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Hydroxychloroquine and mortality in COVID-19 patients: a systematic review and a meta-analysis of observational studies and randomized controlled trials

Augusto Di Castelnuovo [a], Simona Costanzo [b], Antonio Cassone [c], Roberto Cauda [d], Giovanni De Gaetano [b] and Licia Iacoviello [b,e]

[a]Mediterranea Cardiocentro, Naples, Italy; [b]Department of Epidemiology and Prevention, IRCCS Neuromed, Pozzilli (IS), Italy; [c]Polo Della Genomica, Genetica E Biologia, Università Di Siena, Siena, Italy; [d]Fondazione Policlinico Universitario A. Gemelli IRCCS Università Cattolica S. Cuore Roma, Italy; [e]Research Centre in Epidemiology and Preventive Medicine (EPIMED), Department of Medicine and Surgery, University of Insubria, Varese, Italy

ABSTRACT

Background: Hydroxychloroquine (HCQ) was proposed as potential treatment for COVID-19, but its association with mortality is unclear. We reviewed published literature for evidence of an association between HCQ (with or without azithromycin (AZM)) and total mortality in COVID-19 patients.

Methods: Articles were retrieved until April 29th, 2021 by searching in seven databases. Data were combined using the general-variance-based method.

Results: A total of 25 cohort studies (N=41,339 patients) and 11 randomized clinical trials (RCTs; N=8,709) were found. The use of HCQ was not associated with mortality in meta-analysis of RCTs (pooled risk ratio (PRR): 1.08, 95%CI: 0.97-1.20; I^2=0%), but it was associated with 20% lower mortality risk (PRR=0.80, 95%CI: 0.69-0.93; I^2=80%) in pooling of cohort studies. The negative association with mortality was mainly apparent by pooling cohort studies that used lower doses of HCQ (≤400 mg/day; PRR=0.69, 95%CI: 0.57-0.87). Use of HCQ+AZM (11 studies) was associated with 25% non-statistically significant lower mortality risk (PPR=0.75; 0.51-1.10; P=0.15). Use of HCQ was not associated with severe adverse events (PRR=1.12, 95%CI: 0.88-1.44; I^2=0%).

Conclusions: HCQ use was not associated with mortality in COVID-19 patients in pooling results from RCTs (high level of certainty of evidence), but it was associated with 20% mortality reduction when findings from observational studies were combined (low level of certainty of evidence). The reduction of mortality was mainly apparent in observational studies where lower doses of HCQ were used. These findings might help disentangling the debate on HCQ use in COVID-19.

KEYWORDS
SARS-COV-2; COVID-19; hydroxychloroquine; mortality

Introduction

The aminoquinoline hydroxychloroquine (HCQ) is an anti-malaria drug with immunomodulatory and anti-thrombotic properties, currently used in the treatment of autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus and anti-phospholipid syndrome [1,2]. At the beginning of the pandemic, it was proposed as a possible therapy in COVID-19 patients since it could directly inhibit viral entry and spread in several *in vitro* and *in vivo* models [3]. Indeed, HCQ has been used in Ebola virus disease [4], human immunodeficiency virus (HIV) infection [5], SARS-CoV-1 infection and the Middle East Respiratory Syndrome [6].

Despite the lack of evidence of efficacy from few randomized clinical trials, HCQ became very popular and widely used by many clinicians. In Italy over 70% of COVID-19 hospitalized patients were treated with HCQ during the first wave of pandemic [7]. The publication of a very questionable study [8] by one of the most reliable scientific journals showing that the use of HCQ was associated to an increased risk of death, lead to the pausing of several clinical trials, including the Solidarity trial [9]. The study [8] was retracted 13 days after publication [10]. Several national agencies for drug regulation decided to suspend the authorization to use HCQ for COVID-19 treatment or prophylaxis.

Several observational studies and RCTs have been published aimed at investigating the association of HCQ use and mortality in COVID-19 patients [11]. However, a number of questions remain open on the relationship between HCQ treatments in COVID-19 patients: is there a dose issue? Does mortality rate of a population or the severity of the disease affect HCQ efficacy? Is there any interaction with other anti-COVID19 drugs? More recently, at least three large, well-conducted observational studies have been published showing that HCQ decreases mortality risk in hospitalized COVID-19 patients [7,12,13]. All these

CONTACT Licia Iacoviello, MD, PhD licia.iacoviello@moli-sani.org Department of Epidemiology and Prevention, IRCCS Neuromed, via dell'Elettronica 1 86077 Pozzilli (IS), Italy. Phone: +393485108779.
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studies, which have not been included in previous meta-analysis [11], used HCQ doses lower than those administered in randomized clinical trial (RCT), such as the Solidarity or the Recovery trials [9,14].

Therefore, we decided to conduct an updated meta-analysis on observational and RCT studies on HCQ use and the mortality outcome in patients hospitalized for COVID-19. We also performed subgroup analyses to dissect whether treatment effects differ according to characteristics of the primary studies (quality of studies, peer-reviewing status, level of adjustment, sample size, setting and the effects of HCQ dosage).

Methods

This systematic review with meta-analysis was conducted according to the recommendations outlined in the Cochrane Handbook for Systematic Reviews of Interventions, version 5.1.0, and reported in line with the PRISMA statement. Institutional review board approval was not required as the study did not directly involve human participants.

