trial. nRCT=non-Randomised Controled Trial TE=Estimated treatment effect. seTE=Standard error of treatment estimate. RR=Risk ratio. RR were not adjusted for Alberici et al, Ayerbe et al, Barbosa et al, Sanchez-Alvarez et al and Gautret et al. 95%CI= 95% Confidence Interval

Subgroup analysis for hydroxychloroquine alone

Subgroup analysis among all studies (observational and interventional studies) per study design, type of article (peer-reviewed vs unpublished), risk estimated, age, the exclusion of cancer/haemodialysis patients identified a non-significant association in each subgroup (Table 2).

	N	Pooled Relative Risk	Heterogeneity		
			I^2 (%)	P_{within}	$P_{between}$
HCQ alone					
All Studies	15	0.82 [0.62-1.07]	82%	<0.01	
Study Design					
Observational	13	0.78 [0.58-1.05]	82%	<0.01	0.03
Interventional	2	1.10 [0.97-1.25]	0%	0.48	
Type of article					
Peer-reviewed	10	0.78 [0.53-1.16]	83%	<0.01	0.63
Unpublished	5	0.88 [0.63-1.24]	74%	<0.01	
Adjusted estimate					
Yes	9	0.91 [0.67-1.24]	70%	<0.01	<0.0001
No	5	*0.44 [0.35-0.56]*	0%	0.41	
Missing	1	1.10 [0.97-1.25]	Not applicable	Not applicable	
Risk estimated					
Reported in the paper	12	0.86 [0.66-1.12]	73%	<0.01	0.9
Calculated	3	0.91 [0.21-3.93]	48%	0.14	
Risk of bias					
Moderate	5	1.02 [0.88-1.18]	0%	0.9	0.19
Serious	6	0.63 [0.38-1.04]	89%	<0.01	
Critical	3	0.97 [0.23-4.07]	31%	0.23	
Continents					
America	6	1.05 [0.93-1.19]	30%	0.2	0.003
Asia	1	*0.36 [0.18-0.73]*	NA	NA	
Europe	7	*0.62 [0.41-0.93]*	90%	<0.01	
Multiple	1	1.06 [0.51-2.20]	NA	NA	
Mean daily dose					
Not specified	6	*0.58 [0.39-0.85]*	80%	<0.01	0.007
<500 mg/d	4	0.97 [0.55-1.69]	84%	<0.01	
>500 mg/d	5	1.09 [0.98-1.22]	0%	0.88	
Age					
63 years or less	7	0.90 [0.65-1.26]	53%	<0.05	0.1
64 years or more	7	0.69 [0.43-1.10]	88%	<0.01	
Not specified	1	1.10 [0.97-1.07]	NA	NA	
Cancer or haemodialysis patient based-population					
No	12	0.87 [0.64-1.18]	84%	<0.01	0.26
Yes	3	0.61 [0.35-1.06]	43%	0.17	
Influence analysis (exclusion of Yu et al, Magagnoli et al, Membrillo et al, Ayerbe et al)	11	1.00 [0.90-1.12]	29%	0.17	
HCQ+AZI					
All Studies	6	1.33 [0.91-1.91]	75%	<0.01	
Study Design					
Observational	6	1.33 [0.91-1.91]	75%	<0.01	
Interventional	0				

Type of article					
Peer-reviewed	4	1.55 [0.86-2.80]	76%	<0.01	0.2
Unpublished	2	1.07 [0.88-1.30]	0%	0.35	
Adjusted estimate					
Yes	6	1.33 [0.91-1.91]	75%	<0.01	
No	0				
Risk estimated					
Reported in the paper	6	1.33 [0.91-1.91]	75%	<0.01	
Calculated	0				
Risk of bias					
Moderate	3	1.54 [0.80-2.95]	86%	<0.01	0.06
Serious	2	1.22 [0.95-1.57]	0%	0.7	
Critical	1	0.02 [0.00-0.73]	NA	NA	
Continents					
America	3	1.10 [0.91-1.32]	0%	0.48	0.0009
Asia	0				
Europe	2	0.24 [0.00-13.43]	80%	0.02	
Multiple	1	2.93 [1.79-4.79]	NA	NA	
Mean daily dose					
Not specified	3	0.75 [0.08-7.21]	87%	<0.01	0.7
<500 mg/d	3	1.10 [0.87-1.38]	0%	0.43	
>500 mg/d	0				
Age					
63 years or less	2	1.22 [0.94-1.58]	0%	0.69	0.9
64 years or more	4	1.30 [0.62-2.71]	85%	<0.01	
Cancer or haemodialysis patient based-population					
No	6	1.33 [0.91-1.91]	75%	<0.01	
Yes	0				

Table 2. Subgroup analysis for the associations between HCQ alone or HCQ associated with AZI and mortality risk of patients with COVID-19 (observational and interventional studies)
N: number of studies. NA: Not applicable for a single study

Test for subgroup differences (observational vs nRCT vs RCT) was not significant (P=0.09) suggesting no differences in the overall effect according to the design of the studies. The pooled RR for observational studies was 0.78 (95%CI: 0.58-1.05, I^2=82%, $P_{heterogeneity\ within}$ <0.01, n=13) and RR was 3.42 (95%CI: 0.15-77.20, n=1) for non-randomized controlled trial and 1.10 (95%CI: 0.97-1.25, n=1) for the RECOVERY randomized controlled trial (Figure 2).

