Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

European Journal of Internal Medicine 000 (2020) 1–10

Contents lists available at ScienceDirect

European Journal of Internal Medicine

journal homepage: www.elsevier.com/locate/ejinme

Original article

Use of hydroxychloroquine in hospitalised COVID-19 patients is associated with reduced mortality: Findings from the observational multicentre Italian CORIST study

Q1 The COVID-19 RISK and Treatments (CORIST) Collaboration[1,*]

ARTICLE INFO

Keywords:
Hydroxychloroquine
COVID-19
Disease severity
Mortality
Inflammation

ABSTRACT

Background: Hydroxychloroquine (HCQ) was proposed as potential treatment for COVID-19.

Objective: We set-up a multicenter Italian collaboration to investigate the relationship between HCQ therapy and COVID-19 in-hospital mortality.

Methods: In a retrospective observational study, 3,451 unselected patients hospitalized in 33 clinical centers in Italy, from February 19, 2020 to May 23, 2020, with laboratory-confirmed SARS-CoV-2 infection, were analyzed. The primary end-point in a time-to event analysis was in-hospital death, comparing patients who received HCQ with patients who did not. We used multivariable Cox proportional-hazards regression models with inverse probability for treatment weighting by propensity scores, with the addition of subgroup analyses.

Results: Out of 3,451 COVID-19 patients, 76.3% received HCQ. Death rates (per 1,000 person-days) for patients receiving or not HCQ were 8.9 and 15.7, respectively. After adjustment for propensity scores, we found 30% lower risk of death in patients receiving HCQ (HR=0.70; 95%CI: 0.59 to 0.84; E-value=1.67). Secondary analyses yielded similar results. The inverse association of HCQ with inpatient mortality was particularly evident in patients having elevated C-reactive protein at entry.

Conclusions: HCQ use was associated with a 30% lower risk of death in COVID-19 hospitalized patients. Within the limits of an observational study and awaiting results from randomized controlled trials, these data do not discourage the use of HCQ in inpatients with COVID-19.

1. Introduction

The aminoquinoline hydroxychloroquine (HCQ) has been extensively used in the treatment of malaria and is currently widely used to treat autoimmune diseases like rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) and anti-phospholipid syndrome (APS), due to its immunomodulatory and anti-thrombotic properties [1]. More recently, a promising role of HCQ has been suggested in viral infections [2], since it directly inhibits viral entry and spread in several *in vitro* and *in vivo* models. Due to these properties, HCQ has been used in Ebola virus disease [3,4], human immunodeficiency virus (HIV) infection [5], SARS-CoV-1 infection and the Middle East Respiratory Syndrome (MERS) [6,7] and gained worldwide attention as a possible therapy in COVID-19 patients [8].

HCQ might inhibit the intracellular glycosylation of ACE 2, the receptor used by the SARS-CoV-2 virus to enter the cells, resulting in a reduced ligand recognition and internalization of the virus [7] and exerting a possible protective role in SARS-CoV-2 infection. Moreover, due to its immunomodulatory, anti-inflammatory and anti-thrombotic effects, HCQ could also modulate the severity of the disease. However, the exact mechanism for the potential benefit in COVID-19 is largely speculative [9] and might be counterbalanced by adverse effects, mainly cardiovascular [10,11], so that the net balance of this drug's use remains to be established.

The American Food and Drug Administration (FDA) allowed Chloroquine (CQ) phosphate and HCQ to be provided to certain hospitalized patients because these drugs may possibly help patients with severe COVID-19 [12]. The European Medicines Agency (EMA) authorized the use of CQ and HCQ for COVID-19 in clinical trials or as emergency use [13], while the Italian Drug Agency (AIFA) stated in this emergency phase that therapeutic use of HCQ might be considered in COVID-19 patients, both in those with mild presentation managed at home and in

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https://doi.org/10.1016/j.ejim.2020.08.019
Received 28 June 2020; Received in revised form 19 August 2020; Accepted 20 August 2020
Available online xxx

hospitalized patients [14]. In clinical practice, HCQ rather than chloroquine has been used because of its more potent antiviral properties and better safety profile [15].

However, in the light of a recent publication [16], that was later retracted [17], on the lack of safety and efficacy of HCQ in the treatment for COVID-19 patients the Executive Group of the Solidarity Trial decided to implement a temporary pause of the HCQ arm within the trial as a precaution, while the safety data is being reviewed [18]. Similarly, the Italian drug Agency AIFA decided to suspend the authorization to use HCQ for COVID-19 treatment outside clinical trials [19].

Recent reviews of clinical trials or observational studies [20–24] have reported insufficient and often conflicting evidence on the benefits and harms of using HCQ to treat COVID-19 and concluded that as such, it was impossible to determine the balance of benefits to harm. Until now, although several trials had been started on the use of CQ and HCQ in COVID-19, only few of them have been published [25] on small numbers of patients or on surrogate endpoints or in exposed subjects for prophylaxis use [26].