Search strategy and data extraction

Flow diagram for study selection is reported in Figure 1. Articles published in English were retrieved from inception to March 17th, 2021 by searching in Medline, Embase, PubMed, Web of Science, Cochrane Central Database, MedRvix and Preprints.org, with the search terms: '(COVID-19 OR Cov-Sars-2) AND (hydroxychloroquine OR chloroquine)'. In addition, the reference lists of relevant articles for potential studies were also manually reviewed. After initial search, the duplicate results were removed. The remaining articles were screened for relevance by their titles and abstracts by two of us independently (SC and ADC). All selected potential articles were then reviewed by the remaining investigators to ensure their eligibility for inclusion. Disagreements about eligibility of the literature were resolved by consensus based on the agreements of all investigators.

To be included in this meta-analysis, the study had to meet the following criteria: (1) clinical trials or cross-sectional studies or cohort studies; (2) quantitatively investigating the difference in mortality risk in unselected COVID-19 patients according to use or not of HCQ. We only included studies in which HCQ was being used therapeutically, and excluded studies of prophylaxis.

Forty-two articles were identified [7,9,12–51]. For 11 of them [12,15,17,19,21,23,25,31,32,34,47] it was possible to extract data necessary for comparing HCQ+AZM *versus* no HCQ+AZM. For all other studies, it was not possible to systematically distinguish if HCQ therapy was complemented or not with AZM. Two investigators (SC and ADC) independently assessed the methodological quality of each included study by using the *Joanna Briggs Institute Critical Appraisal Checklists* [52], developed to assess quality of non-randomized studies such as cohort and cross-sectional studies. Each item of the checklist scored '0' if it was answered 'no', it scored '1' if it was answered 'unclear', if the item was answered 'yes', it scored '2'. A score higher than 80% of the total has been used as indicator of a better methodology quality and a low risk of bias [52]. Certainty of evidence was

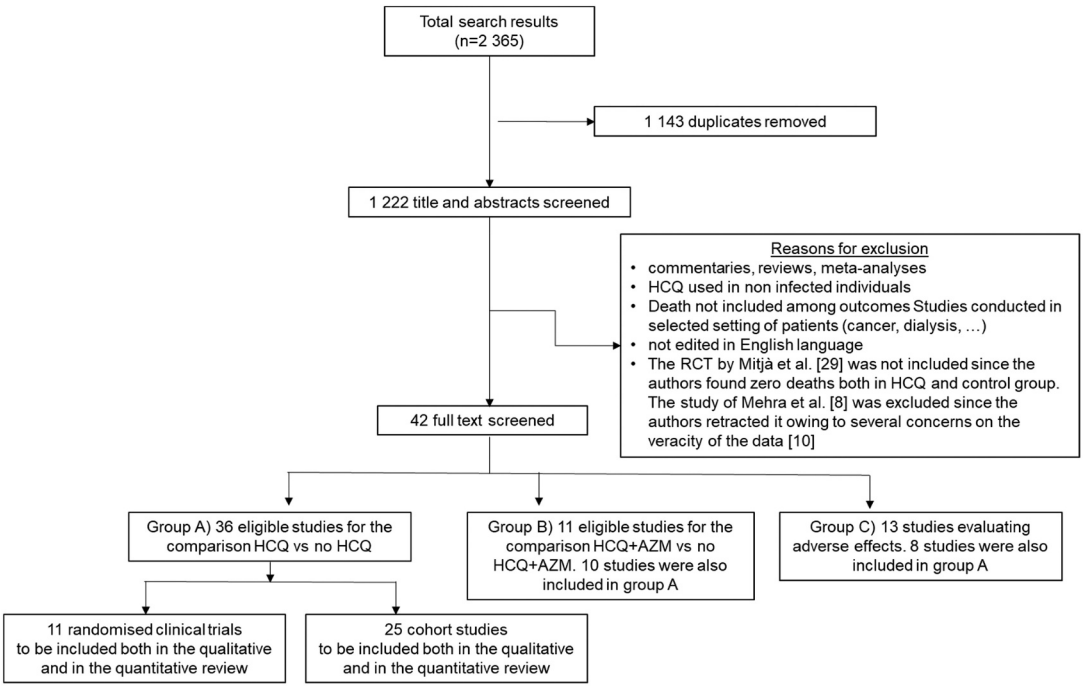

Figure 1. Flow diagram for study selection. AZM means azithromycin; HCQ means hydroxychloroquine

assessed using the GRADE (Grading of Recommendations Assessment, Development, and Evaluation) approach [53]. This method evaluates the certainty of evidence by assessing the following domains: study limitations, directness, consistency, precision, and publication bias and, as additional domains, dose-response association, plausible confounding that would decrease observed effect and strength of association. Disagreements were resolved by consensus or by a third investigator (LI), if consensus could not be reached.