After stratification by the level of bias from ROBIN-I evaluation, the association between hydroxychloroquine and COVID-19 mortality remained non-significant. The broadness of 95% CI and

heterogeneity increased with the risk of bias: moderate risk of bias (RR=1.02 [0.88-1.18], I^2=0, $P_{heterogeneity\ within}$ =0.9, n=5), serious risk of bias (RR=0.63, 95% CI: (0.38-1.04, I^2=89%, $P_{heterogeneity\ within}$ <0.01, n=6)) and critical risk of bias (RR=0.97, 95% CI: (0.23-4.07, I^2=31%, $P_{heterogeneity\ within}$ =0.2, n=3)) (Figure S4).

In our stratified analysis by continents (Figure S5), interestingly, we found a significant decreased risk of mortality with HCQ alone among Asian (RR_{Asia}=0.36, 95%CI: 0.18-0.73, n=1) and European studies (RR_{Europe}=0.62 (95%CI: 0.41-0.93, I^2=90%, $P_{heterogeneity\ within}$ <0.01, n=7)) but there was no significant association among American studies, with heterogeneity detected across continent ($P_{heterogeneity\ between}$=0.003).

Furthermore, we found no association between HCQ alone and mortality by HCQ daily mean dose. The pooled RR was 1.09 (95%CI: 0.98-1.22, I^2=0%, $P_{heterogeneity\ within}$=0.9, n=5), for studies with >500mg, (RR=0.97 (95%CI: 0.55-1.69, I^2=84%, $P_{heterogeneity\ within}$<0.01, n=4) for HCQ dose<500 mg and (RR=0.58 (95%CI: 0.39-0.85, I^2=80%, $P_{heterogeneity\ within}$<0.01, n=6) for an unspecified dose of HCQ, with heterogeneity detected across HCQ dose categories ($P_{heterogeneity\ between}$=0.007).

In our stratified analysis by studies which reported adjusted effect sizes (vs non-adjusted), the pooled RR for adjusted estimates was RR=0.91 (95%CI: 0.67-1.24, I^2=70%, $P_{heterigeneity\ within}$<0.01, n=9) and for non-adjusted estimates RR=0.44 (95%CI: 0.35-0.56, I^2=0%, $P_{heterigeneity\ within}$<0.41, n=5), suggesting differences in the overall effect according to the presence of adjustment on potential confounders.

Influence analysis showed that Yu et al, Membrillo et al, Ayerbe et al, Magagnoli et al are influent studies (Figure S7). Removing these studies make heterogeneity decrease at I^2=0% but the results remained non-significant (RR=1.00 (95% CI: 0.0-1.13, I^2=29%, n=11) (Table 2).

All the results remained similar after exclusion of the two interventional studies (Table S1).

Hydroxychloroquine with azithromycin and mortality

The pooled RR for COVID-19 mortality was 1.33 (95% CI: 0.91-1.921, n=6) (Figure 3) indicating no significant association between hydroxychloroquine with azithromycin and survival. There was significant high heterogeneity across the included studies (I^2 =75%, p<0.01). Egger's test (p= 0.9) and Begg's test (p=0.6) were not significant but the asymmetry in the funnel plot indicates that there could be a publication bias. However, the number of included studies is small.

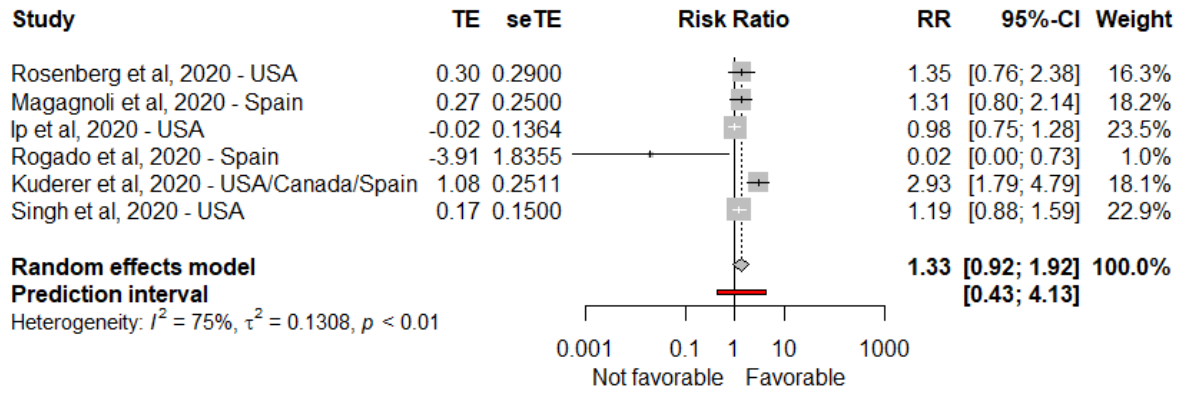

Figure 3: Meta-analysis showing association between hydroxychloroquine with azithromycin and COVID-19 mortality. TE=Estimated treatment effect. seTE=Standard error of treatment estimate. RR=Risk ratio. 95%CI= 95% Confidence Interval

Subgroup analysis for hydroxychloroquine with azithromycin

In all the subgroup analysis (type of article, effect size, risk of bias, continent, mean daily dose, age, exclusion of cancer and haemodialysis patients, influence analysis), no significant association between hydroxychloroquine with azithromycin and mortality was found (Table 2). Nevertheless, in our stratified analysis by continents, we found no significant association with COVID-19 survival risk among American studies (RR=1.10, 95%CI: 0.91-1.32, I^2=0%, $P_{heterogeneity\ within}$=0.48, n=3) and European studies (RR=0.24 (95%CI: 0.00-13.43, I^2=80%, $P_{heterogeneity\ within}$ <0.02, n=2)) but there was a significant increased risk of mortality in the multiple countries (RR=2.93, 95%CI: 1.79-4.79, n=1), with heterogeneity detected across continent ($P_{heterogeneity\ between}$=0.0009).