While waiting the results from ongoing randomized clinical trials (RCT) to define the efficacy in preventing hard endpoints of this treatment so widely used during the emergency phase of the COVID-19 pandemic, powered retrospective observational studies performed in different geographical and disease conditions may still be useful to shed light on this debate. Two retrospective observational studies, both conducted in the New York metropolitan region, did not report any significant association between HCQ use and rates of intubation or death [27,28].

No data are presently available from large cohorts of patients in Italy, which represents one of the most affected countries in terms of total deaths for COVID-19 in the world [29]. We undertook a multicenter Italian collaboration [30] to investigate the relationship between underlying risk factors and COVID-19 outcomes, and to evaluate the association between different drug therapy and disease severity and/or mortality. We report here the results obtained in 3,451 hospitalized COVID -19 patients receiving or not HCQ treatment.

2. Material and methods

2.1. Setting

This national retrospective observational study was conceived, coordinated and analysed within the CORIST Project (ClinicalTrials.gov ID: NCT04318418, 30]. The study was approved by the institutional ethics board of the Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Neuromed, Pozzilli, and of all recruiting centres. Data for the present analyses were provided by 33 hospitals distributed throughout Italy (listed in the supplementary file). Acceptance to participate in the project or to provide data for the present analysis was not related to the use of CQ/HCQ. Each hospital provided data from hospitalized patients who had a positive test result for the SARS-CoV-2 virus at any time during their hospitalization from February 19 to May 23, 2020. The follow-up continued through May 29, 2020.

2.2. Data sources

We developed a cohort comprising 3,971 patients with laboratory-confirmed SARS-CoV-2 infection in an in-patient setting. The SARS-CoV-2 status was declared based on laboratory results (polymerase chain reaction on nasopharyngeal swab) from each participating hospital. Clinical data were abstracted at one-time point from electronic medical records or charts, and were collected using either a centrally designed electronic worksheet or a centralized web-based database. Collected data included patients' demographics, laboratory test results, medication administration, historical and current medication lists, historical and current diagnoses, and clinical notes. In addition, specific information on the most severe manifestation of COVID-19 occurred during hospitalization was retrospectively captured. Maximum clinical severity observed was classified as mild pneumonia; or severe pneumonia; or acute respiratory distress syndrome (ARDS) [31]. Specifically, we obtained the following information for each patient: hospital; date of admission and date of discharge or death; age; sex; the first recorded inpatient laboratory tests at the entry (creatinine, C-reactive protein); past and current diagnoses (myocardial infarction, heart failure, diabetes, hypertension, respiratory disease and cancer) and current drug therapies for COVID-19 – HCQ, lopinavir/ritonavir or darunavir/cobicistat, remdesevir, tocilizumab or sarilumab, corticosteroids, heparin, and for comorbidities (insulin, anti-hypertensive treatments, aldosterone receptor antagonists, diuretics, statins, sacubitril/valsartan). A diagnosis of pre-existing cardiovascular disease was based on history of myocardial infarction or heart failure. Chronic kidney disease was classified as: stage 1: kidney damage with normal or increased glomerular filtration rate (GFR) (>90 mL/min/1.73 m^2); stage 2: mild reduction in GFR (60-89 mL/min/1.73 m^2); stage 3a: moderate reduction in GFR (45-59 mL/min/1.73 m^2); stage 3b: moderate reduction in GFR (30-44 mL/min/1.73 m^2); stage 4: severe reduction in GFR (15-29 mL/min/1.73 m^2); stage 5: kidney failure (GFR <15 mL/min/1.73 m^2 or dialysis). For statistical analysis, stages 3a and 3b and stages 4 and 5 were combined. GFR was calculated by the Chronic Kidney Disease Epidemiology Collaboration (CKD-Epi) equation. Patients were defined as receiving HCQ if they were receiving it at admission to hospital or received it during the follow-up period. According to the AIFA guidance [14], HCQ was administered at dose of 400 mg x 2/day or x4/day the first day, and 200 mg x 2/day from the second day onwards for at least 5 to a maximum of 10 days, according to the clinical evolution of the disease.

2.3. Statistical analysis

The study index date was defined as the date of hospital admission. Index dates ranged from February 19, 2020 to May 23, 2020. The study end point was the time from study index to death. The number of patients who either died, or had been discharged alive, or were still admitted to hospital as of May 29, 2020, were recorded, and hospital length of stay was determined. Patients alive had their data censored on the date of discharge or as the date of the respective clinical data collection. Data were censored at 35 days of follow up in n=330 (8.3%) patients with a follow up greater than 35 days.