Data analysis

For each study, odds ratio (OR) or hazard ratio (HR) and/or number of events (number of deaths and number of total COVID-19 patients) in both the HCQ (or HCQ+AZM) and respective control groups were extracted. If available, measure of association adjusted for covariates were retrieved. Number of events were used to calculate relative risk and 95% confidence intervals (CIs) when other measures of association were not available from the primary study. The following information was also extracted: study design, if the article was not peer-reviewed, region, level of adjustment, sample size, mortality rate in the entire cohort, percentage of patients treated with HCQ, mean duration of the treatment, mean daily dose after the first day and mean total dose of HCQ used. The total dose of HCQ was calculated as the sum of the amount of drug used in the first day plus daily dose multiplied by number of days of treatment after the first.

Pre-specified subgroup analyses have been conducted for all the additional characteristics retrieved.

All analyses were performed using standard statistical procedures provided in RevMan5.1 (The Cochrane Collaboration, Oxford, United Kingdom). Data were combined using the general variance-based method, that requires information on the relative risk (or OR or HR) estimate and their 95% CI for each study. Heterogeneity was assessed using the Higgin's I^2 metric. Fixed and random effects were considered, but due to the large heterogeneity observed in cohort studies, findings from random effects were considered as primary analysis. Five studies [29,40–42,50] reported zero deaths in HCQ and/or control group, or mortality was not the outcome; these studies were only included in the meta-analysis of adverse effect. The hypothesis that publication bias might have affected the validity of the estimates was visually tested by a funnel plot–based approach.

Results

Characteristics of the studies

The workflow of the process of study selection is reported in Figure 1. A total of 42 articles were found in the search. Thirty-six of them were enrolled for analyzing the association with mortality of HCQ use in patients with COVID-19 (11 RCTs and 25 cohort studies), 11 were valuable for analyzing the association of HCQ+AZM and 13 for evaluating adverse effects of HCQ (Figure 1).

The main characteristics of the studies included in the meta-analyses are shown in Table 1. Data from 3 independent cohorts were extracted from the study of Kim et al. [46]; 7 articles were not published in peer reviewed journals; 4 observational studies reported unadjusted relative risks for the association between HCQ and mortality; 13 studies have been conducted in Europe, 17 in North America (Canada, USA or Mexico), 3 in Asia (China, Saudi Arabia) and three in other countries. The outcome considered was total mortality, with the exception of Geleris et al. [18] in which the authors used a combined endpoint formed by intubation or death; the mortality was intra-hospital, with the exception of the studies [28,33,35,36], in which death was all-cause, all-location. All studies included hospitalized patients, with the exclusion of three ones which included ambulatory [28,36] or non-hospitalized individuals [35]. In all studies the control group was formed by patients without HCQ exposure (HCQ or HCQ+AZM). All studies included adult men and women COVID-19 patients, with the exception of two RCT [9,35], that included a portion of individuals with uncertain positivity to Sars-CoV-2. A total of N = 50,048 COVID-19 patients (including N = 8,709 from the 11 RCTs) were counted in the meta-analysis of HCQ, and N = 2,014 in the meta-analysis of HCQ+AZM. All but two cohort studies [24,48] (Supplementary Table 1) and three RCTs [9,38,49] (Supplementary Table 2) reported more than 80% of positive response to the Joanna Briggs Institute Critical Appraisal Checklists.

HCQ and death: RCT studies

Forest plot on the association between HCQ and mortality is reported in Figure 2, separately for RCTs and cohort studies.

HCQ use was not associated with lower mortality after pooling data from 11 RCTs (pooled risk ratio: 1.08, 95%CI: 0.97 to 1.20; I^2 = 0%). After removal of three RCTs [9,38,49] with an overall appraisal lower than 80%, the findings remain unchanged: pooled risk ratio 1.06, 95%CI: 0.95 to 1.19; I^2 = 0%. The overall strength of evidence grade for the null association of HCQ use with total mortality observed in RCTs was judged high (Supplementary Table 3).

HCQ and death: cohort studies

Data pooling from 25 observational studies (27 cohorts) shows that the use of HCQ is associated with 20% lower mortality risk (pooled risk ratio: 0.80, 95%CI: 0.69 to 0.93;

Table 1. Characteristics of the studies included in the meta-analysis.