Discussion

This meta-analysis summarized the results of 14 observational studies, 1 non-randomised study and 1 unpublished randomised controlled trial on hydroxychloroquine with or without azithromycin and COVID-19 survival (Table 1). The results indicated that hydroxychloroquine with or without azithromycin is ineffective to reduce COVID-19 mortality risk in hospitalized patients (Figure 2 and 3). Eight observational studies reported no advantage for hydroxychloroquine (10,11,13,16,20,21,31,32). One US Veterans study identified an increased risk of death(30). Three Spanish and one Chinese studies reported a protective effect (12,14,15,33) but this benefit on survival was not replicated in two RCT, especially RECOVERY Trial which is one of the largest study. Our meta-analysis reported a high heterogeneity. The use of an adjusted effect size to control confusion bias, the daily HCQ dose, the risk of bias and the localisation of the study (by continents) may explain one part of the heterogeneity observed according to our subgroup analysis.

Subgroup analysis revealed that there was a decreased risk of death among 6 European non-randomised studies, one observation Asian study and for studies which did not specify the treatment dose. However, five (14,15,21,32,33) of these European studies have a serious or critical risk of bias (Figure S1). This significant relationship could be explained by a high risk of confusion bias since these articles did not reported adjusted effect size. These studies also have several biases, such as a selection bias Gautret et al, control and treatment groups did not come from the same hospital. In 3 Spanish studies (14,15,33), there was no information when treatment were administrated and when the follow-up began which may lead to a bias in selection. Studies with an adjusted HR in figure S5 and with a higher quality reported a non-significant higher RR than the other studies. In this meta-analysis, the majority of the included studies had a high or critical risk of bias (10 out of 16) (Figure S1 and S2). Most of them do not always report the concomitant use of antiviral or antibacterial drugs. In our subgroup analysis by study design, we found inconsistent results with a positive but not significant association between hydroxychloroquine alone and mortality among interventional studies and an inverse but not significant association among observational studies (Table 2). Heterogeneity between these subgroups was observed across the study design. However, these findings are limited by the very low number of interventional studies.

Two Chinese randomised controlled trial reported no death in both treatment and control group (38,39) and thus their results were not included in our meta-analysis. A previous review on 8 studies (11–14,20,30,39,40) on COVID-19 concluded that the level of evidence for hydroxychloroquine effect is very weak(41). A preprint meta-analysis, using routinely collected records from clinical practice in Germany, Spain, the UK, Japan, and the USA, compared the use of HCQ vs salfasalazine (42). This study observed an increased risk of 30-day cardiovascular mortality (HR=2.19 [1.22-3.94]) but there was no standard care comparative group. Some previous meta-analyses were also conducted on hydroxychloroquine and various health endpoints including mortality. However these studies did not report all the published and unpublished literature, including a very limiting number of studies: from 3 articles(17,18) to 6 articles(19). These previous meta-analyses did not perform subgroup and

sensitivity analysis to test the effect of pooling RCT and observational study, neither studying the source of heterogeneity. They used unadjusted risk ratio (calculated with the number of events in each group) whereas in our meta-analysis, we used adjusted relative risk (43) and we did sensitivity analysis on the adjustment of effect size. Statistical adjustments for key prognostic variables allow to limit confusion bias, especially in observational studies which are not randomised. Our meta-analysis confirmed the partial preliminary results of these meta-analyses about the absence of effect for HCQ on survival.

Our study has several strengths. To our knowledge, this is the first meta-analysis using adjusted relative risk and including numerous subgroup analysis (by continent, population age, effect size, risk of bias, published articles, mean daily dose of hydroxychloroquine, exclusion of cancer and haemodialysis patients) which found stable and consistent results. This study informs clinicians and patients regarding the efficiency of HCQ in treating COVID-19. We included several unpublished papers to minimize the publication bias. Our subgroup analysis by published studies (vs unpublished studies) identified that the inclusion of preprints did not change the results. Exclusion of grey literature (unpublished studies, with limited distribution) could lead to an exaggeration of the intervention effect by 15% (44). There is limited evidence to identify whether grey studies have a poorer methodological quality than published studies(45). Mortality is a reliable endpoint across studies. Limitations come from the studies which do not report adjusted effect size when mortality was not the primary endpoint. Confounding bias is high in these articles (mainly for the preprints). This meta-analysis was based on aggregated data, without access to original patient data. Most of studies are observational which do not allow to identify a causal association. This meta-analysis did not include results from the European DISCOVERY trial and the WHO SOLIDARITY trial (46). To finish, some of the included studies had very low quality of evidence (missing data, small sample size, confusion bias, bias in classification of intervention and selection bias) but the exclusion of these articles did not change the results.

Few peer-reviewed studies with a comparative group analysed some other endpoints such as virological clearance, clinical improvement and arrhythmia risks. A recent randomized controlled trial with 821 asymptomatic participants in contact with a COVID-19 confirmed case, concluded hydroxychloroquine was not efficient to prevent illness in a prophylactic way (47). However, this trial had a limitation: only 16 participants had a confirmed positive RT-PCR test. A small French non-randomised trial identified a higher proportion of negative RT-PCR tests in the HCQ group (21) but two other RCT did not find any difference between the HCQ and standard care groups for clinical improvement (38,39).