Of the initial cohort of 3,971 patients, 350 patients were excluded from the analysis because they had at least one missing data at baseline or lost to follow up on HCQ use (N=94), other drug therapies for COVID-19 (n=265), time to event (n=59), outcome (death/alive, n=8), COVID-19 severity (n=4), age (n=4 with missing data and n=2 with age<18 years) or sex (n=2). Of the remaining 3,621 patients, 170 patients died or were discharged within 24 hours after presentation, and were also excluded from the analysis.

At the end, the analysed cohort consisted of n=3,451 patients. In patients not included in the analysis (n=520), as unique difference with the analysed group, the prevalence of diabetics (19.9% vs 14.8%, P=0.0066) and, to a less extent, of men (62.3% vs 58.3%, P=0.081) was higher. Out of 3,541 patients, 295 (8.5%) had at least a missing value for covariates. Distribution of missing values was as follows: n=178 for C-reactive protein; n=69 for GFR; n=74 for history of ischemic disease; n=64 for history of chronic pulmonary disease; n=51 for diabetes; n=51 for hypertension and n=56 for cancer. We used multiple imputation techniques (SAS PROC MI, n=10 imputed datasets; and PROC MIANALYZE) to maximize data availability. As sensitivity analysis, we also conducted a case-complete analysis on 3,156 patients.

Cox proportional-hazards regression models were used to estimate the association between HCQ use and death. Since multiple imputation was applied, the final standard error was obtained using the Rubin's rule based on the robust variance estimator in Cox regression [32]. The proportional hazards assumption was assessed using weighed Schoen-

feld residuals, and no violation was identified. To account for the non-randomized HCQ administration and to reduce the effects of confounding, the propensity-score method was used. The individual propensities for receiving HCQ treatment were assessed with the use of a multivariable logistic-regression model that included age, sex, diabetes, hypertension, history of ischemic heart disease, chronic pulmonary disease, GFR, C-reactive protein, hospitals clustering and use of other drug therapies for COVID-19 (lopinavir/ritonavir or darunavir/cobicistat, remdesivir, corticosteroids, tocilizumab or sarilumab). Associations between HCQ treatment and death was then appraised by multivariable Cox regression models with the use of propensity-score and further controlling for hospitals clustering as random effect (frailty model). The use of a frailty model was chosen as suggested in [33]. The primary analysis used inverse probability by treatment weighting; the predicted probabilities from the propensity-score model was used to calculate the stabilized inverse-probability-weighting weight [34]. Stabilized weights were normalized so that they added up the actual sample size. Secondary analyses used propensity-score stratification (n=5 strata) or multivariable Cox regression analysis or multivariable logistic regression analyses comparing death versus alive patients, or accounted for hospitals clustering via stratification or by robust sandwich estimator. Pre-established subgroup analyses were conducted according to age or sex of patients, degree of COVID-19 severity experienced during the hospital stay, C-reactive protein at basal or other drug therapies for COVID-19. Hospitals were clustered according to their geographical distribution, as illustrated in Table 1. To quantify the potential for an unmeasured confounder to render apparent statistically significant hazard ratio non-significant, the E-value was calculated [35]. Analyses were performed with the aid of the SAS version 9.4 statistical software for Windows.

3. Results

We included in the final current analyses 3,451 patients who were hospitalized with confirmed SARS-CoV-2 infection at 33 clinical centres across Italy and either died, had been discharged, or were still in hospital as of May 29, 2020. Of these patients, 2,634 (76.3%, range among hospitals 53.2% to 93.6%) received HCQ. Timing of the first dose of HCQ after presentation to the hospital was 1 day for the large majority of centres, and 2 to 3 days for the others. HCQ was administered in all centres at the dose of 400 mg/day (in one centre however it was used at the dose of 600 mg/day and in another at the dose of 600 mg/day but only in patients younger than 65 years). Duration of treatment ranged from 5 to 15 days (with 10 days as the modal value). The drug used was HCQ in all hospitals.

Baseline characteristics according to HCQ use are shown in **Table 1**. Patients receiving HCQ were more likely younger, men and had higher levels of C-reactive protein and less likely had ischemic heart disease, cancer or stages 3a or greater chronic kidney disease (**Table 1**). Patients receiving HCQ more likely received another drug for COVID-19 treatment (78.4%; lopinavir/ritonavir or darunavir/cobicistat, remdesevir, tocilizumab or sarilumab, corticosteroids), in comparison with non-HCQ patients (46.3%; P<0.0001; Table 1).

The unadjusted differences and differences adjusted by propensity scores between HCQ-treated and non-HCQ treated patients for each variable included in the propensity score are shown in **Fig. 1**. All the pre-treatment differences disappeared after adjustment by propensity score weighting. The C-statistic of the propensity-score model was 0.74.