Study	Country	Type of study	N. of patients	HCQ treatment (%)	Mortality (%)	Duration (day, median)	Daily dose (mg/day)	Total dose (mg)
Abd-Elsalam S[38]	Egypt	RCT	194	50.0	5.7	16	400	6800
Cavalcanti AB[17]	Brazil	RCT	332	47.9	2.6	7	800	5600
Chen J[40]*	China	RCT	30	50.0	NA	5	400	2000
Chen L[41]*	China	RCT	30	60.0	0	10	400	4000
Chen Z[42]*	China	RCT	62	50.0	NA	5	400	2000
Dubée V[43]	France	RCT	247	50.0	6.9	9	400	4000
Gonzalez JLB[44]	Mexico	RCT	70	47.1	11.4	5	400	2400
Hernandez-Cardenas C[45]	Mexico	RCT	214	52.6	39.3	10	400	4000
Horby P[14]	United Kingdom	RCT	4716	33.1	25.6	10	800	9200
Lyngbakken MN[49]	Norway	RCT	53	50.9	3.8	7	800	5600
Mitjà O[29]*	Spain	RCT	293	46.4	0	7	400	3600
Pan H[9]	30 Countries worldwide	RCT	1853	51.1	11.1	11	800	10,000
Self WH[33]	USA	RCT	479	50.5	10.4	5	400	2400
Skipper CP[35]	USA and Canada	RCT	423	50	0.5	5	600	3800
Tang W[50]*	China	RCT	150	46.7	0	17	800	16,400
Ulrich RJ[51]	USA	RCT	128	52.3	10.2	5	400	2400
Albani F[15]	Italy	Cohort	816	25.9	25.7	6	400	2400
Arshad S[11]	USA	Cohort	1611	74.6	18.1	5	400	2400
Ayerbe L[16]	Spain	Cohort	2019	92.0	14.5	5	400	2800
Awad N[39]	USA	Cohort	336	55.9	27.7	5	400	2400
Catteau L[12]	Belgium	Cohort	8075	56.2	21.8	5	400	2400
Di Castelnuovo A[7]	Italy	Cohort	3451	76.3	16.7	10	400	4400
Geleris J[18]	USA	Cohort	1376	58.9	16.8	5	400	2800
Ip A[19]	USA	Cohort	2256	84.8	22	5	400	2400
Kalligeros M[20]	USA	Cohort	108	33.3	0.9	5	NA	NA
Kim EJ[46]	USA	Cohort	A: 576	33.3	14.9	NA	NA	NA
			B: 2816	50.0	21.7			
			C: 528	33.3	14.7			
Lagier JC[21]	France	Cohort	400	25.3	0.9	10	600	6000
Lamback BE[47]	Brazil	Cohort	193	52.3[§]	11.4	5	400	2400
Lammers AJJ[22]	The Netherlands	Cohort	689	27.4	18	NA	NA	NA
Lauriola M[23]	Italy	Cohort	80	21.3	38.7	10	600	6000
Lecronier M[24]	France	Cohort	80	47.5	31.0	NA	400	NA
Lotfy SM[48]	Saudi Arabia	Cohort	202	49.0	5.5	6	400	2800
Magagnoli J[25]	USA	Cohort	277	51.1	17.3	5	400	2000
Mahévas M[26]	France	Cohort	173	50.8	9.4	2	600	1200
Membrillo FJ[27]	Spain	Cohort	166	73.5	28.9	5	400	2800
Mikami T[28]	USA	Cohort	2820	73.7	21.7	5	NA	NA
Paccoud O[30]	France	Cohort	84	45.2	6.2	10	600	6000
Rosenberg ES[31]	USA	Cohort	492	55.1	20.3	NA	800	NA
Sbidian E[32]	France	Cohort	4415	14.1	21.4	10	400	4200
Singh S[34]	USA	Cohort	1402	50	11.7	NA	NA	NA
Sulaiman T[36]	Saudi Arabia	Cohort	5541	32.8	1.1	5	400	2400
Yu B[37]	China	Cohort	550	8.7	44.9	8	400	3200

*Since in this study zero deaths in HCQ and/or control group has been observed, or mortality was not the outcome, it was included in the meta-analyses of adverse effect only. [§] prevalence of HCQ+AZM treatment. AZM: azithromycin; HCQ: hydroxychloroquine; RTC means randomized clinical trial; NA means not available

high level of heterogeneity: I^2 = 80%, random effects; Figure 2). After the exclusion of 2 cohort studies [24,48] with an overall appraisal lower than 80% (Supplementary Table 1) the HCQ association with lower mortality remains unchanged (pooled risk ratio for cohort studies: 0.80, 95%CI: 0.68 to 0.94; I^2 = 82%,) as it does after exclusion of the study by Geleris et al. [18] (which used a combined outcome of intubation and death; pooled risk ratio: 0.83, 95%CI: 0.72 to 0.95; I^2 = 77%), or of the study by Membrillo et al. which appears as outlier (0.81, 95%CI: 0.70 to 0.94; I^2 = 80%).

Since large heterogeneity in pooling data from cohort studies was found (I^2 = 80%), as an alternative, we provide here a narrative description of results from individual observational studies, the same included in the quantitative review and whose characteristics are illustrated in Table 1. Namely, 8 studies reported a statistically significant association of HCQ use with lower mortality, with a relative risk ratio ranging from 0.07 to 0.70 among studies. Eight studies found that HCQ use was associated with a non-statistically significant reduced relative risk of mortality (range 0.62 to 0.99) and 11 cohorts reported a positive, non-statistically significant association with death (range 1.04 to 1.67). No studies found a positive, statistically significant association of HCQ use with mortality.

As the body of evidence has several deficiencies (Supplementary Table 3), the overall strength of evidence grade for the association of HCQ use with total mortality observed in cohort studies was judged low.