Several studies raised concerns about an increase of the QTc interval with HCQ use in an intensive care unit (48) and hospitalized patients (11,49). However, this side effect was not found in Tang et al. RCT. Several national health organisations (US FDA Food and Drug Administration(50), French Agency for the Safety of Health Products ANSM (51), European Medecine Agency EMA(52)) raised concerns about using this unapproved drug for COVID-19. ANSM et US FDA removed the authorization for its use outside of clinical trials. The Indian Council of Medical Research took an opposite position and recommend chemoprophylaxis with hydroxychloroquine for asymptomatic cases (53). In an open label, randomised controlled trial with hydroxychloroquine in patient with mild and moderate symptoms, no death were reported (38). Finally, in the comparative peer-reviewed studies, a clear conclusion on hydroxychloroquine is not possible due to the small sample size, the lack of well-performed randomised controlled trials (mainly non-randomised and retrospective studies) and inconsistent results. Many preprints without comparative group and without randomization bring confusion in this highly politicised topic. There is a gap between the speed of clinical research and the expectation of a clear solution to treat COVID-19 patients. Indeed, producing robust clinical trials is necessarily time-consuming. Results from large RCT are needed to shut down the controversy.

Conclusion

In conclusion, there is no strong evidence supporting a benefice for hydroxychloroquine with or without azithromycin to improve survival of COVID-19 hospitalized patients. Conversely, there is no strong evidence supporting an increased mortality associated with HCQ or HCQ + AZ intake.

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List of figures and tables

Supplementary tables and figures

Supplementary tables and figures

S1 Fig. Summary of risk of bias analysis for non-randomised studies (ROBIN-I)

S2 Fig Assessment of quality of studies using ROBIN-I for non-randomised studies.

S3 Fig.: Funnel plot for hydroxychloroquine alone and COVID-19 mortality risk

S4 Fig. Forest plot for hydroxychloroquine alone and COVID-19 mortality risk, subgroup analysis per risk of bias

S5 Fig. Forest plot for hydroxychloroquine alone and COVID-19 mortality risk, subgroup analysis per continent

S6 Fig. Forest plot for hydroxychloroquine alone and COVID-19 mortality risk, subgroup analysis per hydroxychloroquine dose

S7 Fig. Influence analysis for hydroxychloroquine and COVID-19 mortality

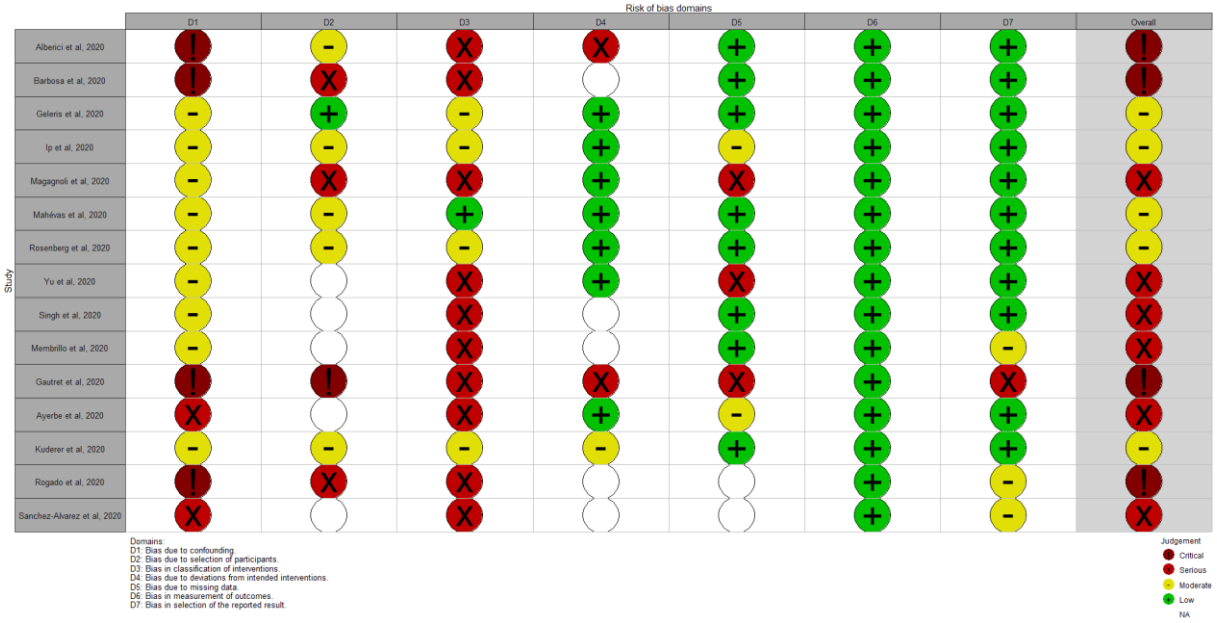
S1. Full electronic search strategy

S2: PRISMA Checklist

S1 Table. Subgroup analysis for the associations between HCQ+AZI and mortality risk of patients with COVID-19 (observational studies)

Figure S1: Summary of risk of bias analysis for non-randomised studies (ROBIN-I)

729 Figure S2: Assessment of quality of studies using ROBIN-I for non-randomised studies.



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761 Figure S3: Funnel plot for hydroxychloroquine alone and COVID-19 mortality risk