3.1. Primary outcome

Out of 3,628 patients, 576 died (16.7%), 2,390 were discharged alive (69.3%) and 485 (14.1%) were still at the hospital. The median follow-up was 14 days (interquartile range 8 to 22; range 2 to 35; 55,388 person-days). Death rate (per 1,000 person-days) was 8.9 in HCQ and 15.7 in non-HCQ patients (**Table 2**). At univariable analysis, hazard ratio for mortality was 0.56 (95%CI: 0.47 to 0.67). In the primary multivariable analysis with inverse probability weighting according to the propensity score, HCQ use was associated with a 30% (95%CI: 16% to 41%) reduction in death risk (**Fig. 2**, **Table 2**, E-value=1.67). Secondary multivariable analyses yielded very similar results (**Table 2**), as well as case-complete analyses restricted to the 3,156 patients without missing data (**Table 2**). Considering secondary multivariable analyses overall, HR for mortality associated with HCQ ranged between 0.64 to 0.70, according to type of analyses. Control of hospitals clustering with different approaches also yielded similar results for the primary analysis (HR=0.71, 95%CI: 0.59 to 0.85 when hospitals clustering was stratified for and HR=0.69, 95%CI: 0.54 to 0.88 with the robust sandwich estimator).

Subgroup analyses are presented in **Table 3**. HCQ use remained consistently associated with reduced mortality in almost all subgroups. The inverse association of HCQ with inpatient mortality is slightly more evident in women, elderly and in patients who experienced a higher degree of COVID-19 severity. It was absent in-patient with C-reactive protein <10 mg/L and clearly confined to patients with elevated C-reactive protein (**Table 3**).

4. Discussion

In a large cohort of 3,451 patients hospitalized for COVID-19 in 33 clinical centers all over Italy, covering almost completely the period of the hospitalization for COVID-19, the use of HCQ was associated with a significant better survival. In-hospital crude death rate was 8.9 per 1,000 person-day for patients receiving HCQ and 15.7 for those who did not. After adjustment for known possible confounders, we observed a 30% reduction in the risk of death in patients receiving HCQ therapy as compared with those who did not.

Our findings provide clinical evidence in support of guidelines by Italian and several international Societies suggesting to use HCQ therapy in patients with COVID-19. However, the observed associations should be considered with caution, as the observational design of our study does not allow to fully excluding the possibility of residual confounders. Large randomized clinical trials in well-defined geographical and socio-economic conditions and in well-characterized COVID-19 patients, should evaluate the role of HCQ before any firm conclusion can be reached regarding a potential benefit of this drug in patients with COVID-19.

Over 76% of patients received HCQ either alone or in combination with other drugs. They were more likely to be younger, men and with higher levels of C reactive protein at entry, while less likely had pre-existing comorbidities such as ischemic heart disease, cancer and severe chronic kidney disease, as compared to patients not receiving the drug. We adjusted our analyses for possible confounders, including age, sex, diabetes, hypertension, history of ischemic heart disease, chronic pulmonary disease, chronic kidney disease, C-reactive protein and additional treatments for COVID-19, and took into account possible differences across centres by either adjustment or stratification. To minimize bias due to the observational design, we used different analytical approaches aiming at creating an overall balance between comparison groups. Finally, we tried to limit bias due to missing data by using a multiple imputation approach, but in no case, the result was changed. Despite all these precautions, we recognize the possibility, however, of residual unmeasured confounders affecting results.

Systematic reviews of small clinical trials had reported contrasting results that were however scarcely reliable because of poor designs [20–25]. The HCQ doses tested in a Chinese randomized clinical trial [25] were approximately double as compared to that used in our study (1200 mg vs 800 mg as loading dose, 800 mg vs 400 mg as maintenance dose) for twice the time (14-21 days versus 7-10 days). National guidelines in Italy suggest to use HCQ 200 mg

Table 1
General characteristics of COVID-19 patients at baseline, according to hydroxychloroquine use.