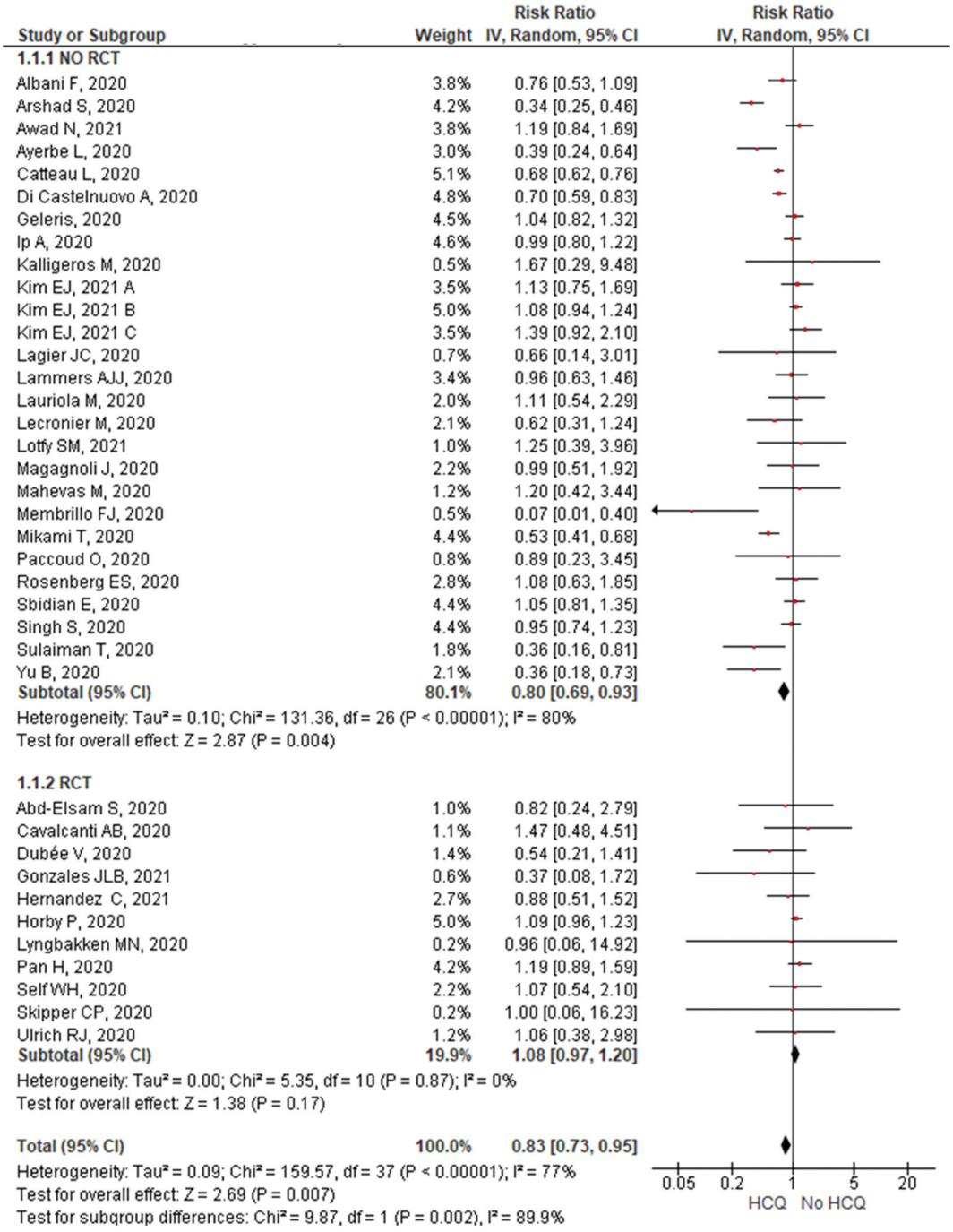

Figure 2. Forest plot for association of hydroxychloroquine use with COVID-19 mortality (random effects). HCQ means hydroxychloroquine; RCT means randomized clinical trial; SE means standard error. Data from 3 independent cohorts were extracted from the study of Kim et al. [46]

HCQ and death: subgroup analyses

Subgroup analyses according to the main features of primary studies are presented in Table 2, separately for RCT and cohort studies. In cohort studies, the association of HCQ with lower mortality was observed with very low differences in all subgroups, with the exception of dose grouping. The reduced mortality observed in cohort studies was in fact confined to studies that used a daily dose ≤400 mg (as estimated in days of treatment after the first, in which a higher (double for most) dose of drug was administered); pooling n = 5 studies which used more than 400 mg of HCQ daily resulted in an overall measure of association equal to 1.05 (95%CI: 0.73 to 1.53; Table 2 and Supplementary Figure 1A). Also, pooling studies which used more than 4,000 mg of HCQ during the entire phase of treatment set an overall measure of association equal to 0.86 (95%CI: 0.66 to 1.14) in comparison with studies which used ≤4,000 mg (0.67; 95%CI: 0.52 to 0.87) (Table 2 and Supplementary Figure 2A). Subgroup analysis according to both dosing (≤400 mg/d or >400 mg/d) and duration of treatment (≤5 days or

Table 2. Pooled analysis in subgroups of studies.