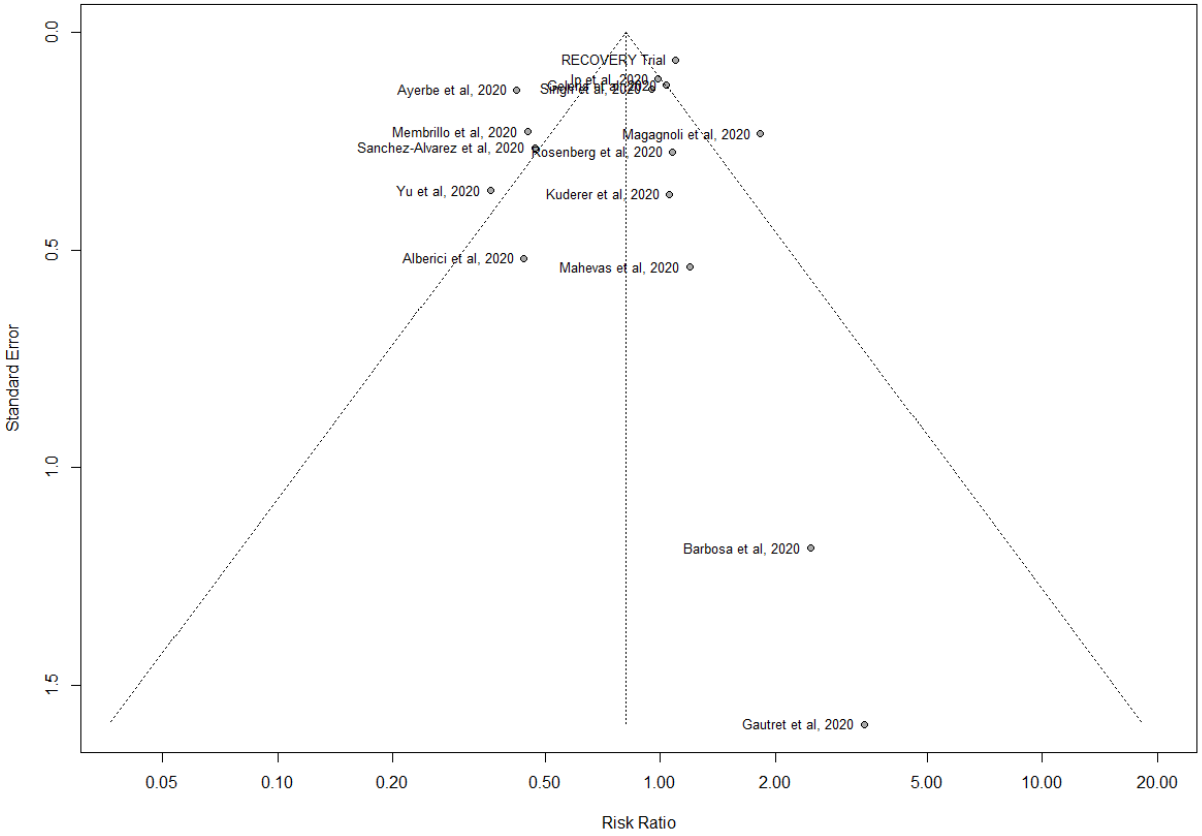

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Figure S4: Forest plot for hydroxychloroquine alone and COVID-19 mortality risk, subgroup analysis per risk of bias