	Hydroxychloroquine		
Characteristic	No (N=817)	Yes (N=2,634)	P-value unadjusted*
Age-median (IQR-yr.)	73 (58-83)	66 (55-77)	<.0001
Gender- no (%)			<.0001
Women	361 (44.2%)	940 (36.7%)	
Men	456 (55.8%)	1,694 (64.3%)	
Diabetes- no (%)			0.71
No	633 (77.5%)	2,090 (79.3%)	
Yes	162 (19.9%)	515 (19.6%)	
missing data	22 (2.7%)	29 (1.1%)	
Hypertension - no (%)			0.31
No	378 (46.3%)	1,294 (49.1%)	
Yes	416 (50.9%)	1,312 (49.8%)	
missing data	23 (2.7%)	28 (1.1%)	
Ischemic heart disease- no (%)			<.0001
No	610 (74.7%)	2,190 (83.1%)	
Yes	179 (21.9%)	398 (15.1%)	
missing data	28 (3.4%)	46 (1.8%)	
Chronic pulmonary disease- no (%)			0.21
No	666 (81.5%)	2,225 (84.5%)	
Yes	127 (15.5%)	369 (14.0%)	
missing data	24 (2.9%)	40 (1.5%)	
Cancer- no (%)			0.036
No	694 (84.9%)	2,338 (88.8%)	
Yes	101 (12.4%)	262 (9.9%)	
missing data	22 (2.6%)	34 (1.3%)	
CKD stage**- no (%)			<.0001
Stage 1	241 (29.5%)	970 (36.8%)	
Stage 2	281 (34.4%)	991 (37.6%)	
Stage 3a or stage 3b	180 (22.0%)	487 (18.5%)	
Stage 4 or stage 5	89 (10.9%)	143 (5.4%)	
missing data	26 (3.2%)	43 (1.6%)	
C Reactive Protein- no (%)			0.0003
<1 mg/L	104 (12.7%)	256 (9.7%)	
1-3 mg/L	120 (14.7%)	301 (11.4%)	
>3 mg/L	549 (67.2%)	1,943 (73.8%)	
missing data	44 (5.4%)	134 (5.1%)	
Lopinavir or Darunavir use			<.0001
No	621 (76.0%)	1,203 (36.7%)	
Yes	196 (24.0%)	1,431 (64.3%)	
Tocilizumab or Sarilumab use			<.0001
No	755 (92.4%)	2,160 (82.0%)	
Yes	62 (7.6%)	474 (18.0%)	
Remdesivir use			0.0015
No	808 (98.9%)	2,551 (96.9%)	
Yes	9 (1.1%)	83 (3.1%)	
Corticosteroids use			<.0001
No	596 (73.0%)	1,655 (62.8%)	
Yes	221 (27.0%)	979 (37.2%)	
Clusters of hospitals			<.0001
Northern regions (except Milan) (n)	169 (20.7%)	616 (23.4%)	
Milan (m)	161 (19.7%)	525 (19.9%)	
Center regions (except Rome) (c))	303 (37.1%)	747 (28.4%)	
Rome (r)	94 (11.5%)	390 (14.8%)	
Southern regions (s)	90 (11.0%)	356 (13.5%)	

(n) include hospitals of Novara, Monza, Varese, Pavia, Cremona and Padova; (m) include Humanitas Clinical and Research Hospital, Centro Cardiologico Monzino, and hospitals of San Donato Milanese (Milano) and Cinisello Balsamo (Milano); (c) include hospitals of Modena, Ravenna, Forlì, Firenze, Pisa, Chieti and Pescara; (r) include National Institute for Infectious Diseases "L. Spallanzani" and Università Cattolica del Sacro Cuore; (s) include hospital of Napoli, Pozzilli (Isernia), Acquaviva delle Fonti (Bari), Foggia, Taranto, Catanzaro, Catania and Palermo *Chi-square test. **Stage 1: Kidney damage with normal or increased glomerular filtration rate (GFR) (>90 mL/min/1.73 m^2); Stage 2: Mild reduction in GFR (60-89 mL/min/1.73 m^2); Stage 3a: Moderate reduction in GFR (45-59 mL/min/1.73 m^2); Stage 3b: Moderate reduction in GFR (30-44 mL/min/1.73 m^2); Stage 4: Severe reduction in GFR (15-29 mL/min/1.73 m^2); Stage 5: Kidney failure (GFR < 15 mL/min/1.73 m^2 or dialysis).

twice daily for at least 5-7 days in patients over 70 years and/or with co-morbidities (chronic obstructive pulmonary disease, diabetes, cardiovascular disease) even with mild respiratory symptoms or with radiographically documented pneumonia or in severe patients [36]. The lower doses of HCQ used in our centers, as suggested by Italian official guidelines [19,36], may have been both more effective and safer.

Two recently published large observational studies, both from large hospitals in New York City, showed no association between HCQ use and in-hospital mortality [27,28], and deserve specific discussion. In the

Table 2
Incidence rates and hazard ratios for death in COVID-19 patients, according to hydroxychloroquine use.