	Cohort studies				RCT			
	N	I^2	Pooled RR* [95% CI]	P values§	N	I^2	Pooled RR* [95% CI]	P values§
ALL STUDIES	27#	80%	0.80 [0.69, 0.93]		11	0%	1.08 [0.97, 1.20]	
Peer-reviewed Studies								
Pre-printed studies	4	80%	0.68 [0.41, 1.12]	0.51	3	0%	0.73 [0.46, 1.15]	0.08
Peer-reviewed studies	23	81%	0.81 [0.69, 0.95]		8	0%	1.10 [0.99, 1.23]	
Adjustment								
Not adjusted	4	4%	1.02 [0.74, 1.41]	0.15	-	-	-	
Adjusted	23	82%	0.78 [0.67, 0.92]		11	0%	1.08 [0.97, 1.20]	
Sample size								
<1,000 COVID-19 patients	16	42%	0.93 [0.75, 1.15]	0.08	9	0%	0.89 [0.64, 1.23]	0.21
≥1,000 COVID-19 patients	11	90%	0.72 [0.58, 0.88]		2	0%	1.10 [0.99, 1.24]	
Mortality incidence in the sample								
≤20%	15	79%	0.80 [0.62, 1.03]	0.93	9	0%	1.08 [0.85, 1.36]	0.99
>20%	12	83%	0.81 [0.66, 0.99]		2	0%	1.08 [0.96, 1.22]	
HCQ treatment in the sample								
≤33.3%	10	54%	0.89 [0.69, 1.14]	0.02	1	-	1.09 [0.96, 1.23]	0.73
33.3–66.6%	11	74%	0.95 [0.79, 1.16]		10	0%	1.04 [0.84, 1.29]	
>66.6%	6	89%	0.52 [0.35, 0.75]		-	-	-	
HCQ treatment, duration								
≤ 5 days	12	85%	0.67 [0.52, 0.87]	0.35	4	0%	0.94 [0.56, 1.59]	0.61
> 5 days	8	46%	0.79 [0.63, 1.00]		7	0%	1.08 [0.97, 1.21]	
HCQ treatment, total dose								
≤4,000 mg	13	84%	0.67 [0.52, 0.87]	0.19	6	0%	0.85 [0.60, 1.21]	0.16
>4,000 mg	5	80%	0.86 [0.66, 1.14]		5	0%	1.10 [0.99, 1.24]	
HCQ treatment, daily dose								
≤400 mg	15	83%	0.69 [0.57, 0.85]	0.050	6	0%	0.85 [0.60, 1.19]	0.14
>400 mg	5	0%	1.05 [0.73, 1.53]		5	0%	1.11 [0.99, 1.24]	
Country				0.14				0.81
Asia	3	46%	0.48 [0.24, 0.95]		-	-	-	
Europe	12	59%	0.74 [0.62, 0.89]		3	2%	1.06 [0.89, 1.27]	
North America	12	86%	0.91 [0.72, 1.15]		5	0%	0.94 [0.56, 1.59]	
Others	-	-	-		3	0%	1.18 [0.90, 1.56]	

*Relative risk for mortality in HCQ versus non HCQ patients. #Three independent cohorts have been included in the meta-analysis from the article of Kim et al.[46] §for difference among subgroups.
HCQ means hydroxychloroquine; NA means Not Applicable; RCT means randomized clinical trial.

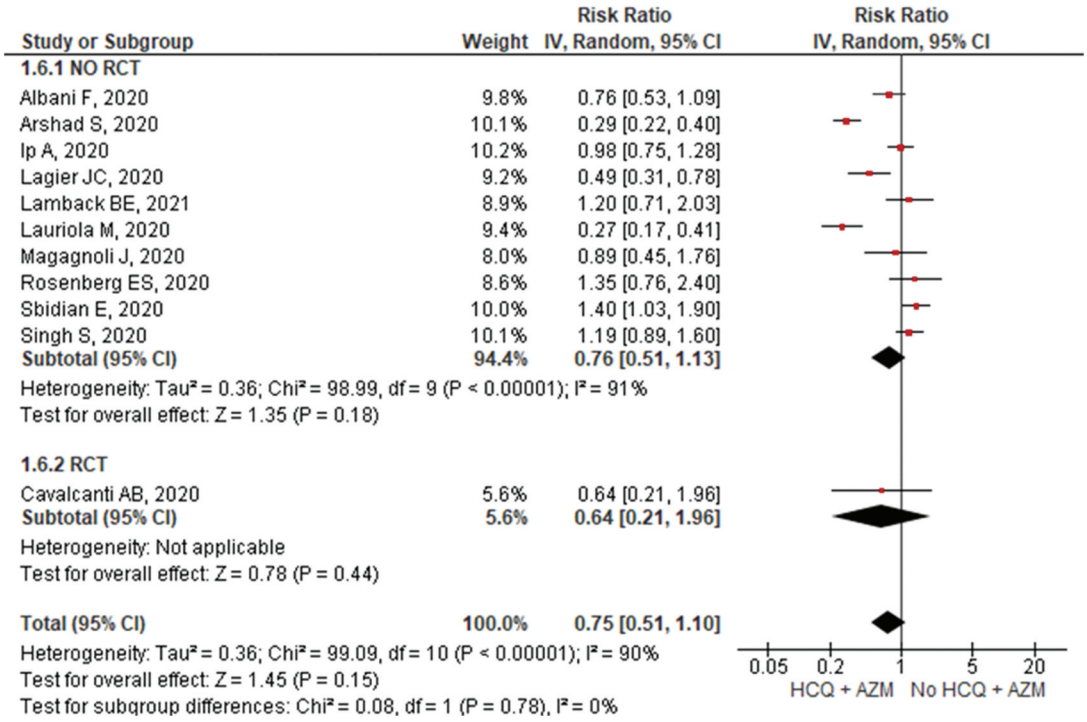

Figure 3. Forest plot for association of hydroxychloroquine + azithromycin use with COVID-19 mortality (random effects). AZM means azithromycin; HCQ means hydroxychloroquine.