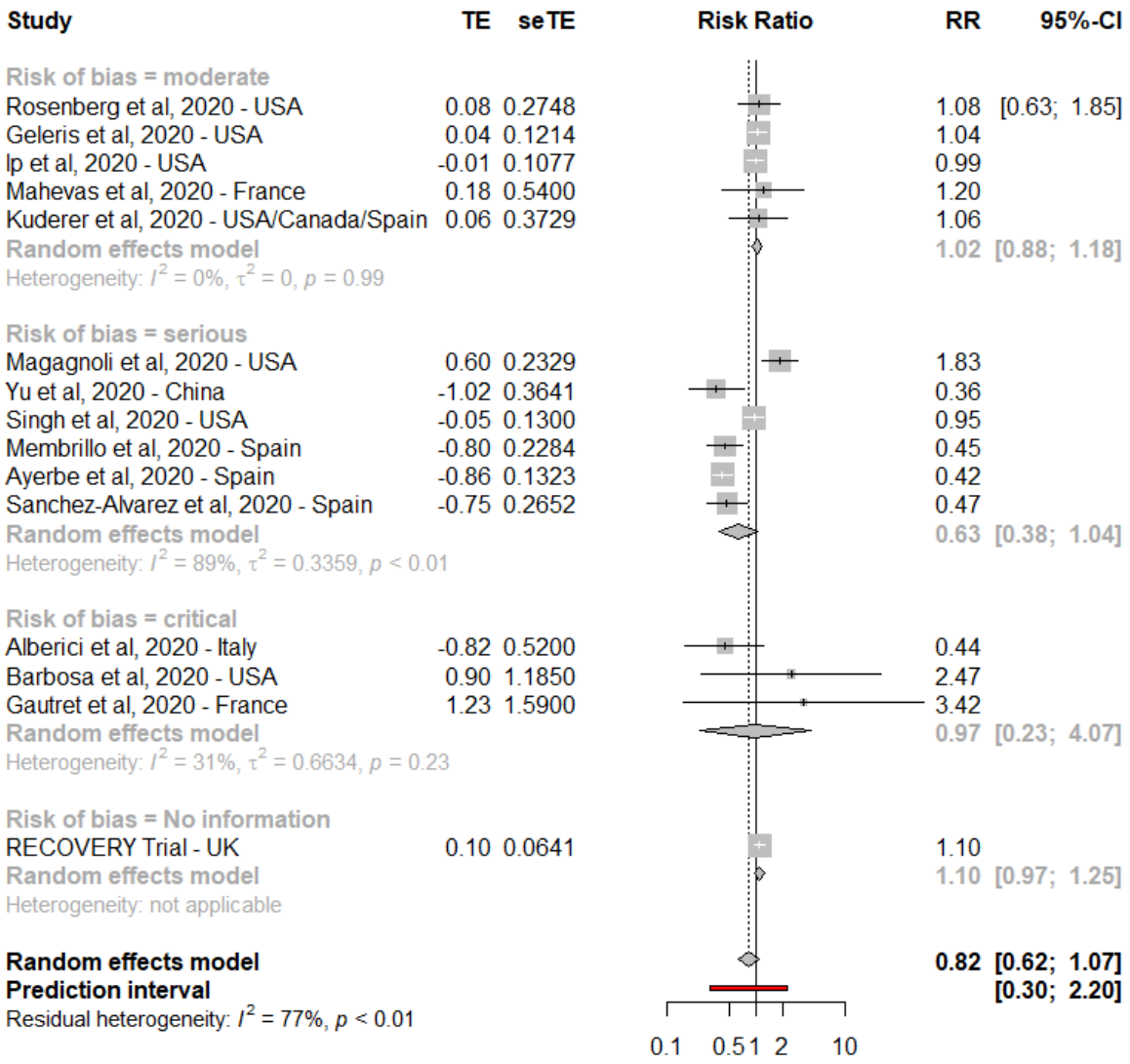


Figure S5: Forest plot for hydroxychloroquine alone and COVID-19 mortality risk, subgroup analysis per continent