Multiple imputation analysis (N=3,451)				
	Death (N=576)	Patient at risk (N=3,451)	Person-days	Death Rate (x1,000 person-days)
Hydroxychloroquine				
No- no. (%)	190 (23.3%)	817 (100%)	12,084	15.7
Yes- no. (%)	386 (14.7%)	2,634 (100%)	43,304	8.9
Hazard ratio for death (HCQ *versus* non HCQ)				**HR (95% CI)**
Crude analysis				0.56 (0.47 to 0.67)
Multivariable analysis*				0.70 (0.58 to 0.85)
Propensity score analysis, inverse probability weighting** (primary analysis)				0.70 (0.59 to 0.84)
Propensity score analysis, stratification (n=5 strata)**				0.67 (0.56 to 0.81)
Odds ratio for death (HCQ *versus* non HCQ)				**OR (95% CI)**
Propensity score analysis, inverse probability weighting**				0.67 (0.54 to 0.82)
Case Complete Analysis (N=3,156)				
	Death (N=510)	**Patient at risk (N= 3,156)**	**Person-days**	**Death Rate (x1,000 person-days)**
Hydroxychloroquine				
No- no. (%)	170 (22.9%)	741 (100%)	11,050	15.4
Yes- no. (%)	340 (14.1%)	2,415 (100%)	39,274	8.7
Hazard ratio for death (HCQ *versus* non HCQ)				**HR (95% CI)**
Crude analysis				0.56 (0.46 to 0.67)
Multivariable analysis*				0.71 (0.59 to 0.86)
Propensity score analysis, inverse probability weighting**				0.64 (0.53 to 0.76)
Propensity score analysis, stratification (n=5 strata)**				0.68 (0.56 to 0.82)
Odds ratio for death (HCQ *versus* non HCQ)				**OR (95% CI)**
Propensity score analysis, inverse probability weighting**				0.67 (0.54 to 0.82)

Abbreviations: HR, hazard ratios; CI, confidence intervals. *Controlling for age, sex, diabetes, hypertension, history of ischemic heart disease, chronic pulmonary disease, chronic kidney disease, C-reactive protein, lopinavir/ritonavir or darunavir/cobicistat, tocilizumab or sarilumab, remdesivir or corticosteroids use as fixed effects and hospitals clustering as random effect. **Including hospitals clustering as random effect covariate.

study of Geleris et al. [27], the percentage use of HCQ was lower than in Italy; moreover, in both US studies [27,28] the drug was more frequently administered to patients with previous illnesses and a more severe presentation of the disease. Our cohort included milder pneumonia patients than the US population, due to between-country differences in indications to the drug for the beginning of therapy (*e.g.,* mild pneumonia in Italy versus only severe pneumonia and ARDS in the US). Concomitant use of other drugs for COVID-19 was very low in one study [27] and was not reported in the other study [28]. In our cohort, patients receiving HCQ were more likely treated with another drug for COVID-19 treat-

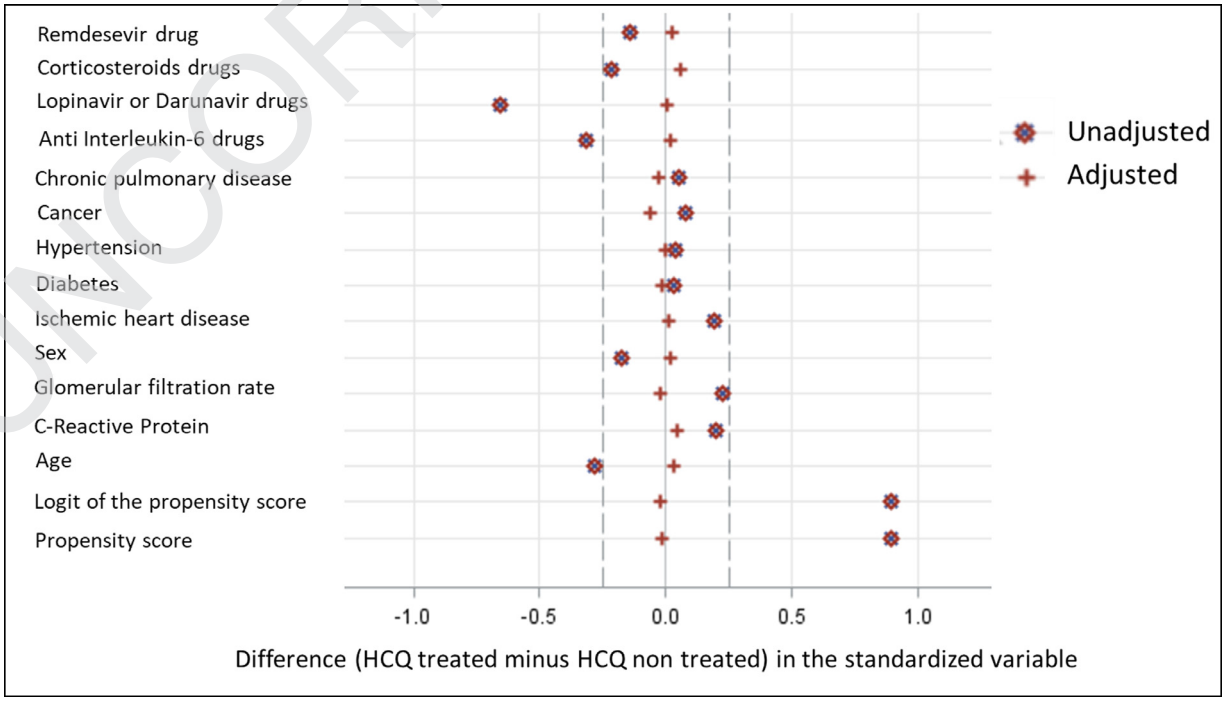

Fig. 1. The unadjusted standardized differences and standardized differences adjusted by propensity scores between HCQ-treated and non-HCQ treated patients for the variables included in the propensity score. All differences for the matched observations are within the recommended limits of –0.25 and 0.25, which are indicated by reference lines.