>5 days) confirms the modification of effect by dosing of HCQ in cohort studies, with a maximum protection observed in studies with ≤400 mg/d and ≤5 days (0.65, 95%CI: 0.48 to 0.89; Supplementary Figure 3A) as opposed to studies with >400 mg/d and >5 days (0.98, 95%CI: 0.54 to 1.77; Supplementary Figure 3A).

Although not statistically significant, a modification of effect by dosing was also observed in RCTs: the association with mortality for HCQ versus non HCQ use was 0.85 (95 CI%: 0.60 to 1.19) in 6 RCTs which used ≤400 mg/day and 1.11 (95 CI%: 0.99 to 1.24) in 5 RCTs which used >400 mg/day (Table 2 and Supplementary Figure 1B). Similar findings have been observed according to total dose of HCQ (Table 2 and Supplementary Figure 2B).

HCQ+AZM and death

Figure 3 reported random forest for 11 studies (one RCT) comparing HCQ+AZM. Use of the combination HCQ +AZM was associated with 25% lower mortality risk, with very large uncertainty (pooled risk ratio: 0.75, 95% CI: 0.51 to 1.10; P for testing of overall effect = 0.15; high level of heterogeneity: I^2 = 90%, random effects; Figure 3).

By visual inspection of funnel plots (Supplementary Figure 4), we failed to observe any selection bias for both meta-analyses.

Adverse events of HCQ

Description of adverse effects as investigated in RCTs is reported in Supplementary table 4. Pooled analyses of the relationship between HCQ and incidence of adverse effects are reported in Supplementary Figure 5. HCQ use was associated with an increased risk of adverse effects of any type (pooled risk ratio: 1.95, 95% CI: 1.25 to 3.04; P for testing of overall effect = 0.003; Supplementary Figure 5A). On the contrary, patients treated with HCQ in RCTs showed a similar rate of serious adverse events, as that non-treated with HCQ (pooled risk ratio: 1.12, 95%CI: 0.88 to 1.44; P for testing of overall effect = 0.36; Supplementary Figure 5B).

Discussion

In a meta-analysis of 11 RCTs, the use of HCQ was not associated with mortality, but it was

associated with a 20% lower risk of mortality in a meta-analysis of 25 observational studies.

The potential for selection bias in observational studies is an important issue. The decision from the clinicians to utilize or not a drug may depend on comorbidities and baseline risk of the patient. However, in the pandemic, and in the absence of guidelines and specific anti COVID-19 drugs, allocation of HCQ in observational studies was not associated systematically with a lower or higher baseline risk profile. For example, in the CORIST study [7] patients receiving HCQ were more likely younger and less likely had ischemic heart disease, cancer or chronic kidney disease, but, on the contrary, they were more likely men and had higher levels of C-reactive protein. As a consequence, it is not clear if in that particular study HCQ patients were potentially at higher or lower risk of a negative prognosis. In attempting to account for baseline differences between patients who received HCQ and those who did not, we used the results for adjusted measure of association for each study, and this was possible for 23 out of 27 studies included in the meta-analysis of observational studies. After the exclusion of 4 unadjusted studies [21,24,39,48], the strength of the overall association of HCQ with mortality was unchanged. Although we attempted to control for potential confounding factors inherent to patient and clinical characteristics, it is possible that unmeasurable confounding still remains, and this may explain the different findings between the results of observational and RCT studies. However, it is hard to determine which are, if any, the unmeasured characteristics that have confused so strongly the association between HCQ and mortality in COVID-19 that was observed in observational studies. In fact, these features must be a) unmeasured in observational studies; b) associated with mortality in COVID-19 and c) associated with HCQ use, in a way that when the risky conditions are present, the clinicians tend systematically to avoid using HCQ. For example, HCQ is contraindicated in patients with cardiomyopathy but this condition has been mostly measured in observational studies and was not identified as a risk factor for mortality in COVID-19 patients.

The dissimilar findings between observational and RCTs we found might also be explained by differences in HCQ dosage [54]. Interestingly, we observed that the reduced mortality associated with HCQ treatment in observational studies was actually confined to studies that used a daily dose ≤400 mg, or a total dose ≤4,000 mg. Obviously these two conditions largely overlapped in studies, that we can now designate as 'at low HCQ dosing'. Remarkably, 6 over 11 RCTs are in this category, but the others 5 are not, including the RECOVERY [14] and the SOLIDARITY study [9] which used 800 mg/day for 9 or 10 days (after the first), respectively, and a total dose of 9200 or 10,000 mg of HCQ (including the dose at first day) respectively, a very high dose regimen as compared to the rest of studies, particularly of the observational ones. Interestingly, though not statistically significant, the use of HCQ was associated with a 15% lower mortality in the six RCTs which used HCQ low doses, but a 10% higher mortality in the five RCTs which used high doses of the drug.