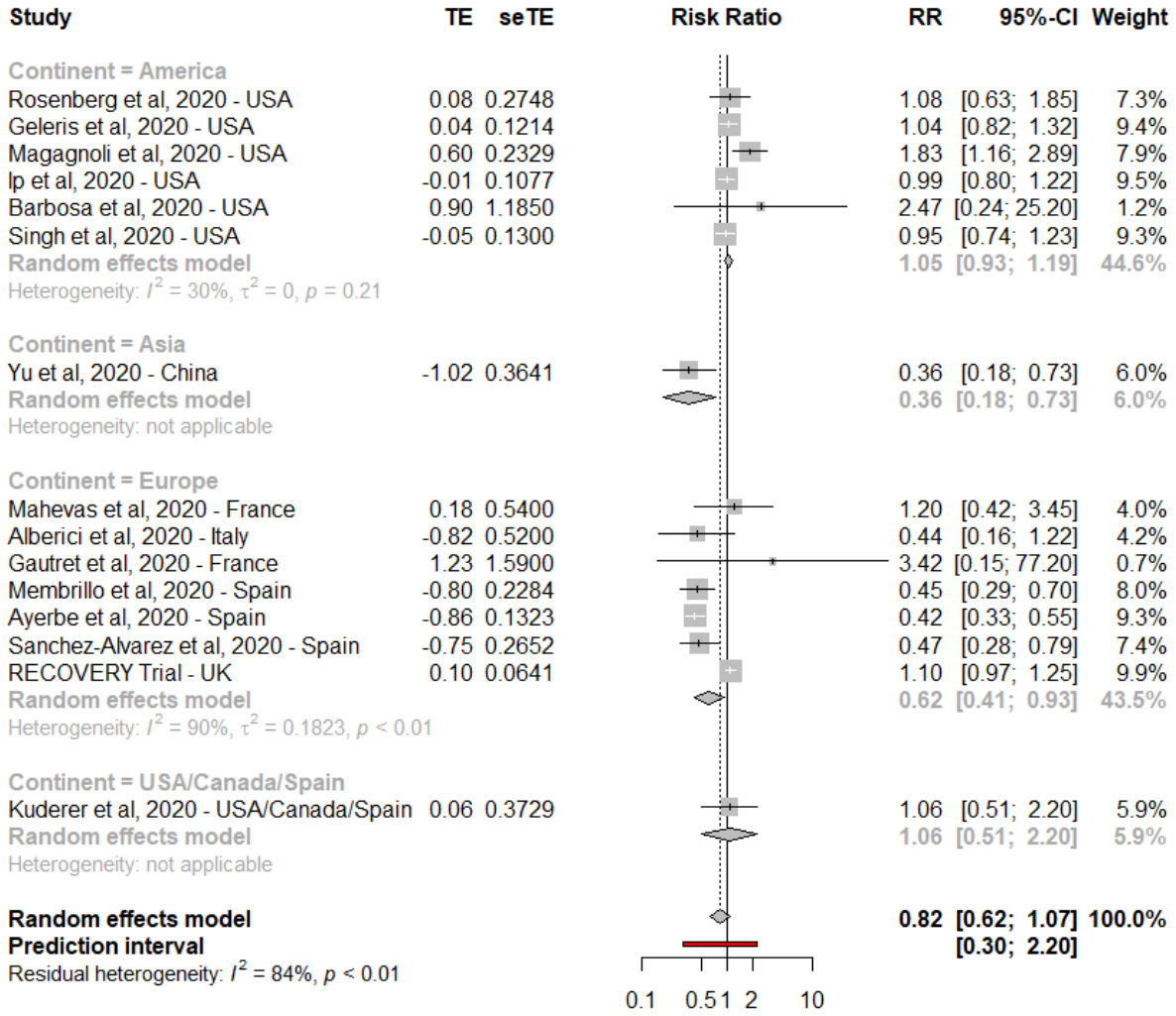
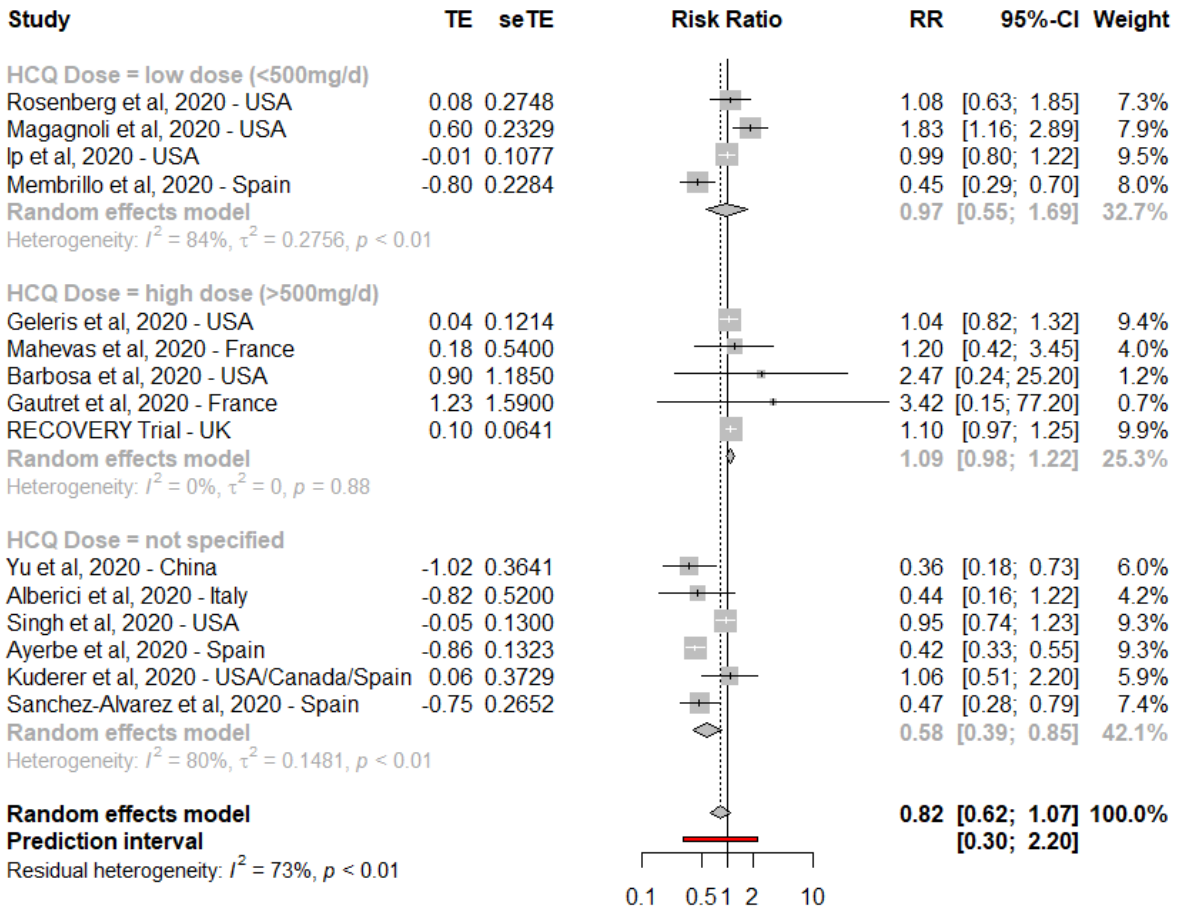
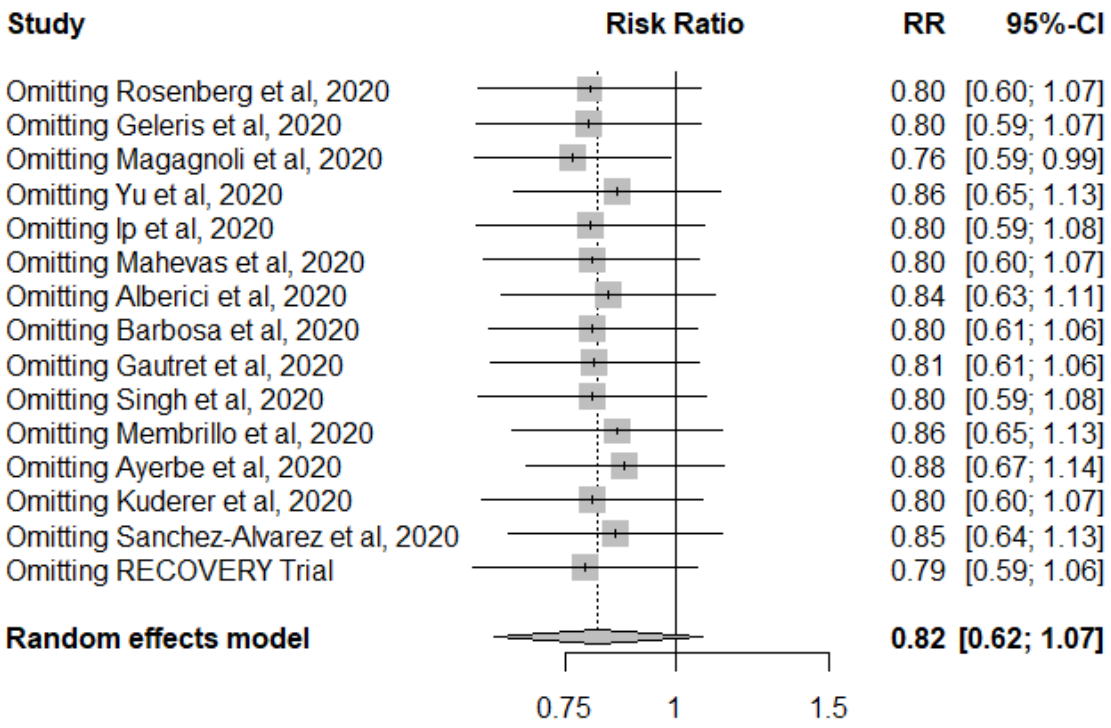