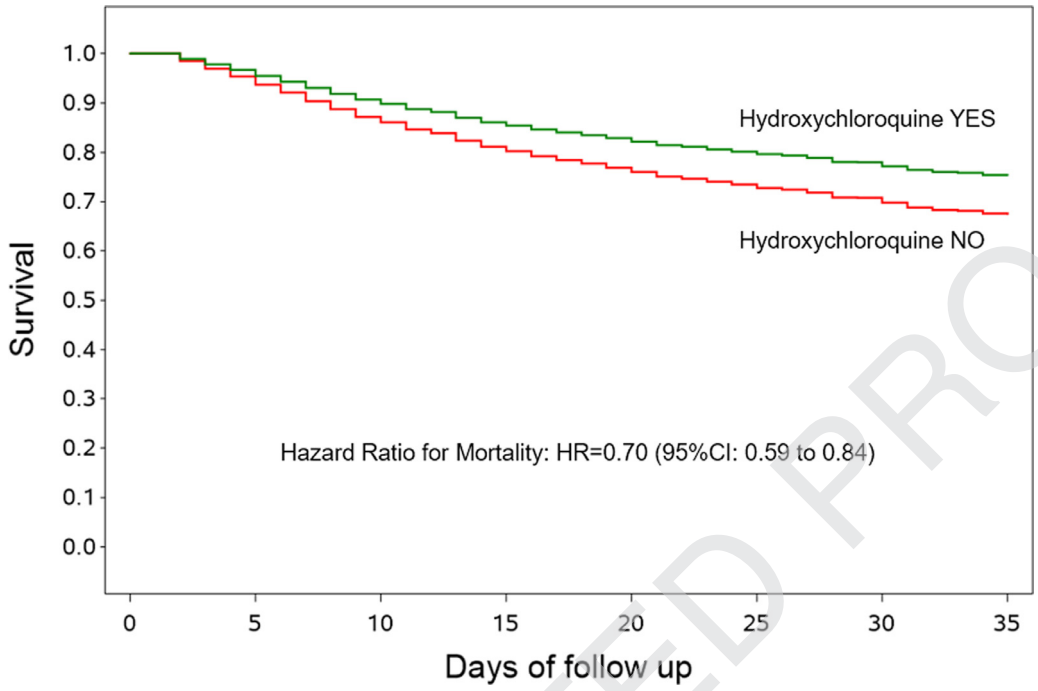

Fig. 2. Survival curves according to hydroxychloroquine use. The curves are adjusted by propensity score analysis (inverse probability for treatment weighting) and hospital index as random effect, and are generated using the first imputed dataset. The other imputed datasets are similar and thus omitted.

Table 3
Hazard ratios for mortality according to hydroxychloroquine use in different subgroups.

	Hydroxychloroquine NO (N=817)	Hydroxychloroquine YES (N=2,634)	
Subgroups	**No. death/patient at risk**	**No. death/patient at risk**	**HR (95% CI)***
Women	80/361	116/940	0.63 (0.46 to 0.86)
Men	110/456	270/1,694	0.74 (0.60 to 0.93)
Age <70 years	22/357	93/1,542	0.76 (0.50 to 1.16)
Age ≥70 years	168/460	293/1,092	0.68 (0.56 to 0.83)
Highest degree of COVID-19 severity experienced at hospital			
Mild pneumonia or less	28/424	40/1,358	0.70 (0.41 to 1.18)
Severe pneumonia	80/253	172/764	0.76 (0.58 to 0.99)
Acute respiratory distress syndrome	82/140	174/512	0.68 (0.52 to 0.90)
Use of other COVID-19 treatments^			
No	101/439	64/570	0.63 (0.45 to 0.88)
Yes	89/378	322/2,064	0.77 (0.61 to 0.99)
C-Reactive Protein at basal**			
<10 mg/L	56/412	125/1,138	1.23 (0.86 to 1.77)
≥10 mg/L	123/361	241/1,362	0.59 (0.47 to 0.73)

Abbreviations: HR, hazard ratios; CI, confidence intervals; *Propensity score analysis, inverse probability weighting, including hospital clustering as random effect covariate; multiple imputed analysis.
^Lopinavir/ritonavir or darunavir/cobicistat or tocilizumab or sarilumab or remdesivir or corticosteroids.
**Missing data for N=178. Frequencies and hazard ratios are based on a case complete analysis (N=3,273) without missing data for C-reactive Protein; multiple imputed analysis (N=3,451) yielded very similar results.

ment (78.4%), in comparison with non-HCQ patients (46.3%). Anyway, our findings are adjusted for concomitant other drugs use.