The possibility that HCQ reduced the risk of negative prognosis in COVID-19 patients when only

administered at 'low dose' cannot be here undoubtedly proven, but it is a plausible hypothesis that may explain, at least in part, the different results between observational and RCT studies. More importantly, it might be useful in disentangling the debate on HCQ use in COVID-19. If our hypothesis of 'low doses-short duration' is correct, it follows that immunomodulation and inflammation occurs quite early after infection with SARS-CoV-2, as also suggested by the benefit of HCQ treatment in patients with higher C-reactive protein [7]. In this line, it is of interest that the studies with low doses and long duration of HCQ treatment also provided an overall negative association with mortality. This finding would also support the immunomodulatory hypothesis as potential mechanism of HCQ action [55] as it implies a cytokine rebound when the treatment is stopped. If this happens at a critical moment, it could worsen the patient clinical condition, thus vanishing the (potential) beneficial effect of HCQ [56].

High levels of HCQ administration were used in RCTs to maximize the antiviral activity of the drug that was considered to be the main mechanism of action of HCQ in this context. In some studies, the inverse association of HCQ with inpatient mortality was more evident in elderly, in patients who experienced a higher degree of COVID-19 severity or having elevated C-reactive protein levels [7], suggesting that the anti-inflammatory potential of HCQ may have had a more important role than its antiviral properties. HCQ, indeed, besides antiviral activity, may have both anti-inflammatory and anti-thrombotic effects [3]. This can justify its effect in reducing mortality risk, since Sars-Cov-2 can induce pulmonary microthrombi and coagulopathy, that are a possible cause of its severity [57,58] and the lack in preventing SARS-CoV-2 infection after exposure [59]. On the other end, national guidelines suggested to use HCQ 200 mg twice daily for 5–7 days probably to maintain a better risk/benefit profile hypothesizing that low doses could be more effective and safer. Indeed, non-sigmoidal, bell-shaped dose-response curves are possible with drugs having complex biological effects, multiple-binding sites or cellular and organ targets. On the other hand, anti SARS-2-CoV-2 activity of HCQ has been confirmed in Vero cells [60]. HCQ is also reported to reduce secretion of IFN-γ and IL-17 in activated Th1 and Th17 cells, respectively [61–62].

The concomitant use of azithromycin seems neither to increase nor decrease the effect, if any, of the HCQ since the combination of the two drugs was associated with a lower mortality risk to a very similar extent to that observed for HCQ alone; but the assumption is inconclusive because of the very large uncertainty in the findings.

A main concern with HCQ treatment have been its side effects, in particular, a severe cardiovascular toxicity. Indeed, HCQ can cause prolongation of the QT interval on electrocardiogram [61], which could be exacerbated by coadministration with azithromycin, widely prescribed as co-treatment in Covid-19 treatment. Our meta-analysis of data from RCTs, that allowed a proper evaluation of side effects, shows that use of HCQ was associated with an increase in side effects of any type, but not of major type, including cardiovascular events. This despite the high prevalence of cardiovascular disease in patients with COVID-19 or the high dose used in RCTs.

This meta-analysis has the strength of including all available studies that had not been included in previous meta-analyses, especially cohort studies [11,63]; findings on RCTs are in line with that from previous meta-analyses [63,64]. As a novelty, our meta-analysis considers modification of effect by dosing of HCQ. We recognize that the results obtained in observational or RCT studies were different and have discussed the possible implications of the difference observed. The pooled findings from cohort studies suffer of a high degree of heterogeneity, possibly depending from various factors, including setting, size of the study, dosing of HCQ and type of patients (in some studies HCQ was started right after the diagnosis but in others a large number of patients treated were already in intensive care).

Overall, the data of our meta-analysis suggest, though not proving, that a proportion of hospitalized COVID-19 patients might benefit of a treatment with low-dosage HCQ that, interestingly, is the same dosage as that currently used in some immuno-inflammatory and autoimmune diseases [65]. Research aimed to define the category of potentially HCQ-benefited patients may be worth being done in view of the persistent poverty of active anti-COVID-19 pharmacological treatments.

Conclusions

In conclusion, HCQ was not associated with decreased mortality in COVID-19 patients when RCTs studies were pooled (high level of certainty of evidence), but it was associated with 20% mortality reduction when cohort studies were combined (low level of certainty of evidence). The negative association with mortality was mainly apparent by pooling observational studies using lower doses of HCQ. Use of HCQ was not associated with severe adverse events.

Finding from cohort studies should be considered with caution because the overall strength of evidence grade was judged to be low.

At present, this is the largest comprehensive quantitative overview on the association of HCQ with mortality in COVID-19 patients, and our findings underscoring HCQ dosage effects might help

disentangling the debate on HCQ use and encourage the planning of RCTs using low doses of HCQ (not necessarily with a short duration of the treatment) in hospitalized COVID-19 patients.

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ORCID

Augusto Di Castelnuovo http://orcid.org/0000-0001-9767-7998
Simona Costanzo http://orcid.org/0000-0003-4569-1186
Antonio Cassone http://orcid.org/0000-0002-0434-0761
Roberto Cauda http://orcid.org/0000-0002-1498-4229
Giovanni De Gaetano http://orcid.org/0000-0002-7823-1402
Licia Iacoviello http://orcid.org/0000-0003-0514-5885

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Data availability

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