Figure S6: Forest plot for hydroxychloroquine alone and COVID-19 mortality risk, subgroup analysis per hydroxychloroquine dose



841 Figure S7: Influence analysis for hydroxychloroquine and COVID-19 mortality



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S1. Full electronic search strategy

Cochrane Library
Website: https://www.cochranelibrary.com/advanced-search
Cochrane Review matching (Hydroxychloroquine or HCQ) in Title Abstract Keyword AND (mortality or death) in Title Abstract Keyword AND (COVID-19 or SRAS-CoV-2) in Title Abstract Keyword - (Word variations have been searched)

PubMed
Website:
https://pmlegacy.ncbi.nlm.nih.gov/pubmed/?term=(hydroxychloroquine+or+HCQ)+AND+(COVID-19+OR+SARS-CoV-2+OR+coronavirus)+AND+(Mortality+OR+death)
((hydroxychloroquine or HCQ) AND (COVID-19 OR SARS-CoV-2 OR coronavirus) AND (Mortality OR death)

Web of Science
Website:
http://apps.webofknowledge.com.proxy.insermbiblio.inist.fr/Search.do?product=UA&SID=F6KgcWI7K6kjXJwhAoH&search_mode=GeneralSearch&prID=9a27b347-ecf8-4832-9206-db1bbd2cc9a8
You searched for: TOPIC: (covid-19 OR SRAS-CoV-2) AND TOPIC: (hydroxychloroquine or HCQ) AND TOPIC: (mortality or death)

Manual additional searches:
MedRxiv
https://www.medrxiv.org/
Search: Hydroxychloroquine COVID-19 mortality
Google scholar:
https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=hydroxychloroquine+COVID-19&btnG=
Search: Hydroxychloroquine COVID-19 mortality

903 S2. PRISMA Checklist

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Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	p.1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	p.2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	p.3 Lines 110-138
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	p.3 Lines 139-141
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	3 Line 154
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	p.4 Lines 170-187
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	p.3 Lines 146-152
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	p.3 lines 147-152 p.29 S1.
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	p.4 lines 159-164
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	p.4 lines 166-169
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	p.4 lines 170-186

Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	p.4 lines 184-186
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	p.4 lines 171-183
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	p.4-5 lines
Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	p.4 line 202-203
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	p.5 lines 208-218
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	p.5 Fig. 1
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	p. 6-10 Table 1
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	p.23 Supplemantary Figures S1 and S2
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	5-7
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	p.11-16 Fig.2-3 Table 2
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	p.11 lines 266-273 p.15 lines 371-374 Figure S3
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	p.13-15 Lines 313-367 p.16 lines 381-389 Table S1
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	p.16 lines 393-437
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g.,	p.17 lines 439-454

		incomplete retrieval of identified research, reporting bias).	
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	P.17 lines 465-486
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	Funding information is entered in the financial disclosure section of the submission system

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907 Table S1: Subgroup analysis for the associations between HCQ+AZI and mortality risk of
908 patients with COVID-19 (observational studies)

Subgroup analysis for the associations between HCQ and mortality risk of patients with COVID-19 (excluding interventional studies)					
	N	RRpooled	Heterogeneity		
			I^2 (%)	P_{within}	$P_{between}$
HCQ alone					
All Studies	13				
Type of article					
Peer-reviewed	9	0.76 [0.51-1.13]	85%	<0.01	0.84
Unpublished	4	0.81 [0.52-1.27]	72%	0.01	
Adjusted estimate					
Yes	9	0.91 [0.67-1.24]	70%	<0.01	0.0001
No	4	*0.44 [0.35-0.55]*	0%	0.52	
Risk estimated					
Reported in the paper	11	0.83 [0.61-1.11]	72%	<0.01	0.82
Calculated	2	0.69 [0.15-3.25]	54%	0.14	
Risk of bias					
Moderate	5	1.02 [0.88-1.18]	0%	0.9	0.18
Serious	6	0.63 [0.38-1.04]	89%	<0.01	
Critical	2	0.75 [0.16-3.58]	44%	0.18	
Continents					
America	6	1.05 [0.93-1.19]	30%	0.2	<0.0001
Asia	1	*0.36 [0.18-0.73]*	NA	NA	
Europe	5	*0.45 [0.37-0.55]*	0%	0.47	
Multiple	1	1.06 [0.51-2.20]	NA	NA	
Mean daily dose					
Not specified	6	0.58 [0.39-0.85]	80%	<0.01	0.029
<500 mg/d	4	1.06 [0.84-1.33]	0%	0.75	
>500 mg/d	3	0.58 [0.39-0.85]	80%	<0.01	
Age					
63 years or less	6	0.89 [0.64-1.24]	59%	0.03	0.39
64 years or more	7	0.69 [0.43-1.10]	89%	<0.01	

Cancer or hemodialysis patient based-population					
No	10	0.83 [0.59-1.18]	85%	<0.01	0.34
Yes	3	0.61 [0.35-1.06]	43%	0.17	
Influence analysis (exclusion of Yu et al, Magagnoli et al, Membrillo et al, Ayerbe et al)	9	0.95 [0.84-1.08]	27%	0.20	