While the US studies were confined to one hospital only or a defined relatively small area in the Country, our study included 33 hospitals distributed all over Italy, covering regions with a high number of cases and a high intra-hospital mortality and regions with a lower burden of the disease. The participating Italian clinical centers have different healthcare facilities, different size, specialization, and ownership, and therefore quite closely represent the real-life Italian approach to COVID-19. Moreover, they differed for the percentage of use of HCQ and for the rate of in-hospital mortality that ranged between 34.1 and 1.5 per 1,000 persons/day. To consider this variability, we adjusted the analysis for recruiting center and performed a number of subgroup analyses. In all circumstances, the association between HCQ use and a reduced risk of death of about 30% was maintained. Quite interestingly, 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 especially having elevated C-reactive protein, suggesting that the anti-inflammatory potential of HCQ may have had more important role rather than its antiviral properties. HCQ, indeed, beside an antiviral activity, may have both anti-inflammatory and anti-thrombotic effects [8]. 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 [37,38] and the lack in preventing SARS-CoV-2 infection after exposure [26]

Nevertheless, large randomized clinical trials on the efficacy of HCQ on hard end-points are still lacking and the largest observational study showing no effect in reducing mortality has been retracted [16,17], Agencies have suspended clinical trials on the efficacy of HCQ on COVID-19 disease or have restricted its use only to patients included

in clinical trials, in the absence of an ample, serene and balanced discussion at international level.

Very recently, a large RCT has become available as a pre-print publication [39], reporting no beneficial effect of HCQ in patients hospitalized with COVID-19. However, the dose of HCQ used in that trial was almost the double of that administered in our real life conditions. A reduced mortality was also observed by other observational studies using low or intermediate doses of HCQ [40,41].

Moreover, in our study patients taking HCQ more frequently received other anti-COVID drugs, whose interaction in reducing mortality cannot be completely ruled-out. Of note, despite the higher dosage used, the RCT did not show any excess in ventricular tachycardia or ventricular fibrillation in the HCQ arm (39).

Therefore, it will be very important to compare results of studies with different mode of use and doses of HCQ, different characteristics of treated and untreated patients and different academic or real-world conditions.

4.1. Strengths and limitations

A major strength of this study is the large, unselected patient sample from 33 hospitals, covering the entire Italian territory. Patient sampling covered all the overt epidemic period in Italy. Several statistical approaches were used to overcome biases due to the observational nature of the investigation.

This study has however, several recognized limitations. The study population pertains to Italy, and the results obtained may not be applicable to other populations with a possibly different geographical and socio-economic conditions and natural history of COVID-19. Due to the retrospective nature of the study, some parameters were not available in all patients, and all in-hospital medications might have been not fully recorded. Moreover, although guidelines on the use of HCQ in COVID-19 patients had been published in Italy since the first phase of the pandemic, individual centers could have deviated from recommendations and used different doses or treatment schemes. We have no information on the HCQ doses used individually nor of their possible association with azithromycin. Moreover, adverse events possibly related to drug therapy were not collected, thus we cannot exclude bias due to therapy interruption because of side effects; we do not know whether some deaths could have been due to cardiovascular complications of HCQ. However, recent data on Italian wards showed that COVID-19 patients receiving HCQ and azithromycin had a QTc-interval longer than before therapy, but did not experience, during their hospital stay, any arrhythmic complications, such as syncope or life-threatening ventricular arrhythmias [42], a finding also reported by the RCT mentioned above (39).

Finally, the possibility of unmeasured residual confounding cannot be completely ruled-out. However, the E-value for the lower boundary of the confidence interval of our main result is 1.67, indicating that the confidence interval could be moved to include the null by a strong unmeasured confounder associated with both HCQ treatment and death with a risk ratio of 1.67-fold for each, above and beyond all the measured confounders. Weaker confounders, however, could not do so.

5. Conclusions

Our study, including a large real life sample of patients hospitalized with COVID-19 all over Italy, shows that HCQ use (200 mg twice/day) was associated with a 30% reduction of overall in-hospital mortality. In the absence of clear-cut results from controlled, randomized clinical trials, our data do not discourage the use of HCQ in inpatients with COVID-19. Given the observational design of our study, however, these results should be transferred with caution to clinical practice.

Source of funding

None.

Declaration of Competing Interests

None.

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Acknowledgments

We thank the participating clinical centres included in this cohort. This Article is dedicated to all the patients who suffered or died, often in solitude, due to COVID-19; their tragic fate gave us moral strength to initiate and complete this research.

The Authors alone are responsible for the views expressed in this Article. They do not necessarily represent the views, decisions, or policies of the Institutions with which they are affiliated.

Appendix 1

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ejim.2020.08.019.

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